LIVING ON THE INTERTIDAL / SUBTIDAL EDGE

ECOLOGY OF THE STALKED BARNACLE *Pollicipes pollicipes* AND OF THE SEA URCHIN *Paracentrotus lividus*

David Jacinto

Tese apresentada à Universidade de Évora
para obtenção do Grau de Doutor em Biologia

ORIENTADORA: *Professora Doutora Teresa Cruz*

ÉVORA, DEZEMBRO DE 2016

INSTITUTO DE INVESTIGAÇÃO E FORMAÇÃO AVANÇADA

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To my peers

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Abstract

This thesis contributes to the knowledge of temperate rocky shore ecology, with direct implications in the management and conservation of two important local marine resources that inhabit the very edge of subtidal and intertidal habitats on wave-swept rocky shores: the sessile filter feeding stalked barnacle *Pollicipes pollicipes* and the mobile keystone herbivore sea urchin *Paracentrotus lividus*. Research was conducted along gradients of wave exposure on rocky shores of Southern Europe.

The abundance of *P. lividus* was four times higher in SW Portugal than in NW Italy. Most of the variation in urchin abundance occurred at small spatial scales, probably shaped by habitat complexity. In SW Portugal, sea urchin attachment forces while in burrows were measured and related to burrow shape, urchin size and habitat. Burrowing behaviour enhances sea urchin attachment force and might be an adaptive response to hydrodynamic stress.

Abundance of *P. pollicipes* in SW Portugal is highly and positively related to wave exposure at local and regional scales. Predation and recruitment processes seem to be important drivers of these abundance patterns. A distribution model of *P. pollicipes* abundance in relation to wave exposure was developed for the SW coast of Portugal and might be used for improvement of its management and conservation.

Growth of *P. pollicipes* was studied by applying a novel method using fluorescent calcein for marking and to estimate growth. Growth rate decreased with barnacle size and was highly variable amongst individuals, particularly in smaller barnacles. No effect of shore level on barnacle growth was detected.

An assessment of the state of the fishery, conservation and management of the stalked barnacle in different regions of continental Portugal was made, highlighting an overall negative tendency of this state and recommending a change into a co-management system.

Resumo

Viver no limite intertidal / subtidal: ecologia do percebe *Pollicipes pollicipes* e do ouriço-do-mar *Paracentrotus lividus*

Esta tese contribui para o conhecimento da ecologia do litoral rochoso e tem implicações diretas na gestão e conservação de dois recursos marinhos locais que habitam o interface subtidal/intertidal de costas rochosas sujeitas a agitação marinha: o percebe *Pollicipes pollicipes*, animal séssil e filtrador e o ouriço-do-mar *Paracentrotus lividus,* animal móvel e herbívoro. Foram realizados vários estudos ao longo de gradientes de hidrodinamismo em costas rochosas do sul da Europa.

A abundância de *P. lividus* foi quatro vezes superior no sudoeste de Portugal relativamente ao noroeste de Itália. Grande parte da variação na abundância de *P. lividus* ocorreu a pequenas escalas espaciais, provavelmente influenciada pela complexidade do habitat. A força com que o ouriço-do-mar se fixa ao substrato foi medida no terreno no sudoeste de Portugal, tendo esta sido relacionada com a forma da depressão que ocupa, o tamanho individual e o habitat. O comportamento escavador desta espécie aumenta a sua força de fixação ao substrato e poderá ser uma resposta adaptativa ao hidrodinamismo..

A abundância de *P. pollicipes* na costa sudoeste de Portugal, a diferentes escalas espaciais, está relacionada de forma positiva com a agitação marinha, e é influenciada pela predação e pelo recrutamento desta espécie. Foi desenvolvido um modelo de distribuição e abundância de *P. pollicipes* para esta costa baseado na relação com a agitação marinha, cujos resultados podem ser usados para melhorar a gestão e conservação deste recurso.

Um novo método com recurso a calceina fluorescente foi desenvolvido para marcar percebes e estudar o seu crescimento. A taxa de crescimento diminuiu com o tamanho do animal, sendo altamente variável entre indivíduos, sobretudos nos de menores dimensões.

O estado da apanha, conservação e gestão do percebe em diferentes regiões de Portugal Continental apresentou uma tendência global negativa, e recomenda-se uma alteração para um sistema de cogestão deste recurso.

Contents

Chapter 1. General introduction

1.1 Wave-swept rocky shores, life in an environment of extremes

When visiting the shoreline, we are often struck by conflicting emotions and signals that puzzle our senses and reactions to the amazingness of such a landscape. The violence imposed by breaking waves on a stormy day, which seem capable of breaking apart the hardest bedrock and engulf the visitor in the most deadly manner, clearly contrasts to the appealing and gentle swipe of a translucent sea inviting one to a refreshing bath by the shore on a rather benign condition of a bright summer morning. Adding on to the continuous alteration of the wave regime that strikes the coast throughout the year, a predictable tidal cycle imposes a set of physical conditions upon life on the seashore at smaller temporal scales. Twice a day, the passing tidal wave exposes plants and animals to both terrestrial and marine worlds. When the tide is in, seashore life is bathed by incoming seawater that supplies nutrients and food, redistributes propagules and larvae, exposes them to marine predators, moderates ambient temperature and imposes a huge range of hydrodynamic forces. When the tide is out, the same rocky shore marine communities are exposed to air, desiccation, severe temperature fluctuations, intense solar radiation, and both marine and terrestrial predators.

Additionally, concentration of human activities on coastal areas carries a great stress to biological entities, either by coastal development, harvesting and fisheries pressure or pollution levels, amongst others. Despite all adversity, the coastal zone is one of the most productive and biodiverse marine ecosystems (Suchanek 1994), where communities maintain a dynamic equilibrium driven by biological interactions under variable environmental stressors (Menge 2000).

Marine organisms that inhabit wave-swept rocky shores have different mobility and feeding strategies. For example, fishes use fins and crabs use jointed legs to move fast and agilely in detriment of being relatively easily dislodged or pushed by breaking waves and having the need for ceaseless hiding and sheltering. Molluscs like limpets and nudibranchs, on the other hand, move slowly and crawl on a single ventral foot secreting mucus glue that provides adhesion to the substratum. Echinoderms, like sea urchins and sea stars, attach strongly and temporarily to the substratum through specialized organs, the podia or tube feet, which are the external appendages of the water-vascular system (Flammang 1995, Santos and Flammang 2005). Many sea urchin species use their spines and teeth to excavate holes or cavities in the rock providing them added adhesion potential (Otter 1932, Santos and Flammang 2007). Many other rocky shore species, however, never evolved the ability to move or have secondarily lost it. Sessile organisms like sponges, bryozoans, gorgonians, bivalves and barnacles rely on strong adhesives to withstand hydrodynamic forces impinging on them (Kamino 2008). Both stalked barnacles and acorn barnacles attach almost permanently to the substratum upon metamorphosis (Anderson 1994). Other than some gradual sliding in response to crowding by other barnacles, or microhabitat readjustment, stalked barnacles do not change much their position (Kugele and Yule 2000).

The ability to move is closely related with the feeding and foraging strategies used by different shore species (Owen 1980). Predatory life style is usually complemented by the ability to move about actively. Common predators such as fishes, crabs and lobsters are among the faster moving and more agile animals in the wave-swept environment and their predatory potential is tightly related with the hydrodynamic context of their feeding grounds (Burrows and Hughes 1991, Etter 1996, Robles et al. 2001). Likewise, herbivores such as sea urchins and limpets, that feed on sessile macroalgae or on the biofilm that coats many marine surfaces must also be able to move to reach their foraging grounds.

Sea urchins are often the dominant herbivore in temperate coastal regions and their foraging behavior may strongly limit the distribution of macroalgae (Lawrence 1975). Sea urchin foraging displacements might require large movements across the surface or contrarily, might be restrained to the animal's immediate vicinity. For example, sea urchins of the species *Strongylocentrotus droebachiensis* may form dense aggregations at the lower limit of the kelp beds that can advance into the kelp zone at rate up to 4 m/month (Scheibling et al. 1999, Gagnon et al. 2004). Under such context, intensive grazing by urchins often maintains extensive denuded zones known as urchin barrens (Lawrence 1975). Studies have also shown an ontogenetic change in the sea urchin *S. droebachiensis* foraging behaviour, as individuals smaller than 15–20 mm are sedentary and cryptic, whereas larger individuals may actively move about to forage (Dumont et al. 2004, 2006). Adult sea urchins (*S. droebachiensis*) move up to 5 m/day on barrens, where food is rare, but tend to move less when close to the kelp bed, where food is more abundant (Dumont et al. 2006). Under different ecological context such as under heavy wave exposure, sea urchins might adopt other feeding strategies, becoming facultative suspension feeders squatting in a burrow and relying on their tube feet to catch drift algae (e.g. Boudouresque and Verlaque 2007).

Suspension and filter feeding is normally associated with sessile organisms like stalked barnacles which rely on specialized appendages, the cirri, they hold against the water flow to catch drifting larvae and other food particles. Holding their feeding structures into the flows, barnacles are exposed to mechanical damage or predation (Marchinko and Palmer 2003).

Mobility and feeding strategies provide faculties for wave-swept animals to explore their ecological niches and to cope with the extremely variable hydrodynamic regimes imposed on the coastal areas they inhabit (Denny 2006).

1.2 Effects of wave exposure on coastal marine populations

It has long been recognized that wave exposure is one of the most important abiotic factors affecting marine communities in coastal areas (Ballantine 1961, Kingsbury 1962, Lewis 1964, Stephenson and Stephenson 1972). Stress caused by hydrodynamic forces is an important process that affects ecological structure and community dynamics in wave-swept rocky shores (Denny 1988).

The biological and ecological consequences of wave exposure are ubiquitous, intense, and highly relevant to our understanding of a variety of patterns and processes in rocky shores. Algae and animals that inhabit very exposed rocky shores and their interactions might be different than those found in nearby sites protected from the waves. Wave-induced hydrodynamic force gradients occur at a wide range of both spatial and temporal scales, affecting processes and resulting patterns from centimetres to kilometres and from seconds to years apart.

Hydrodynamic forces represent the tendency of water to push on organisms as it flows past them. On rocky shores, these forces result predominantly from fluid motions associated with ocean waves breaking on the shore. The intertidal and shallow subtidal zones of wave-swept shores are characterized by intense water motion. Water velocities from breaking waves regularly reach 10 m/s, while storm waves can generate water velocities larger than 25 m/s (Denny et al. 2003) and accelerations larger than 400 m/s^2 (Denny et al 1985). Wave-swept organisms confront forces mainly associated with the water velocities around them (lift and drag) but also with the water's acceleration (impingement force). The magnitudes of hydrodynamic forces depend on organism shape, size and properties of their tissues (Boller and Carrington 2006). Lift force acts perpendicular to the direction of flow. Lift arises from differences in pressure between two sides of the organism as induced by differences in flow speed and has been demonstrated to impose force on a variety of sessile invertebrates (e.g. Denny 1987, Denny and Gaylord 1996). Drag, on the other hand, acts in the direction of the flow pushing organisms downstream, as a direct consequence of both skin friction and upstream-downstream pressure difference and it has been found to affect invertebrates and macroalgae (Denny and Gaylord 2002). The impingement force, generated by the impact of the face of the wave on an organism, can also be considerable in the intertidal zone (Gaylord 2000).

Wave-induced hydrodynamic forces operate at different spatial and temporal scales. The relative magnitudes of the total and individual forces changes seasonally and daily as the tide rises and falls. During the low tide, hydrodynamic forces are absent over most of the emergent section of the coast. Where waves crash, high impingement forces are imposed on organisms. During the high tide and on shallow subtidal habitats, marine organisms are fully immersed and drag and lift are the major forces acting on them. Maritime storms increase the severity of flows faced by organisms at larger temporal scales.

Coastal topography and geometry, largely influences the hydrodynamic forces. At small scales, the substrate rugosity and complexity, its biota, crevices and holes may all provide protection from rapid water motions (O'Donnell 2008, O'Donnell and Denny 2008). At the scale of meters, water velocities are increased wherever topographic features accelerate flows, for example in converging channels and along the sides of boulders (Denny et al. 2003). At larger scales, coastal features like headlands, focus waves and increase their sizes, leading to faster wave-induced water velocities and larger hydrodynamic forces (Denny et al. 2004).

Wave-induced hydrodynamic forces can break or dislodge organisms, opening patches of substratum for settlement and invasion (Paine and Levin 1981) and may, directly or indirectly, limit the local abundance and distribution of species (Siddon and Witman 2003), the morphology and body size of organisms (Kitching and Lockwood 1974, Denny 1999), the foraging behaviour (Kawamata 1998), growth (Etter 1989), reproduction (McCarthy et al. 2003), recruitment and survival of organisms (Jonsson et al. 2006). The underlying context and scales of variability regarding wave-exposure in coastal habitats may alter intra- and inter-specific interactions and influence trophic and non-trophic interactions such as predation, herbivory, parasitism, intra- and interspecific competition, mutualism, commensalism and amensalism (Ebling et al. 1964, Menge and Sutherland 1987, Robles et al. 2001, Wernberg and Connell 2008).

How hydrodynamic forces on wave-swept rocky shores influence biological interactions, larval ecology and biology, and set the physical environmental conditions may be determinant to the patterns of distribution, abundance, patchiness, and size structure of marine populations. Comparable patterns can be explained by different factors (e.g. vertical patterns of distribution of intertidal species may be set by desiccation, competition or predation), and different patterns may be triggered by the same factor (e.g. predation can influence vertical patterns of distribution and diversity of intertidal species) (Menge and Sutherland 1987, Robles and Desharnais 2002). Also, different processes, such as predation or competition, can be important in some times or places but not in others. For example, at wave-exposed headlands, predation might be ineffective in controlling prey abundance (e.g. mussels and barnacles), while predation and grazing can be strong at more sheltered sites (Menge 1976). Consequently, prey abundances might be controlled by competition for space and physical disturbance at wave-exposed sites, and by predation or grazing at wave-protected sites (Menge and Sutherland 1976). Besides, the influence of an environmental factor on one species might not necessarily be the same for another. Strong wave action, for example, may inhibit grazers and predators, but be favourable for filter feeders (Etter 1996, Sebens et al. 1998, Robles et al. 2001, Robles and Desharnais 2002).

Interpreting variation in community structure as a consequence of predictable changes in the relative importance of species interactions and physical stress or disturbance are important for simplifying the enormous context dependency of species interactions across environmental gradients (Hart and Marshall 2013). Understanding how changes in the environment influence species biology and ecology is crucial for predicting population and community dynamics, either for conservation or resource management purposes. When research is directed towards key species or marine resources, the relevance of field observations and experimental studies goes beyond the discussion of ecological concepts and may also provide a handful of applied knowledge for management and conservation purposes. Here, the focus is directed to two common and important marine resources targeted in regional artisanal fisheries on temperate rocky shores of Southern Europe, the sessile filter-feeder stalked barnacle (*Pollicipes pollicipes*) and the mobile herbivore sea urchin (*Paracentrotus lividus*), to study ecological patterns and processes affecting these common inhabitants of the very edge of intertidal and subtidal wave-swept habitats.

1.3 Stalked barnacles and sea urchins, two important ecological and economical resources inhabiting wave-swept temperate rocky shores

The stalked barnacle *Pollicipes pollicipes* (Crustacea: Cirripedia) is a common inhabitant of extremely exposed intertidal and shallow subtidal rocky shores from Brittany to Senegal (Fig. 1.1), where it forms clusters of sessile individuals attached to each other and to the primary substratum within a complex biological matrix and frequently in association with other conspicuous species like mussels, algae, limpets and acorn barnacles (Barnes 1996). It is a very important economic resource in the North East Atlantic coast, particularly in Spain and Portugal, and is intensively harvested on intertidal and shallow subtidal rocky shores wherever it is abundant (Molares and Freire 2003, Jacinto et al. 2010, Parada et al. 2012, Boukaici et al. 2012, Sousa et al. 2013). In Portugal and Spain, its commercial value can range from 20 to 200 euros per kg in restaurants.

Along the west Iberian coastal area, where stalked barnacle exploitation is more intense, the current management strategies for *P. pollicipes* fishery can be divided into bottom-up approaches as the co-management systems in practice in Galicia (Molares and Freire 2003) and in Asturias (Rivera et al. 2014), Spain, and into top-down regional (Basque Country, Spain; Borja et al. 2006) and national (Portugal; Sousa et al. 2013) systems. In Galicia and Asturias, fishermen and regional authorities participate in management and data gathering processes (co-management), and there are official and representative data about this fishery (Macho et al. 2013, Molares and Freire 2003, Rivera et al. 2014). On the other hand, in Portugal, the National Administration centralizes the management and, most of the catch is sold directly to intermediaries or to the final consumer, without regulation (Jacinto et al. 2010). Consequently, official data about *P. pollicipes* fishery are scarce and do not estimate the real pressure upon this resource (Castro and Cruz 2009, Cruz 2000, Jesus 2006). Also, specific regulations (including spatial and temporal closures, limited number of harvest licenses, size and bag limits, catch reporting, among others) on the fishery of *P. pollicipes* in Portugal are recent (mostly since 2000), different along the coast and have frequently changed (reviewed by Sousa et al. 2013). Yet, despite the socio-economic relevance of *P. pollicipes* fishery in Portugal and the respective diversity of management regulations, there are no recent studies leading to an evaluation of the state of the fishery, management and conservation of *P. pollicipes* along the Portuguese coast.

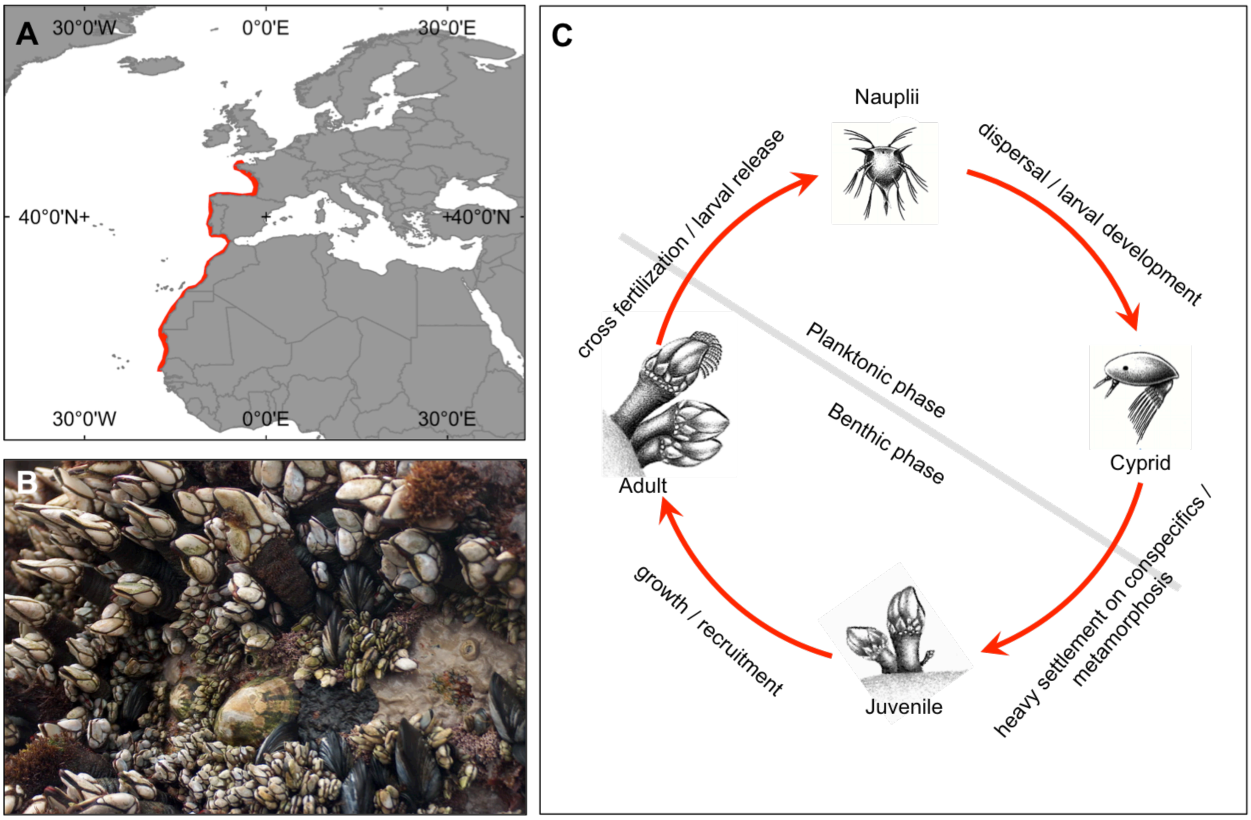

Fig. 1.1 *Pollicipes pollicipes* distribution range (A); barnacle clumps in the intertidal zone of SW Portugal (B) and life cycle schematic (C; adapted from an illustration by Telma Costa)

The external morphology of the adult (and juvenile) stalked barnacle presents two main features: a leathery flexible stalk, the peduncle, which supports the capitulum. The peduncle is muscular and contractile, contains the ovarian tissue and cement glands and is covered by a flexible cuticle with embedded calcareous scales. *P. pollicipes* is attached to the substrate by a cement extruded at the base of the peduncle (Rocha 2015). The rest of the animal's body, including its thoracic appendages and most other organs are within the mantle cavity in the capitulum protected by a bivalve carapace formed by several conspicuous calcareous plates like the rostrum (R), the scutum (S; pair), the tergum (T; pair), the rostrolatum (RL; pair), the latera (L; pair), the carinolatera (CL; pair), the carina (C) and several other imbricating smaller plates between and below the previously mentioned plates. The numbers of imbricating plates in the capitulum generally increases with barnacle age, being added from below as they are formed in the growth zone at the base of the capitulum (Barnes 1996). The maximal rostral-carinal length (RC) is often used as a size descriptor in stalked barnacles (Cruz 2000).

The body enclosed by the capitulum consists of a prosoma, thorax from which cirri arise and a vestigial abdomen (Anderson 1994). The cirri have two arms each, the rami, supported by a common segment, a pedicel. As specialized suspension feeders, barnacles use their six pairs of highly modified thoracopods (cirri) as a highly specialized basket for food capture (Barnes 1996). The mouth consists of the labrum with mandibles, maxillae, and outer maxillae.

When under water, *Pollicipes pollicipes* extends its cirri and spreads them out in the current like a net. It uses the front pair of legs to manipulate the objects it catches and move them into its mouth. Its diet mainly consists of zooplankton (Barnes 1996).

P. pollicipes is a simultaneous hermaphrodite. Each barnacle is capable of fertilising other nearby individuals by transmitting sperm through a penis and also of brooding its own eggs in its mantle cavity. Breeding is more intense from April to September on SW

Portugal (Cardoso and Yule 1995, Cruz and Hawkins 1998, Cruz 2000) and similar patterns were observed in NW Spain (Molares et al. 1994b, Macho 2006). Brooding patterns differ between size classes and are similar across tidal levels (Cruz and Araújo 1999). Adults produce approximately 30000 – 130000 eggs per brood and have multiple broods per year (Molares et al. 1994b, Barnes 1996, Cruz and Hawkins 1998, Cruz and Araújo 1999, Cruz 2000). Embryo development occurs inside the mantle cavity of adults and when the eggs hatch, the larvae are released into the sea (Fig. 1.1). Embryo and larval development time of *P. pollicipes* reared in the laboratory under controlled conditions is about 1.5 to 2 months (Molares et al. 1994a, 1994b, Kugele and Yule 1996). The planktonic larva undergoes six naupliar stages (Molares et al. 1994a) during which food reserves are built up and one non-feeding cyprid stage which seeks out a suitable hard surface on which to settle. It then fixes itself to an appropriate substrate, undergoes metamorphosis and becomes a sessile juvenile. Cyprids of *Pollicipes* settle heavily on conspecifics and so recruitment can be monitored with relative ease (Hoffman 1989, Barnes 1996, Cruz et al. 2010). Settlement onto conspecifics might help to create a benign habitat with reduced physical and biological stresses (Barnes 1996, Cruz et al. 2010) and promotes gregariousness which facilitates mating with conspecifics in barnacle species (Knight-Jones and Stevenson 1950, Clare 2010, Elbourne and Clare 2010, Rocha 2015).

Barnacle recruitment is dependent upon processes acting at a wide range of spatial and temporal scales that affect cyprid settlement and early juvenile survival (Pineda 1994, 2000). Considerable inter-annual variation in the timing and duration of the recruitment season was observed in SW Portugal, but the most consistent period of recruitment was from mid-summer to mid-autumn (Cruz et al. 2010).

Growth of *Pollicipes* spp. is dependent on food availability and environmental conditions that affect feeding behaviour and is highly variable in space and time (Barnes 1996). Factors affecting barnacle growth may include immersion time and food availability, light, season, water temperature, microhabitat and population density. Stalked barnacles grow by increasing the length of the peduncle, by periodic moults of the exoskeleton of the prosoma, cirri and thorax, and by the successive accretion of material to the calcareous plates of the capitulum (Chaffee and Lewis 1988, Anderson 1994, Barnes 1996, Helms 2004). Growth in stalked barnacles is highly variable and very difficult to study. Previous observations of *P. pollicipes* growth patterns on the SW coast of Portugal suggest that growth rates vary (between 0.11 to 0.66 mm RC per month) with barnacle size, season and intertidal level (Cruz 1993, Cruz et al. 2010). Adult size varies considerably between individuals and environmental conditions, and can be larger than 100 mm in total length and than 30 mm RC (personal observations). Longevity estimates suggest that stalked barnacles may live up to 20-30 years in the wild (Barnes 1996).

Along its distribution range, juvenile and adult *P. pollicipes* occurs mostly in wave exposed locations such as headlands (Barnes 1996), but within such locations its abundance varies at small spatial scales (meters apart) being more abundant in areas orientated to the prevailing income wave direction (Borja et al. 2006). On less exposed areas, *P. pollicipes* occurrence is rare and mainly restricted to a few individuals occupying small shelters like cracks and crevices. Recent observations made on the SW coast of Portugal suggest that top-down control via predation is one of the main processes shaping *P. pollicipes* abundance patterns, and may vary along wave exposure gradients at a multitude of spatial and temporal scales. Despite its economic and ecological importance several aspects of its biology and ecology are yet to be studied, most likely as a result of the difficulty associated with conducting ecological studies in the extreme exposed habitats where this species occurs (Barnes 1996, Cruz 2000, Cruz et al. 2010)

The sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) is a common rock-boring echinoid that inhabits intertidal rock pools and shallow subtidal reefs throughout the Mediterranean Sea (Benedetti-Cecchi et al. 1998, Bulleri et al. 1999, Tomas et al. 2004, Boudouresque and Verlaque 2007) and the northeastern Atlantic, from Scotland to southern Morocco, including the Macaronesian archipelagos (Pérez-Ruzafa et al. 1999, Boudouresque and Verlaque 2007) (Fig. 1.2). *P. lividus* is an important economic resource, that is intensively harvested for its highly appreciated gonads, in France, Spain, Italy (Guidetti 2004, Boudouresque and Verlaque 2007) and, to a lesser extent, in Portugal (Castro 2004, Castro and Cruz 2009, Costa 2012, Bertocci et al. 2014, Mamede 2014). *P. lividus* plays an important ecological role, since its grazing activity can determine the structure of macroalgal assemblages and, in particular, trigger the transition from erect macroalgal stands to encrusting coralline barrens (reviewed by Boudouresque and Verlaque 2007). The effects of *P. lividus* on macroalgal communities are density dependent and vary at a hierarchy of spatial and temporal scales (Benedetti-Cecchi et al. 1998, Bulleri et al. 1999, Cebrian and Uriz 2006, Guidetti and Dulčić 2007).

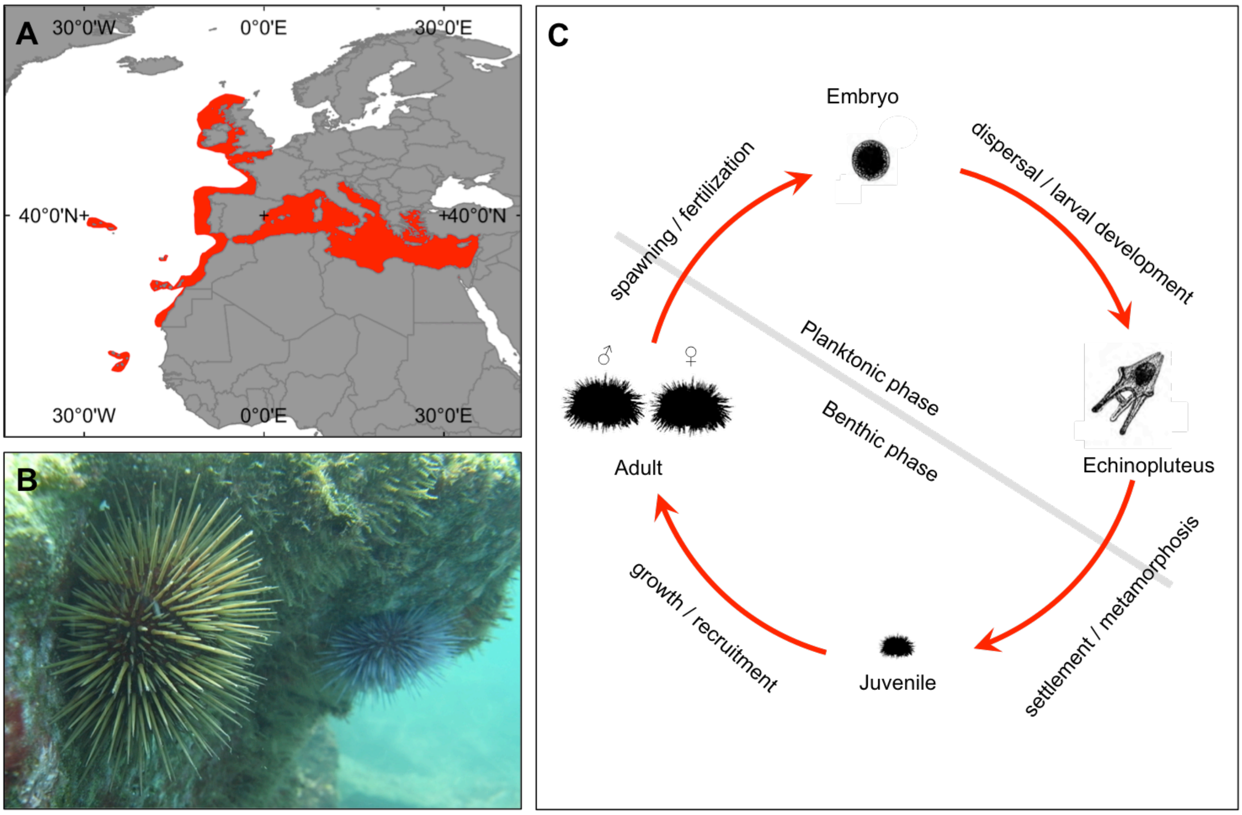

Fig. 1.2 *Paracentroutus lividus* distribution range (A); Sea urchins foraging the subtidal zone of SW Portugal (B) and life cycle schematic (C)

Like most echinoids, *P. lividus* has a simple life cycle (Fig. 1.2). Reproduction is confined to the adult stages and involves the spawning of eggs and sperm freely into the seawater, followed by external fertilization (Lawrence 2007). Sexes are separate although some degree of hermaphroditism has been observed (Byrne 1990). Gametes are shed into the water column where fertilization occurs producing vast numbers of small, yolk-poor eggs that develop into planktonic, feeding larvae known as echinoplutei (Lawrence 2007). Size at onset of sexual maturity in *P. lividus* varies along its geographic range (from 11 to 50 mm in test diameter), between habitats and regions, and can be the result of different energetic investments into reproduction or different growth rates, so that individuals may have the same age but a different size when they become reproductive (Ouréns et al. 2011). Spawning in *P. lividus* occurs in spring and summer in the coast of Portugal (Gago et al. 2003). The duration of the planktonic life of larvae of *P. lividus* has been estimated to be 23–29 days *in situ* (Pedrotti 1993, but see Lozano et al. 1995). Larval development involves growth and elaboration of the larval body, as well as formation of the rudiment of the juvenile echinoid. Development is arrested when metamorphic competence is achieved. Induction of settlement and metamorphosis occur in response to environmental factors that signal the availability of suitable benthic habitat (Lawrence 2007). Settlement peaks normally occur during summer, but is highly variable in space and time (Lozano et al. 1995, López et al. 1998, Hereu et al. 2004, Tomas et al. 2004, Boudouresque and Verlaque 2007). Post-metamorphic development involves growth and sexual maturation to yield a reproductive adult.

A globosely shaped animal with a rigid body wall (test) surrounded by multiple rows of spines characterizes the post-metamorphic urchin phase (Lawrence 2007). The test is made of many calcite plates sewn together at the edges by collagen fibers (Ellers et al. 1998). The spines are attached to projections called tubercles on the outer surfaces of the test plates by layers of collagen and muscle (Lawrence 1987). A unique organ system, the water vascular system, found on echinoderms, can be seen externally in the form of water-filled tube feet, or podia, used for locomotion and feeding (Lawrence 1987). The tube feet project through pores present in the test plates which allow continuity of the fluid in the tube feet with the rest of the water vascular system (Lawrence 1987, Santos and Flammang 2005, Santos et al. 2005). Like all sea urchins, *P. lividus*, has an elaborate jaw apparatus (known as the Aristotle's lantern) that is composed of calcite ossicles and muscles that support five teeth used for biting prey, algae and to erode the rocky substrate (Lawrence 1987, 2007). *P. lividus* is a dominant grazer in shallow-water environments, and at high densities it can turn complex algal communities into overgrazed communities with a dominance of encrusting coralline algae, known as barren grounds (Boudouresque and Verlaque 2007). Under certain ecological contexts, *P. lividus* might adopt a sit-and-wait feeding strategy, and instead of actively foraging the substrate for food, it uses its teeth and spines to bore into the rocky substrate, creating cavities, which it occupies more or less permanently, and feed mainly on drifting algae while sheltered from physical stressors and predators (Boudouresque and Verlaque 2007). Due to its burrowing activity, *P. lividus* is an important agent of bioerosion and tidepool deepening in coastal habitats (Otter 1932, Trudgill et al. 1987).

1.4 Aims and structure of the thesis

This thesis aims at contributing to the knowledge of temperate rocky shore ecology, with direct implications in the management and conservation of important local marine resources. Research was conducted along gradients of wave exposure on both subtidal and intertidal habitats of temperate wave-swept rocky shores of Southern Europe, focusing on different aspects of patterns and processes of distribution of two important marines resources: the sea urchin *Paracentrotus lividus* and the stalked barnacle *Pollicipes pollicipes*.

The contents of the present thesis are organized in eight chapters.

Chapter 1 comprises the present general introduction and aims, underlining some of the main aspects of life and physical processes in wave-swept rocky coasts and a summary on the ecology and life cycles of the stalked barnacle *Pollicipes pollicipes* and the sea urchin *Paracentrotus lividus*, the two focal species under research in this thesis.

Chapters 2 through 7 cover the experimental work done and each chapter encloses a research topic published in, submitted to, or in preparation for publication in marine ecology related scientific journals.

Chapter 2 is entitled "Patterns of abundance, population size structure and microhabitat usage of *Paracentrotus lividus* (Echinodermata: Echinoidea) in SW Portugal and NW Italy" (Jacinto et al. 2013), and pens a study aimed to assess how variation in hydrodynamic conditions at multiple spatial scales may generate different ecological contexts and influence patterns of density, size structure and microhabitat usage of the common sea urchin *P. lividus*, and whether these effects are consistent between regions that are markedly different for oceanic climate, such as the coasts of SW Portugal and NW Italy.

The observations and results obtained on microhabitat usage of sea urchin populations of the Atlantic wave exposed coasts led to the work presented in Chapter 3, entitled "*Paracentrotus lividus* (Echinodermata: Echinoidea) attachment force and burrowing behavior in rocky shores of SW Portugal" (Jacinto and Cruz 2012). This chapter constitutes a descriptive study of experimental, hypothesis-testing nature to assess the importance of urchin burrows in wave-swept habitats and presents one of the first attempts to measure, *in situ,* the attachment force of the rock boring sea urchin *P. lividus*, in different habitats (tidepools, low shore intertidal channels and in the shallow subtidal), and to test the model that burrowing behaviour enhances sea urchin attachment force and ability to resist dislodgement in wave-swept habitats, and thus, might be an adaptive response to hydrodynamic stress.

From this point forward the focus is turned to key aspects of stalked barnacle ecology, which present other suite of strategies to thrive on extremely exposed rocky shore habitats of the northeastern Atlantic coasts.

In Chapter 4, “Small scale patterns and processes of distribution of the stalked barnacle *Pollicipes pollicipes* at Cabo de Sines (SW Portugal)”, hypotheses derived from models potentially explaining the indirect effect of wave exposure on the highly variable small-scale distribution patterns in stalked barnacle populations were tested. *P. pollicipes* abundance, predation pressure and recruitment patterns were expected to vary along small spatial scale wave exposure gradients. It was hypothesized that *P. pollicipes* abundance would be positively correlated with wave exposure measured in sites few meters apart from each other, possibly as a result of lower predation pressure and/or higher recruitment rates at the most exposed sites.

The results and observations that were made led to the reasoning behind the study presented in Chapter 5, entitled “Predicting *Pollicipes pollicipes* (Crustacea: Cirripedia) abundance in intertidal rocky shores of SW Portugal: a multi-scale approach based on a simple fetch-based wave exposure index” (Jacinto and Cruz 2016). Here, the abundance of *P. pollicipes* was modelled as a function of a simple wave exposure index based on fetch estimates from digitized coastlines at different spatial resolutions and wave energy estimated from oceanographic and meteorological data provided from nearby stations. This study aimed at predicting *P. pollicipes* abundance along a stretch of coast in SW Portugal and discussing the potential use of the inference model as a simple and cost effective tool that may lead to better assessment of harvesting grounds and dimension of exploitable areas, which may help improving management plans and support decision making regarding conservation, harvesting pressure and surveillance strategies for this highly appreciated and socio-economic important shellfish resource.

In Chapter 6, “Growth rate variation of the stalked barnacle *Pollicipes pollicipes* (Crustacea: Cirripedia) using calcein as a chemical marker” (Jacinto et al. 2015), a novel and promising method to study growth in stalked barnacles is presented and discussed. Growth rates in *Pollicipes* spp. are highly variable in space and time (Barnes 1996, Cruz et al. 2010) and factors affecting barnacle growth may include immersion time and food availability, light, season, water temperature, microhabitat and population density. Previous observations of *P. pollicipes* growth patterns on the SW coast of Portugal (Cruz 1993, 2000, Cruz et al. 2010) suggest that growth rates vary with barnacle size, season and intertidal level. This study describes the use of calcein tagging methods in order to mark multiple juvenile and adult barnacles and to estimate growth rate variation with intertidal vertical level and size of the stalked barnacle *Pollicipes pollicipes* in SW Portugal.

In Chapter 7, entitled "The state of the fishery, conservation and management of the stalked barnacle *Pollicipes pollicipes* in Portugal" (Cruz et al. 2015), an assessment of the state of the fishery, conservation and management of the stalked barnacle *P. pollicipes* in Portugal is made for the first time. Although this species can be considered the most important intertidal economical resource in continental Portugal, official data on this fishery are not representative of the real pressure upon *P. pollicipes*. Additionally, monitoring the abundance of this species is difficult due to the very harsh environments it inhabits. The main goal of this study was the evaluation of the state of the fishery, management and conservation of *P. pollicipes* in Portugal considering three regions with different regulations regarding the fishery of this species. This evaluation was made combining different approaches and sources of data, using recent original and published data (Sousa et al. 2013) and data from past studies (Jesus 2006, Cruz et al. 2008). Despite not being the first author of the study presented in Chapter 7, my contribution was critical to all aspects of the investigation, thus the inclusion of this chapter in the present thesis.

In chapter 8, "General discussion and final remarks", the main results attained and information gathered throughout the present thesis are discussed and summarized, some priority conservation/management measures are suggested, and future research objectives are outlined.

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Chapter 2. Patterns of abundance, population size structure and microhabitat usage of *Paracentrotus lividus* (Echinodermata: Echinoidea) in SW Portugal and NW Italy

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Jacinto D., Bulleri F., Benedetti-Cecchi L., Cruz T. 2013. Patterns of abundance, population size structure and microhabitat usage of *Paracentrotus lividus* (Echinodermata: Echinoidea) in SW Portugal and NW Italy. Mar. Biol. 160: 1135–1146.

2.1 Abstract

In coastal habitats, wave exposure influences several aspects of the life history of marine organisms. Here, we assess how hydrodynamic conditions can generate variation in density, size structure and microhabitat usage of *Paracentrotus lividus* and whether these effects are consistent between regions that are markedly different for oceanic climate, such as the coasts of SW Portugal and NW Italy. The abundance of *P. lividus* was 4 times higher in SW Portugal than in NW Italy, but within each region, there was no effect of wave exposure. In SW Portugal, higher urchin abundances were found at shallower depths, while no effect of depth on urchin abundance emerged in NW Italy. Most of the variation in urchin abundance occurred at small spatial scales (metres), and our results suggest that habitat complexity, that is, the presence of cracks and crevices, is an important determinant of patterns of distribution of this species. The population in NW Italy was characterized by a unimodal size distribution, with a higher proportion of medium-sized individuals. In contrast, in SW Portugal, smaller individuals represented a large proportion of the populations. Size structure varied between exposed and sheltered habitats in SW Portugal, suggesting that the proportion of individuals from different size cohorts may vary along wave-exposure gradients as a result of direct or indirect effects of hydrodynamic forces. In SW Portugal, most urchins occurred in burrows, while in NW Italy, urchins were mainly observed in crevices. These results suggest that creating/occupying burrows might be an adaptive behaviour that allows sea urchins to better withstand stressful hydrodynamic conditions and, therefore, are more common on exposed Atlantic coasts. Overall, our study suggests that the effects of hydrodynamic forces on sea urchin populations are context dependent and vary according to background oceanic climate.

2.2 Introduction

Wave exposure plays a key role in determining patterns of distribution and abundance of marine organisms in coastal habitats (Denny 1988, Denny et al. 2003, Siddon and Witman 2003, Lindegarth and Gamfeldt 2005), particularly when the affected species have the potential to modify benthic community structure. Sea urchins are among the most efficient grazers in marine environments, and their ecological importance has been documented in a variety of habitats worldwide (Lawrence 1975), including seagrass meadows (Eklöf et al. 2008), kelp beds (Dean et al. 1984, Tegner et al. 1995), erect macroalgal assemblages (Benedetti-Cecchi et al. 1998, Bulleri et al. 1999, Hernández et al. 2008), coral reefs (Carreiro-Silva and McClanahan 2001) and intertidal rock pools (Benedetti-Cecchi and Cinelli 1995). Recruitment, feeding and sheltering behaviour, predation pressure and abundance patterns of several sea urchin species are, either directly or indirectly, influenced by wave exposure. Observations in tropical and temperate habitats have shown that settlement and recruitment of several sea urchin species vary spatially according to wave exposure (Ebert 1982 in Himmelman 1986, Hereu et al. 2004b). Variation in larval supply, early mortality and predation along wave exposure gradients may account for such patterns (Hereu et al. 2004b). At shallow depths, wave-induced water motion has been shown to inhibit sea urchin grazing activity (Harrold and Reed 1985, Witman 1987, Kawamata 1998, Vanderklift et al. 2009). When hydrodynamic forces are strong, sea urchins can avoid dislodgement by wave forces by taking refuge in deeper habitats or in sheltered microhabitats such as cracks, crevices or burrows, and start feeding on drift algae (Lissner 1983, Witman 1987, Tuya et al. 2007, Clemente and Hernández 2008).

Paracentrotus lividus is a common rock-boring echinoid that inhabits intertidal rock pools and shallow subtidal reefs, throughout the Mediterranean Sea (Benedetti-Cecchi et al. 1998, Bulleri et al. 1999, Tomas et al. 2004, Boudouresque and Verlaque 2007) and the northeastern Atlantic, from Scotland to southern Morocco, including the Macaronesian archipelagos (Boudouresque and Verlaque 2007). *P. lividus* represents an important economic resource in France, Spain, Italy (Guidetti et al. 2004b, Boudouresque and Verlaque 2007) and, to a lesser extent, in Portugal. *P. lividus* plays an important ecological role, since its grazing activity can determine the structure of macroalgal assemblages and, in particular, trigger the transition from erect macroalgal stands to encrusting coralline barrens (reviewed by Boudouresque and Verlaque 2007). The effects of *P. lividus* on macroalgal communities are density dependent and vary at a hierarchy of spatial and temporal scales (Benedetti-Cecchi et al. 1998, Bulleri et al. 1999, Cebrian and Uriz 2006, Guidetti and Dulčić 2007).

In NW Italy, the effects of *P. lividus* on the distribution and abundance of erect algae and limpets are of minor importance as opposed to those of the co-occurring urchin species, *Arbacia lixula*, probably as a result of low densities (Bulleri et al. 1999) or from the prevalence of passive feeding on drifting algae while resting in crevices (Dean et al. 1984, Harrold and Reed 1985, Vanderklift et al. 2009). As other rock-burrowing sea urchins, *P. lividus* can switch from an active (grazing) to a passive (drift-feeding) feeding habit when in burrows (Otter 1932, Boudouresque and Verlaque 2007). *P. lividus* may also be considered as an ecosystem engineer for its ability to create such burrows that increase the complexity of the substratum and the associated biodiversity (Otter 1932, Trudgill et al. 1987, Boudouresque and Verlaque 2007). Burrows, created by the mechanical action of spines and mouthparts (Otter 1932), provide shelter from predators and resistance to dislodgement due to hydrodynamic forces (Boudouresque and Verlaque 2007).

This study was aimed to assess how hydrodynamic conditions can generate variation in

P. lividus density, size structure and microhabitat usage and whether these effects are consistent between regions markedly different for oceanic climate, such as the coasts of SW Portugal and NW Italy. Specifically, we tested the following models and hypotheses: (1) Within each region, *P. lividus* abundance varies according to local levels of hydrodynamic stress, depth and topography of the substratum. We expected lower urchin abundance in more exposed and shallower sites and with less topographical complexity (e.g. due to increased hydrodynamic stress); (2) Hydrodynamic forces affect urchin population size structure, both directly (e.g. dislodgment of larger individuals, inhibition of grazing activity and/or recruitment rates) and indirectly (e.g. reduced predation pressure at more exposed sites). Accordingly, within each region, we expected population size structure to vary between sites with different levels of hydrodynamic stress and along depth gradients. More specifically, we expected to observe a higher proportion of large individuals at less wave-exposed sites and at greater depths; (3) Hydrodynamic stress affects microhabitat usage by *P. lividus*. Within each region, we expected burrowing and sheltering in crevices to occur mostly at more exposed conditions (at shallower depths and more wave-exposed sites), with a higher proportion of free-living individuals in less wave-exposed conditions (deeper and less wave-exposed sites).

2.3 Materials and methods

Study regions and ocean climate

In order to assess the generality in the effects of hydrodynamic conditions on the distribution and population dynamics of *P. lividus* over broad spatial scales, this study was carried out on rocky shores within two markedly different distinct oceanographic regions: the Atlantic ocean (ATL–SW Portugal) and Mediterranean sea (MED–NW Italy) (Fig. 2.1).

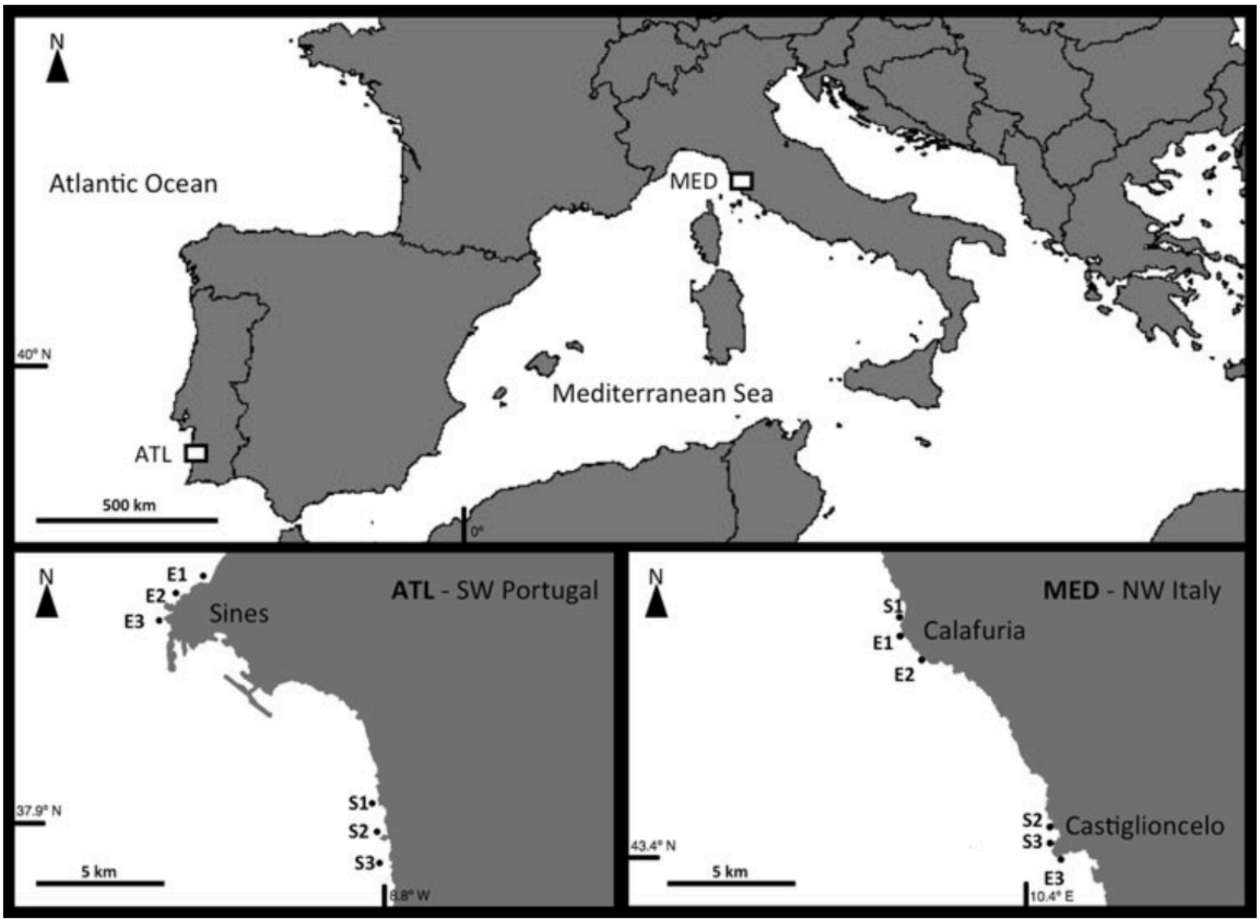

Fig. 2.1 Location of study regions (ATL–SW Portugal and MED–NW Italy) in southern Europe and study sites (1–3) according to exposure level (S sheltered sites, E exposed sites)

The SW coast of Portugal is a highly productive upwelling system, forced by persistent northerlies (upwelling favourable), typically observed in late spring and summer (Wooster et al. 1976, Fiúza et al. 1982, Oliveira et al. 2009). Upwelling is an important oceanographic process that influences phytoplankton productivity cycle (1–2 mg/m^3; SW Portugal annual average of chlorophyll-a concentration based on satellite observations. Source: http://ec.europa.eu/maritimeaffairs/atlas/maritime_atlas), due to additional energy input, associated with the nutrients upwelled into the euphotic zone

(Peliz et al. 2002, Oliveira et al. 2009). Upwelling also affects sea surface temperature (SST) patterns (15–18 ºC; SW Portugal annual average of nearshore SST based on satellite observations. Source: http://ec.europa.eu/maritimeaffairs/atlas/maritime_atlas), due to cross-shore movement of water masses during upwelling/downwelling events (Oliveira et al. 2009), and is an important larval transport and retention mechanism (Queiroga et al. 2007).

Levels of surface chlorophyll are, in contrast, low in the nearshore of the northwest Mediterranean Sea (0.2–0.5 mg/m^3; NW Italy annual average of chlorophyll-a concentration based on satellite observations. Source: http://ec.europa.eu/maritimeaffairs/atlas/maritime_atlas), due to the low river discharge and general pattern of water circulation. The Tyrrhenian and West Corsica Currents, flowing from the south, transport water poor in phytoplankton and nutrients into the Western Mediterranean Basin (Nezlin et al. 2004). The salinity (between 36 and 39‰) and sea surface temperature (18–20 ºC; NW Italy annual average of nearshore SST based on satellite observations. Source: http://ec.europa.eu/maritimeaffairs/atlas/maritime_atlas) in this basin are subjected to wide fluctuations, but they are high when compared to the Atlantic seas along the SW coast of Portugal.

Wave climate along the NW coast of Italy is also very distinct from that of the SW coast of Portugal. Waves are predominantly from SW and are less than 1 m high in ~85% of the observations from 1989 to 2003 at La Spezia (43º 55’ 12’’ N 9º 49’ 06’’ W) oceanographic buoy (APAT 2004, Franco et al. 2004). In this region, significant wave height larger than 1–2 m may result from SW waves generated by storms and wind-driven swell (APAT 2004, Franco et al. 2004). In contrast, the SW coast of Portugal is characterized by heavy swells, winter storms and a high tidal range (0.9–3.5 m).

Significant wave height along the SW coast of Portugal is above 1 m about 95 % of the year and above 4 m in 5 % of the year (Instituto Hidrográfico 2006). The most common wave direction is from NW along the Portuguese coast (Instituto Hidrográfico 2006).

Finally, the two study regions (Fig. 2.1) are also distinct for what concerns local geology. Study sites along the NW coast of Italy are predominantly formed by sedimentary rocks (sandstones and conglomerates), while in the SW coast of Portugal, igneous (gabbros, diorites and syenites) and metamorphic rocks (schists) are prevalent (sources: http://www.pcn.minambiente.it/ and http://geoportal.lneg.pt/, Italy and Portugal, respectively). From a mineralogical point of view, rocky substrates are of intermediate hardness in NW Italy (similar in Calafuria and Castiglioncello sites) and harder in SW Portugal (hard in Cape of Sines sites where igneous rocks prevail and less hard at the southern sites where metamorphic rocks are the most common rocky substrates).

Local hydrodynamic force measurements

Local scales of hydrodynamic force variability (within each study region) were assessed by measuring the maximum force exerted on a drag sphere connected to custom-made dynamometers (Bell and Denny 1994) during two deployment periods (MED: 20 June to 31 July 2008; ATL: 15 August to 31 October 2008).

In both study regions (ATL and MED), two levels of wave exposure (exposed and sheltered) were considered based on coastal configuration and orientation (Fig. 2.1) and information on wave climate (see above). Three sites were randomly identified for each exposure level, and within each site, wave force was measured along 2 transects (100 m apart) by means of dynamometers (N = 5) attached to the rock surface at 6 m depth (~2 m apart).

In SW Portugal, exposed sites were confined to a prominent headland area (Cape of Sines, Fig. 2.1) and sheltered sites to an area ~10 km south of the headland. In NW Italy, sites for each level of exposure were determined based on their relative orientation along 2 stretches of rocky headlands in Calafuria and Castiglioncello coasts (Fig. 2.1). We considered that sites oriented to the N and NW of those headlands would be relatively more sheltered from wave action, when compared to sites S or SW oriented.

Since different dynamometer deployment periods were carried out in ATL and MED, hydrodynamic force data were analysed for each region separately by permutational multivariate analysis of variance, PERMANOVA (Anderson 2001) including three factors: exposure (fixed, with two levels, exposed vs sheltered), site (random, nested in exposure with three levels, with the exception of exposed level in ATL where only data from two sites were collected) and transect (random, nested within site, with two levels). Sampling size ranged from 3 to 5 dynamometers. Analyses were based on Euclidean distances of untransformed data. Unrestricted permutation of raw data and Type III sums of squares were applied (Anderson et al. 2008). We used PERMANOVA to analyse univariate data, since several dynamometers were lost during the field work, resulting in an unbalanced design (Anderson et al. 2008). Homogeneity of univariate dispersion based on Euclidean distance was tested using the PERMDISP routine (Anderson 2006). The software PRIMER 6 and PERMANOVA+ (www.primer-e.com; Anderson et al. 2008) was used to perform all procedures.

Urchin abundance patterns

The density of urchins was quantified at the study sites (see Fig. 2.1) at two depths: 3 and 6 m. Sampling was carried out in July/August 2008 in NW Italy and

September/November 2008 in SW Portugal. At each site and for each depth, 2 horizontal transects were held and 7 quadrates (50 x 50 cm) were randomly sampled along each ~20 m transect.

In each quadrate, all visible urchins (test diameter > 5 mm) were identified (in Italy, 2 species coexist, *P. lividus* and *Arbacia lixula*, while in Portugal, only *P. lividus* was observed), counted, measured with a calliper (test diameter without spines to the nearest mm) and individually described as free living (F), in crevices (C) or in burrows (B; urchins found in excavated circular depressions in the rock).

Inclination (measured with a clinometer) and a topographical complexity index (the ratio between surface contour length and quadrate lateral length, as in rope-and-chain method (Luckhurst and Luckhurst 1978) were also measured for each quadrate.

Urchin abundance data (*P. lividus* only) were analysed by means of a five-factor ANOVA: (1) Region (Re), fixed, with 2 levels (ATL and MED); (2) Depth (De), fixed, with 2 levels (3 and 6 m), crossed with Region; (3) Exposure (Ex), fixed, with 2 levels (sheltered and exposed), crossed with Region and Depth; (4) Site (Si), random, with 3 levels and nested within the interaction Re X Ex; (5) Transect (Tr), random, with 2 levels nested within all factors. Homogeneity of variance was assessed using Cochran's C-test (data were transformed to the squared root) and Student–Newman–Keuls (SNK) tests were used when appropriate. Data analyses were performed according to Underwood (1997) using GMAV5 software (Institute of Marine Ecology, University of Sydney).

Putative relationships between urchin abundance and inclination and topographical complexity index at the quadrate scale were assessed by Spearman's correlation.

Urchin population size structure

Urchin population size structure was analysed by PERMANOVA (Anderson 2001), based on a distance matrix of Whittaker's Index of Association (Legendre and Legendre 1998), calculated from a multivariate data matrix of the absolute frequency of individuals in 8 size classes (10-mm-wide classes) per site. With this index, urchin size structure is compared using the differences in proportion of urchins for each size class per site. All urchins observed on each transect within each site were pooled, in order to have a larger number of individuals per site. The PERMANOVA design included 3 factors: (1) Study Region (Re), (2) Exposure (Ex) and (3) Depth (De). Sampling size was 3 sites. Unrestricted permutation of raw data and Type III sums of squares were applied (Anderson et al. 2008). When appropriate, posteriori pairwise comparisons were conducted.

Principal coordinate analysis, PCO, ordination was performed on the Whittaker's Index of Association resemblance matrix and raw Spearman's correlations of the variables (size classes) with the PCO axes being calculated and overlaid on the PCO graph to visualize potential linear or monotonic relationships between variables (size classes) and PCO axes (Anderson et al. 2008).

Microhabitat usage

Size distribution variation according to microhabitat (*i.e.* urchins found in burrows, in crevices or free over the substrate, B, C and F, respectively) was graphically represented with box and whiskers plots.

To test the hypothesis that wave exposure affects microhabitat usage by *P. lividus*, and since results suggest that the microhabitat usage varies with urchin size (see results; Fig. 2.6), we performed statistical analyses on a specific urchin size range in order to avoid possible confounding effects of sea urchin size on microhabitat usage. In both studied regions, the urchin size range from 40 mm to 60 mm was well represented (see results; Fig. 2.6) and urchins within that size range occurred in burrows, crevices and/or free on the substrate.

Urchin population microhabitat structure was analysed by PERMANOVA (Anderson 2001), based on a distance matrix of Whittaker's Index of Association (Legendre and Legendre 1998), calculated from a multivariate data matrix of the absolute frequency of individuals for the three microhabitats considered (burrows, crevices and free living) per site. Urchin microhabitat structure was compared using the differences in proportion of urchins for each microhabitat. The PERMANOVA design included 3 factors: (1) Study Region (Re); (2) Exposure (Ex) and (3) Depth (De). All individuals observed on each transect within each site (as above) were pooled. Sampling size was 3 sites. Unrestricted permutation of raw data and Type III sums of squares were applied (Anderson et al. 2008). When appropriate, posteriori pairwise comparisons were conducted.

2.4 Results

Hydrodynamic force measurements

Mean maximal forces experienced by dynamometers were highly variable between instruments deployed a few metres apart and varied between 2.5 and 29.8 N in NW Italy (which according to Bell and Denny 1994, is equivalent to a water flow velocity of 2.1 and 7.8 ms^{-1}), and 2.3 and 25.1 N in SW Portugal (2.1 and 7.1 ms^{-1}). In both regions, sheltered sites experienced weaker maximal hydrodynamic forces than exposed sites (6.7 ± 1.12 N and 12.4 ± 1.90 N in NW Italy and 6.3±0.82N and 11.2±1.45N in SW Portugal, mean ± SE; Table 2.1).

Table 2.1 PERMANOVA analysis of the effects of ''Exposure'' (Ex), ''Site'' (Si) and ''Transect'' (Tr) on hydrodynamic force measurements made at 6 m depth in both studied systems

Source	ATL				MED			
	df	MS	Pseudo-*F*	*p*	df	MS	Pseudo-*F*	*p*
Ex	1	234.31	11.379	**0.0264**	1	426.23	21.876	**0.0045**
Si(Ex)	3	20.103	4.9182	0.0579	4	17.37	0.16489	0.9502
Tr(Si(Ex))	5	4.1056	0.15656	0.9759	6	106.21	1.8559	0.1078
RES	33	26.224			42	57.228		

Analyses were based on Euclidean distances of untransformed data. *p* values were obtained using 9,999 random permutations. N = 3–5 replicate dynamometers. PERMDISP tests: F = 1.0079 (ATL; $p > 0.05$) and F = 3.0354 (MED; $p > 0.05$). Significant effects are indicated in bold ($p < 0.05$)

Urchin abundance patterns

Paracentrotus lividus was more abundant in SW Portugal than in NW Italy (7.8 ± 7.33 and 1.8 ± 2.22 individuals per quadrate, respectively; mean ± SD). In NW Italy, *P. lividus* was about 5 times more abundant than *A. lixula* (0.4 ± 0.83; mean ± SD). ANOVA results reveal a significant effect of the interaction term Depth X Site (De X Si (Re X Ex); Table 2.2) on *P. lividus* abundance patterns, and SNK tests to the interaction term suggest that in ATL, abundances of *P. lividus* varied with depth (at most sites, urchin abundances were higher at 3 m depth), while in MED, no variation in urchin abundance with depth at each site was observed (Fig. 2.2; Table 2.2). No effect of wave-exposure levels in P*. lividus* abundance patterns was detected.

Correlations between *P. lividus* abundance and topographical complexity index (1.27 ± 0.224 and 1.37 ± 0.328, ATL and MED, respectively; mean ± SD) and substrate inclination (27 ± 23.1° and 27 ± 30.6°, ATL and MED, respectively; mean ± SD) suggest that *P. lividus* abundance was positively correlated with topographical complexity index in both NW Italy and SW Portugal ($r = 0.575$; $p < 0.001$; $n = 179$ and $r = 0.165$; $p < 0.05$; $n = 167$, respectively). Urchin abundance was negatively correlated with substratum inclination angle in Italy ($r = -0.261$; $p < 0.001$; $n = 179$), but not in Portugal ($r = -0.043$; $p > 0.5$; $n = 167$).

Urchin population size structure

Urchin test diameter in SW Portugal varied from 5.2 to 74.1 mm (median = 20.0 mm). Two modes in urchin size distribution were observed: one mode corresponding to the 10 to 20-mm size class (42.2 % of the observations) and another mode at the 50 to 60-mm size class (12.5 %) (Fig. 2.3).

In NW Italy, *P. lividus* test diameter varied between 10.3 and 65.0 mm (median = 42.0 mm), with a single mode in the 40 to 50-mm size class (36.3 % of the observations) (Fig. 3).

Table 2.2 ANOVA on *P. lividus* abundance in relation to ‘‘Region’’ (Re), ‘‘Depth’’ (De), ‘‘Exposure’’ (Ex), ‘‘Site’’ (Si) and ‘‘Transect’’ (Tr)

Source	Df	MS	*F*	*p*	*F* vs
Re	1	119.0822	54.31	**0.0001**	Si(Re X Ex)
De	1	1.3747	0.35	0.5715	De X Si(Re X Ex)
Ex	1	4.3582	1.99	0.1963	Si(Re X Ex)
Si(Re X Ex)	8	2.1927	2.16	0.0691	Tr(Re X De X Ex X Si)
Tr(Re X De X Ex X Si)	24	1.0147	1.62	**0.0353**	RES
Re X De	1	0.1252	0.03	0.8431	De X Si(Re X Ex)
Re X Ex	1	5.9996	2.74	0.1367	Si(Re X Ex)
De X Ex	1	11.2428	2.85	0.1300	De X Si(Re X Ex)
De X Si(Re X Ex)	8	3.9494	3.89	**0.0045**	Tr(Re X De X Ex X Si)
Re X Re X Ex	1	13.418	3.40	0.1025	De X Si(Re X Ex)
RES	288	0.6249			

N = 7 replicate quadrates. Transformation: Sqrt(X + 1). Cochran’s Test: C = 0.0652 (p > 0.05). Significant effects are indicated in bold (p < 0.05).

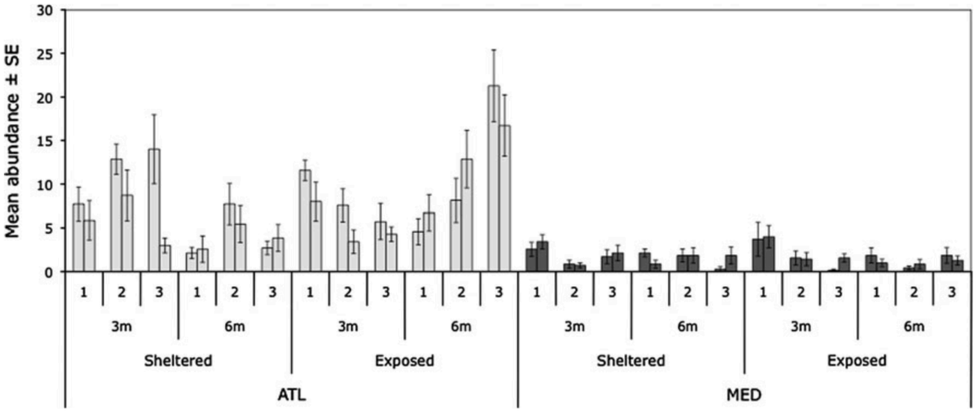

Fig. 2.2 Urchin abundance per 50 x 50-cm quadrate (mean ± S.E.; N = 7) per transect, within site according to depth (3 and 6 m) and two exposure levels (sheltered and exposed), in both study systems (ATL and MED).

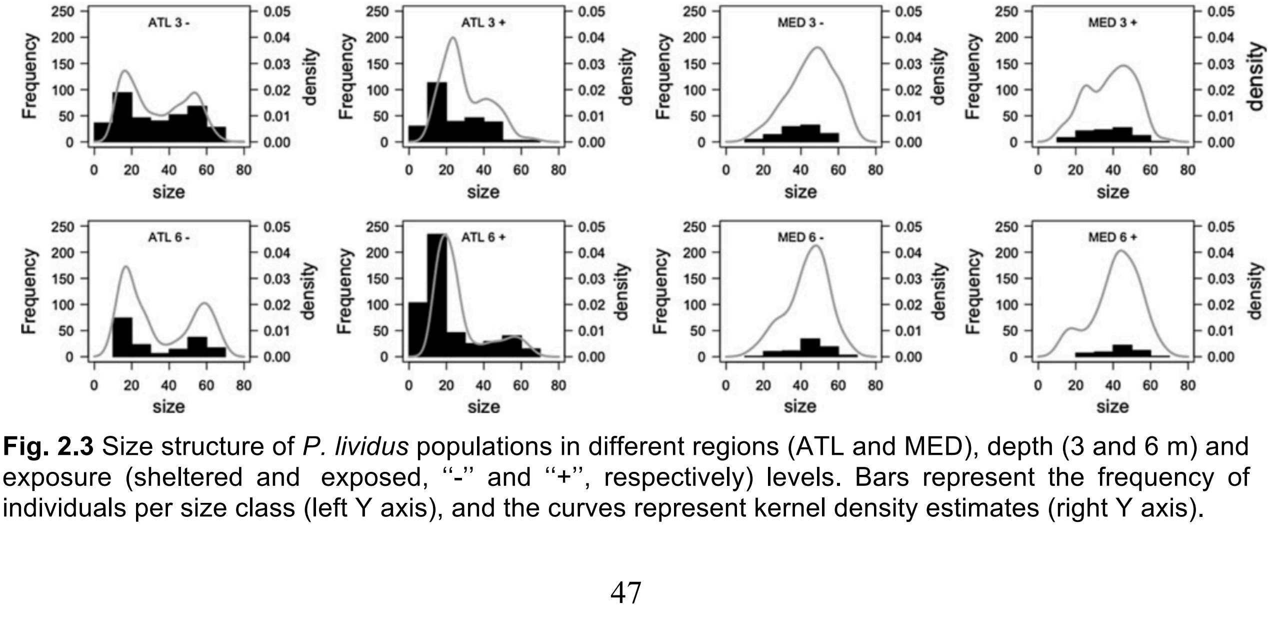

Fig. 2.3 Size structure of *P. lividus* populations in different regions (ATL and MED), depth (3 and 6 m) and exposure (sheltered and exposed, ‘‘-’’ and ‘‘+’’, respectively) levels. Bars represent the frequency of individuals per size class (left Y axis), and the curves represent kernel density estimates (right Y axis).

PERMANOVA results suggest a significant variability in urchin size structure between regions (Table 2.3), mostly driven by the increased proportion of small individuals (test diameter <30 mm) in SW Portugal (Figs. 2.3, 2.4). The effect of the interaction Region X Exposure (Re X Ex; Table 3) was marginally significant ($p = 0.0797$), and posteriori pairwise comparisons were made in order to test for variability between exposure levels in both regions. Pairwise test results suggest a significant effect of wave exposure in sea urchin population size structure in SW Portugal (exposed ≠ sheltered, $p < 0.05$), but not in NW Italy (exposed = sheltered, $p > 0.05$). In SW Portugal, differences in urchin population size structure between exposure levels were probably related to a greater proportion of large individuals in sheltered areas than in exposed area (Figs. 2.3, 2.4).

Microhabitat usage

In both regions, a small fraction of the sampled *P. lividus* urchins were found free on the substrate (ATL: 8.7 %, MED: 10.1 %; Fig. 2.5). In SW Portugal, most urchins were found in burrows (83.7 %), while in NW Italy, most individuals were in crevices (51.7 %). In both regions, urchins in burrows were smaller and free animals were larger (Fig. 2.5).

PERMANOVA results suggest that patterns of microhabitat usage varied between studied regions (Table 2.4; Fig. 2.6). Such variability was mainly due to higher proportion of B urchins in ATL and a higher proportion of C urchins in MED.

Table 2.3 PERMANOVA on *P. lividus* size structure (as a multivariate matrix of 8 size classes) in relation to ‘‘Region’’ (Re), ‘‘Exposure’’ (Ex) and ‘‘Depth’’ (De)

Source	Df	MS	Pseudo-*F*	*p*
Re		1.5385	21.514	**0.0001**
Ex	1	9.5439E-2	1.335	0.2598
De	1	0.17847	2.496	0.0804
Re X Ex	1	0.18032	2.522	0.0797
Re X De	1	3.3039E-2	0.462	0.6848
Ex X De	1	1.4334E-2	0.200	0.8827
Re X Ex X De	16	2.3688E-2	0.331	0.7787
RES	23	7.1515E-2		

Distance measure: Whittaker’s Index of Association. *p* values were obtained using 9,999 random permutations. N = 3 (pooled data from 2 transects per site and 7 quadrates per transect). Significant effects are indicated in bold ($p < 0.05$)

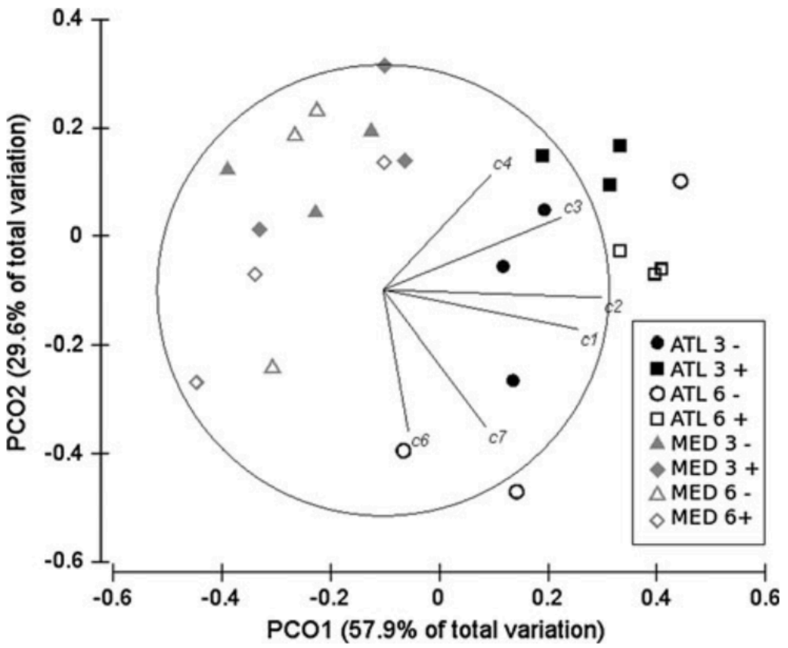

Fig. 2.4 Principal coordinate analysis (PCO) ordination performed on a distance matrix of Whittaker’s Index of Association for *P. lividus* size structure (8 size classes as variables) between replicate ‘‘Sites’’ for each combination of Region (ATL and MED), Depth (3 and 6 m) and Exposure (sheltered and exposed, ‘‘-’’ and ‘‘+’’, respectively). Vector overlay represents the raw Spearman’s correlations of variables (C1–C8, 10-mm adjacent size classes) with the PCO axes

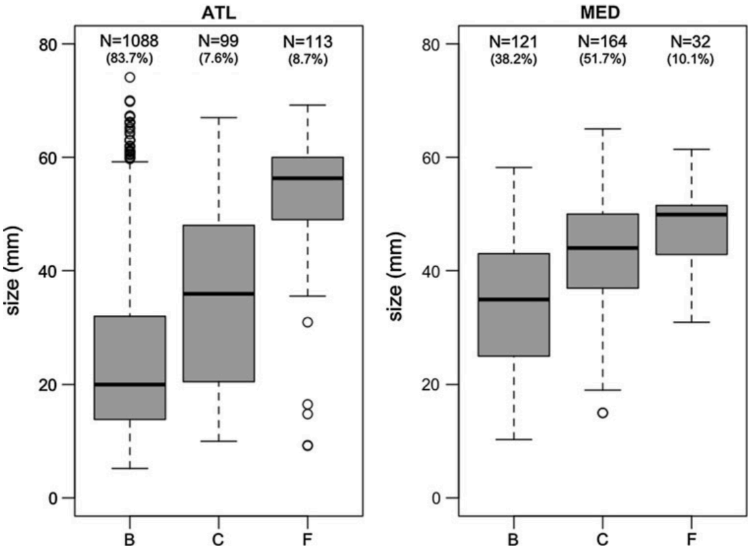

Fig. 2.5 *Paracentrotus lividus* test size (mm) distribution per microhabitat (B in burrow, C in crevice and F free over the substratum) in both studied regions (ATL and MED). Frequency and relative proportion of individuals observed per group are shown above each *box plot*

Table 2.4 PERMANOVA on *P. lividus* microhabitat usage structure (as a multivariate matrix of abundance of urchins per microhabitat: burrows, crevices and free urchins) in relation to ''Region'' (Re), ''Exposure'' (Ex) and ''Depth'' (De)

Source	Df	MS	Pseudo-*F*	*p*
Re		0.90918	10.286	**0.001**
Ex	1	3.9493E-2	0.44682	0.632
De	1	0.26874	3.0405	0.072
Re X Ex	1	0.2251	2.5468	0.102
Re X De	1	9.3067E-2	1.053	0.354
Ex X De	1	0.18373	2.0787	0.148
Re X Ex X De	16	1.7285E-2	0.19556	0.815
RES	23	8.8386E-2		

Distance measure: Whittaker's Index of Association. *p* values were obtained using 9,999 random permutations. N = 3 replicate sites (pooled data from 2 transects per site and 7 quadrates per transect). Significant effects are indicated in bold ($p < 0.05$)

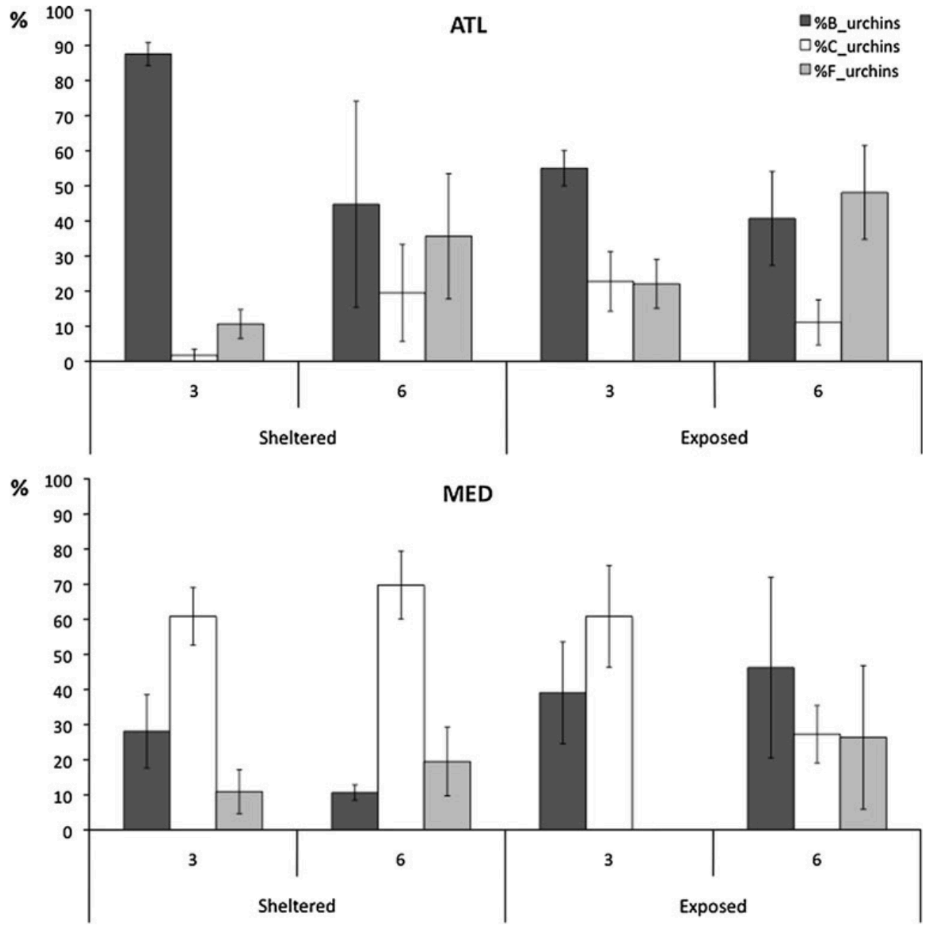

Fig. 2.6 *Paracentrotus lividus*, mean relative frequency of B, C and F urchins (test diameter size range 40–60 mm; mean ± S.E.) in relation to ‘‘Depth’’ (3 and 6 m) and ‘‘Exposure’’ (sheltered and exposed) in both studied regions (ATL and MED). N = 3 replicate sites (pooled data from 2 transects per site and 7 quadrates per transect)

2.5 Discussion

The density of *P. lividus* was 4 times higher in SW Portugal than in NW Italy, probably as a consequence of the larger proportion of juvenile urchins observed in SW Portugal. Several aspects of the study regions, including hydrodynamic stress, SST, productivity, water circulation and substrata characteristics, may interact at different spatiotemporal scales and influence ecological processes affecting different life stages, determining the observed abundance patterns of abundance of *P. lividus* (see Prado et al. 2012).

There was no effect of wave exposure on *P. lividus* abundance neither in NW Italy nor in SW Portugal. Thus, our hypothesis of variation in urchin abundance between sites characterized by different wave exposure was not supported. Data provided by the force transducers used to measure maximal hydrodynamic forces in the field did not reveal major variations in wave climate between regions, at odds with long-term data series from local oceanographic buoys, suggesting that larger waves are more frequent in SW Portugal than in NW Italy (APAT 2004, Franco et al. 2004, Instituto Hidrográfico 2006). Nevertheless, despite a large variability at small spatial scale (metres), hydrodynamic forces were significantly different between levels of exposure in both study regions, validating the classification of exposed and sheltered sites. Although we did not compare vertical variation in wave forces, there is large evidence indicating that they decrease along a depth gradient (Witman 1987, Denny 1988, Siddon and Witman 2003, García-March et al. 2007).

Paracentrotus lividus occurs from intertidal habitats down to 20 m depth, with a trend for abundance to decrease with depth (e.g. Turon et al. 1995, Chellazi et al. 1997, Tuya et al. 2007) and peaks at depths <10 m (Boudouresque and Verlaque 2007). In SW Portugal, *P. lividus* abundance was higher at 3 m depth; this pattern might be related to higher recruitment rates and/or lower predation pressure at shallower depths in this wave-exposed region. In contrast, no variation in urchin abundance with depth was observed in NW Italy. The abundance of *P. lividus* has been found to decrease from shallow to deep habitats (i.e. 1–3 vs 7–9 m), in the Canary Islands (Tuya et al. 2007). Thus, the range of depth investigated in this study (i.e. 3 and 6 m) might have not been wide enough to capture variations related to depth. In addition, *P. lividus* populations are highly variable, both spatially and temporarily (Boudouresque and Verlaque 2007, Hereu et al. 2012), and the ‘‘snapshot-type’’ assessment provided by this study might not be sufficient to take into account temporal variations in patterns of abundance of investigated urchin populations.

Most variation in urchin abundance occurred at smaller scales (metres), and our results suggest that habitat complexity, that is, the presence of cracks and crevices, might be related to higher urchin abundances. Other studies have shown a similar relationship between sea urchin abundance and habitat complexity, suggesting that substrate rugosity and availability of spatial refuges provide shelter from predation (Guidetti et al. 2004a, Hereu et al. 2004a, Clemente et al. 2007) and/or dislodgement by breaking waves (Clemente and Hernández 2008). We only sampled visible urchins, without any effort to locate cryptic individuals (e.g. beneath spine canopy, under boulders, deep inside crevices or cracks). Thus, our observations might be biased towards larger individuals as small individuals (<1–2 cm), which are particularly vulnerable to predation, live permanently in holes, crevices, under pebbles and boulders (Boudouresque and Verlaque 2007). In NW Italy, substrate inclination was also significantly correlated with urchin abundance, suggesting that *P. lividus* is more abundant on horizontal platforms. Bulleri et al. (1999) observed a similar pattern and suggested that it might be related to interspecific interactions between *P. lividus* and *A. lixula*. However, experimental research has failed in supporting such a model (Bulleri et al. 1999).

We observed variation in urchin size distribution between regions and a possible effect of exposure levels in shaping size structure in SW Portugal. The NW Italy populations were characterized by a unimodal size distribution, with a higher proportion of medium-sized individuals. Such patterns are characteristic of regions with low larval supply, low recruitment and/or high juvenile predation rates (Botsford et al. 1994, Sala and Zabala 1996, Vadas Sr et al. 2002). On the contrary, in SW Portugal, smaller individuals represented a large proportion of the populations, suggesting higher recruitment and/or lower juvenile predation pressure, and/or higher selective predation on urchins of intermediate size (Sala and Zabala 1996, Shears and Babcock 2002, Guidetti 2006).

The temporal lag between sampling periods for each region (summer vs. autumn) may have affected the variability observed in the size structure of the urchin populations. *P. lividus* larvae have a peak settlement period during spring and early summer (see references in Boudouresque and Verlaque 2007), so it is possible that the smaller juvenile urchins observed (test diameter, TD < 10 mm) are less than 1 year old. However, it is likely that individuals with a size 10 mm < TD < 20 mm (the modal smaller size class found in Atlantic sites, not present at MED sites) are more than 1 year old (Turon et al. 1995). Sampling season variability may account for the lack of observations of very small juvenile individuals (TD < 10 mm at the MED sites), but not for size classes >10 mm, which are the result of previous settlement episodes, that could not have occurred in between sampling periods.

Variation in urchin population size structure was also observed at smaller scales: size structure was significantly different between exposed and less-exposed areas in SW Portugal, suggesting that the proportion of individuals from different size cohorts vary along wave-exposure gradients. It seems plausible to admit that hydrodynamic forces may be shaping size structure in SW Atlantic urchin populations, supporting our initial hypothesis. Further studies are, however, required in order to assess whether hydrodynamic effects on *P. lividus* population size structure are direct (e.g. affecting local larval supply; dependent on urchin size and attachment strength) or indirect (e.g. acting on sea urchin predators).

We studied microhabitat usage in *P. lividus* populations by considering the number of urchins in shelters, both in natural crevices in the rocks and in urchin-made burrows, and those found freely over the substrate. Results suggest that urchin populations on both studied regions are extremely dependent on shelter as only a small fraction of individuals (10 %) were observed freely over the substrate. Nevertheless, microhabitat usage is different between regions. In SW Portugal, most urchins occurred in burrows, while in NW Italy, urchins were mainly observed in crevices. We ruled out a possible effect of rock type in the observed patterns of urchin microhabitat usage, as there are no evidences of the occurrence of more natural crevices in NW Italy nor that *P. lividus* is more able to excavate burrows in SW Portugal. Despite sea urchins having a higher bioerosion potential in soft substrates (Otter 1932, Trudgill et al. 1987), *P. lividus* burrows were found in all types of rocky substrates, from soft sedimentary and metamorphic substrates to hard igneous ones. Assuming that the investigated populations are under distinct wave climates, our results suggest that creating/occupying burrows might be an adaptive behaviour that allows sea urchins to better withstand stressful hydrodynamic conditions and therefore is more widespread on the exposed Atlantic coast (Otter 1932, Trudgill et al. 1987, Boudouresque and Verlaque 2007). Field data collected in SW Portugal suggest that burrowing enhances urchin attachment force since urchins sampled in burrows required higher forces to be dislodged from the substrate than the ones freely over the substrate (Jacinto and Cruz 2012).

Sea urchin test diameter seems to be the most important factor influencing sheltering behaviour: small individuals are mostly found in crevices or burrows, while larger ones may be found free over the substrate. Such cryptic behaviour of smaller individuals occurs in *P. lividus* and other sea urchin species and is likely an adaptive behaviour that reduces predation pressure and hydrodynamic stress (Nishizaki and Ackerman 2004, Boudouresque and Verlaque 2007, Scheibling and Robinson 2008). Size distribution variability between microhabitats might also be related to feeding behaviour and food quality and/or availability as suggested by other studies where size structure and growth of *P. lividus* populations have been compared between different habitats (e.g. Turon et al. 1995, Gago et al. 2003). Gago et al. (2003) observed that for individuals with the same number of growth rings (an estimate of age), the mean size of urchins from barren habitats (high densities of urchins, most individuals in burrows) was smaller than that from erect algae habitats (less-dense urchin populations), and attributed such size variability to the difference in food quality and availability in both habitats. Similar observations have been made by Turon et al. (1995), which found *P. lividus* growth rates to be smaller in shallower exposed sites, with scarce and low-quality food, when compared to deeper sites, with no food limitation, where sea urchins grew faster and to larger sizes.

When in burrows, sea urchins reduce their cross-sectional area exposed to the water flow, which decreases the lift and drag forces acting on them (Denny 1988, Gaylord et al. 1994), and diminishes the dislodgment risk. Effects of wave exposure on *P. lividus* microhabitat usage were not consistent between regions. The hydrodynamic stress imposed by the wave climate in NW Italy may not be intense enough to trigger a burrowing behaviour response, suggesting that sheltering in natural crevices may enable urchins to withstand wave battering in this area. In contrast, in SW Portugal, where hydrodynamic stress is more severe, urchin burrowing was more widespread.

In summary, our results suggest that the effects of hydrodynamic forces on sea urchins are context dependent and, hence, varying according to background oceanic climate. The mechanisms underpinning effects of hydrodynamic forces on sea urchins are yet to be fully elucidated. Such knowledge is necessary for planning sound strategies for managing *P. lividus* populations.

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Chapter 3. *Paracentrotus lividus* (Echinodermata: Echinoidea) attachment force and burrowing behavior in rocky shores of SW Portugal

Jacinto D., Cruz T. 2012. *Paracentrotus lividus* (Echinodermata: Echinoidea) attachment force and burrowing behavior in rocky shores of SW Portugal. In: Kroh, A. & Reich, M. (Eds.) Echinoderm Research 2010: Proceedings of the Seventh European Conference. Zoosymposia. 7: 231–240.

3.1 Abstract

The rock burrowing sea urchin *Paracentrotus lividus* is a common wave-swept organism that inhabits intertidal rock pools and shallow subtidal temperate shores. Here we present field data on *P. lividus* attachment force, burrow shape and test diameter measured in different rocky habitats (intertidal pools, low shore intertidal channels and shallow subtidal shores) and in two study sites with different wave-exposure conditions. These data were then analysed to look for a possible relationship between the size of sea urchins and their attachment force and burrow shape as well as the influence of wave-exposure and habitat in the measured variables. *P. lividus* test diameter varied among habitats: urchins were smaller in mid-shore intertidal pools and bigger in shallow subtidal shores. We observed in all studied habitats that attachment force was not correlated with test size, while burrow shape index (BSI) was negatively correlated with test size. Results suggest that the attachment force of large and small urchins was similar, but smaller animals occurred in relatively deeper burrows (higher BSI values). Attachment force was positively correlated with BSI, which indicates that burrows may enhance attachment force of *P. lividus* living in rocky shores by allowing the use of spines as anchorage points and reducing the urchin area exposed to drag forces. Our results partially support the hypothesis that attachment force and BSI might be influenced by local variation in wave-exposure. Higher values for both attachment strength and BSI were measured in intertidal rock pools, the most wave-exposed habitat considered in this study, suggesting that urchins living in such habitat are more able to resist dislodgment by wave-induced forces since they occupy relatively deeper burrows and thusattach more firmly to the substrate. However, neither attachment force nor BSI were higher in the more exposed shore.

3.2 Introduction

Stress caused by hydrodynamic forces is an important process that affects ecological structure and community dynamics in wave-swept rocky shores (Denny 1988). Wave induced hydrodynamic forces can break or dislodge organisms, opening patches of substratum for settlement and invasion (Paine and Levin 1981) and may, directly or indirectly, limit the local abundance and distribution of species (Siddon and Witman 2003), the morphology and body size of organisms (Denny 1999), the foraging behaviour (Kawamata 1998), growth (Etter 1989), reproduction (McCarthy et al. 2003), recruitment and survival of organisms (Jonsson et al. 2006). It might also modify intra- and inter-specific interactions (Robles et al. 2001, Wernberg and Connell 2008).

Sea urchins are common mobile organisms in coastal habitats that thrive in a wide range of environmental conditions, from calm bays to exposed capes. Sea urchins rely on their tube feet for locomotion and attachment to the substratum (Lawrence 1987). The strength with which sea urchins attach to a substrate is determined by the tube foot tenacity and the number of tube feet involved (Santos and Flammang 2007). Attachment strength, together with test size and shape influences the maximum hydrodynamic force that different sea urchin species are able to withstand and might account for their distribution patterns (Siddon and Witman 2003, Santos and Flammang 2007, Tuya et al. 2007, Santos and Flammang 2008) and their resistance to predatory attacks (Guidetti and Mori 2005, Gianguzza et al. 2010).

Several echinoid species (*e.g. Stomopneustes variolaris*, *Paracentrotus lividus*, *Echinometra* spp., *Echinostrephus* spp., *Heliocidaris* spp., *Heterocentrotus trigonarius* and *Strongylocentrotus* spp.) exhibit a specialized behaviour that provides extra protection from hydrodynamic stress: rock burrowing by the mechanical action of both spines and teeth (Otter 1932). It has been shown that *Paracentrotus lividus* uses the teeth of its Aristotle's lantern to bore and not its spines (Märkel and Meier 1967 in Asgaard and Bromley 2008). By sheltering into burrows, sea urchins largely reduce their cross-sectional area exposed to the water flow, which decreases the drag forces acting on them (Gaylord et al. 1994) and diminishes the risk of dislodgement. At the same time, burrows may enhance attachment force since both tube feet and spines may be used to anchor individual urchins to the substratum (Otter 1932, Trudgill et al. 1987).

The rock burrowing sea urchin *Paracentrotus lividus* has a wide geographical distribution, occurring mostly on intertidal rock pools and shallow subtidal shores despite exhibiting highly variable spatial and temporal distribution patterns (Boudouresque and Verlaque 2007). In SW Portugal, *P. lividus* abundance and size structure patterns vary at different spatial scales. Smaller sea urchins occur at very high densities in burrows in mid-shore intertidal pools (~300 ind./m^2), while larger individuals occur in moderate densities (~30 ind./m^2; D. Jacinto, unpublished data) in shallow subtidal shores (5–8 meters depth). The proportion of sea urchins in burrows is smaller in shallow subtidal shores when compared to mid-shore intertidal pools (D. Jacinto, unpublished data).

P. lividus is an important ecological and economical resource. As an herbivore, its impact on the surrounding communities might be a density dependent process, which may profoundly affect benthic communities inducing the formation of bare patches dominated by encrusting algae (Benedetti-Cecchi et al*.* 1998). Biological and physical factors such as predation pressure (Hereu 2005) and hydrodynamic stress (Kawamata 1998) influence sea urchin foraging behavior, reducing its grazing activity and dictating the outcome of interactions with local algae populations. Like other rock burrowing species, *P. lividus* can switch from mobile (grazing) to sedentary (drift-feeding) feeding when in burrows (Boudouresque and Verlaque 2007, Asgaard and Bromley 2008).

Being an agent of bioerosion, *P. lividus* can significantly effect on the topography, complexity of the substratum and associated biodiversity. *Paracentrotus* bioerosion is an important process responsible for pool deepening in the mid-intertidal zone and weakening the intertidal rock mass (Trudgill et al. 1987).

Rock burrowing behaviour in *P. lividus* populations is frequently observed in the highly energetic Atlantic shores (Otter 1932, Trudgill et al*.* 1987; Gago et al*.* 2003) but scarce in the Mediterranean (D. Jacinto pers. obs.), probably due to differences in the oceanographic conditions and wave regimes. Such observations suggest that rock burrowing behaviour in *P. lividus* might be an adaptive response to hydrodynamic forces, enhancing their attachment force and ability to resist dislodgement in wave-swept habitats as proposed by Otter (1932).

In the present study, we collected data on *P. lividus* test diameter, attachment force, surface rugosity and burrow shape in different rocky habitats (mid-shore intertidal pools, low-shore intertidal channels and shallow subtidal shores), in order to test the following hypotheses:

Urchin size affects attachment force. Within each studied habitat we expect a positive and significant relationship between attachment force and urchin size.

Burrows enhance attachment force. Within each studied habitat we expect a positive and significant relationship between attachment force and burrow shape index (BSI; a measure of the rugosity or surface profile of the substrate from where the sea urchin was dislodged). We also test for a possible relationship between BSI and urchin size.

Wave-exposure variation between shores and habitats influences *P. lividus* attachment force and BSI. We expect a larger attachment force and a larger BSI in more wave-exposed shores and habitats. Our study is one of the first attempts to measure attachment force of the sea urchin *P. lividus* in the field (but see Guidetti and Mori 2005, Santos and Flammang 2007, Gianguzza et al. 2010) and to assess the importance of urchin burrows as an adaptive strategy to resist dislodgment in wave-swept habitats.

3.3 Materials and methods

The field study was carried out between August to October 2009 on intertidal and shallow subtidal rocky shores of SW Portugal (Fig. 3.1). Two shores were considered, *Cabo de Sines* (CAB) and *Praia do Burrinho* (BUR), about ten kilometres apart (Fig. 3.1). CAB is a headland, mainly consisting of igneous rock formations (gabbro) with a complex topography, while BUR is a metamorphic rocky shore (mainly schist) situated southwest from CAB in the lee of the headland. Location of the shores relatively to the prevalent income wave and wind direction (from NW) and previous observations and measurements on the variability of subtidal hydrodynamic forces in SW Portugal (D. Jacinto, unpublished data) suggest that hydrodynamic stress is higher in CAB. Three habitats were considered in each shore: mid-shore intertidal pools (IP), low-shore intertidal channels (<1 m deep; IC), and shallow subtidal shores (6 m depth; SS). Empirical data show that wave induced flow decreases with depth (Siddon and Witman 2003). Here, we assume that the studied habitats are subjected to variable wave-induced hydrodynamic stress (higher in mid-shore intertidal pools and lower in shallow subtidal shores).

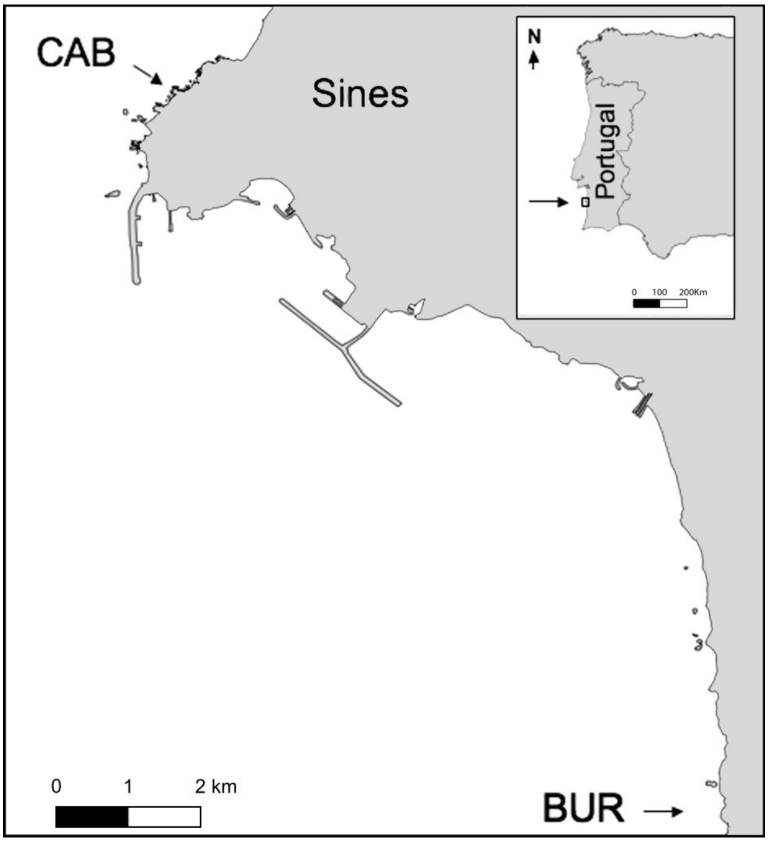

Fig. 3.1 Location of study area in relation to mainland Portugal (inset). Two shores (~ 10 km apart) were sampled: CAB = Cabo de Sines and BUR = Praia do Burrinho.

In each shore and habitat, individual sea urchins (n = 50) were sampled. For each individual, we measured the following variables: 1) attachment force; 2) test diameter; and 3) Burrow Shape Index (BSI), a measure of the rugosity or surface profile of the substrate from where the sea urchin was dislodged (regardless of urchins being sampled inside or outside of burrows).

Attachment force was measured by clamping a metal grab (a steel compass modified as a grabbing claw that allows a proper grip to sea urchins even when inside tight burrows; Fig. 3.2) to the sea urchin’s test, connected by a hook to a spring dynamometer, and pulled normally to the substratum at an approximate constant speed. The maximum force (Newton) required to dislodge each sea urchin was recorded.

Test diameter of the dislodged urchin was measured with a calliper to the nearest millimetre.

A plastic profile copy gauge, or profilometer, was used to measure BSI, the topographical rugosity of the substrate from where the sea urchin was dislodged. By pressing the profile gauge against the surface where the urchin was laying on, we were able to transfer the profile of the surface or burrow where the urchins were found to an underwater slate, and later analyse it with image analysis software (ImageJ; Abràmoff et al*.* 2004). Two perpendicular profile measurements were made for each surface or burrow. We considered the variable burrow shape index (BSI) as the average ratio between the length of the surface or burrow profile (BP) and the length of the burrow opening (BO), *i.e.*, contoured-to-linear distance of the topography of the surface or burrow where the urchins were found (BSI=BP/BO). BSI is higher than one when urchins occur in burrows (concave surfaces) and approaches the unity when urchins are found on very shallow burrows or outside burrows (flat surfaces).

The hypotheses of a relation between attachment force and BSI with urchin size, and of attachment force with BSI were tested by non-parametric Spearman's rank correlation (r_S) for each habitat. The hypothesis that wave-exposure variation between shores and habitats influences *P. lividus* attachment force and BSI was tested by ANOVA in an experimental design with 2 factors: 1) habitat (H), a fixed factor with 3 levels (IP, IC and SS); 2) Shore (S), a fixed factor with 2 levels (CAB = *Cabo de Sines* and BUR = *Praia do Burrinho*) (n=50). Homogeneity of variance was assessed using Cochran's C-test and Student-Newman-Keuls (SNK) tests were used when appropriate (Underwood 1997). The package GAD (Sandrini-Neto and Camargo 2011) for R software (R Development Core Team 2011) was used to perform the analysis (according to Underwood 1997).

Fig. 3.2 Metal grab used to clamp *P. lividus* and measure attachment force when connected by a hook to a spring dynamometer. The gear consists of a steel compass modified as a grabbing claw that allows a proper grip to sea urchins even when inside tight burrows.

3.4 Results

Urchin size (test diameter) distribution varied amongst studied habitats and shores (Fig. 3.3A; Fig. 3.4D–F; Tables 3.1 and 3.2). Overall, smaller urchins were sampled in mid-shore intertidal pools (34.6 ± 4.88 mm, mean ± SD; range: 25–47 mm), medium sized urchins in low-shore intertidal channels (48.0 ± 6.57 mm, mean ± SD; range: 29–66 mm) and larger individuals in shallow subtidal shores (59.8 ± 8.42 mm, mean±SD; range: 29–82 mm). Differences on sea urchin mean test diameter were also detected between shores, suggesting that sea urchins sampled in CAB were smaller than the ones sampled in BUR (Tables 3.1 and 3.2; Fig. 3.3A).

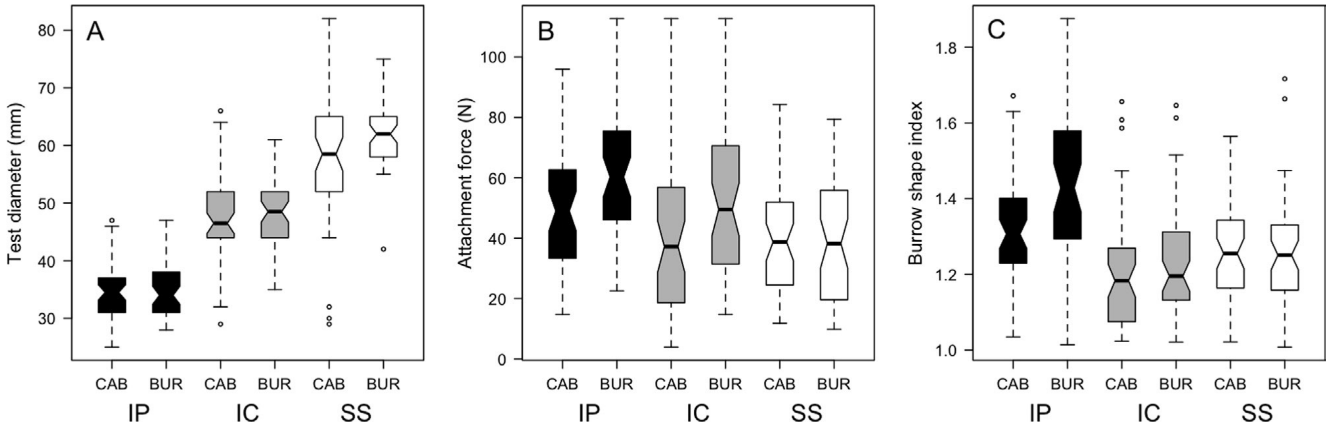

Fig. 3.3 *Paracentrotus lividus*. Boxplots of (A) Test diameter (mm), (B) attachment force (N) and (C) Burrow shape Index per habitat (IP = mid-shore intertidal pools; IC = low-shore intertidal channels and SS = shallow subtidal shores) and study site (CAB and BUR) in SW Portugal (n = 50).

P. lividus attachment force was highly variable within each habitat and shore (Fig. 3.3B; Fig. 3.4A–C; Tables 3.1 and 3.2): in IP, it varied between 14.7 N and 112.7 N (mean ± SE: 49.0 ± 2.54 N and 62.2 ± 2.92 N, CAB and BUR, respectively); in IC, it varied between 3.9 N and 112.6 N (mean ± SE: 38.5 ± 3.47 N and 53.5±3.62 N, CAB and BUR, respectively); and in SS, it varied between 9.8 N and 84.3 N (mean±SE: 39.3±2.49 N and 38.3±2.87 N, CAB and BUR, respectively). Results show a strong interaction effect ($p < 0.01$; Tables 3.1 and 3.2) between the two main factors, habitat and shore, on the distribution patterns of mean attachment force values (SNK tests for the interaction H x S, Table 3.2). Both in CAB and BUR, the force required to dislodge sea urchins from the substrate was on average higher in IP than any other habitat. However, while in BUR significant differences were found between average attachment forces measured in intertidal channel and shallow subtidal rocky shores (IP > SS), no such differences were found on CAB (IP=SS). Higher mean attachment force values were measured in intertidal pools (IP) of BUR than those of CAB. The same was true for urchins sampled in intertidal channels (IC) while on shallow subtidal rocky habitat (SS) no differences were found between study shores.

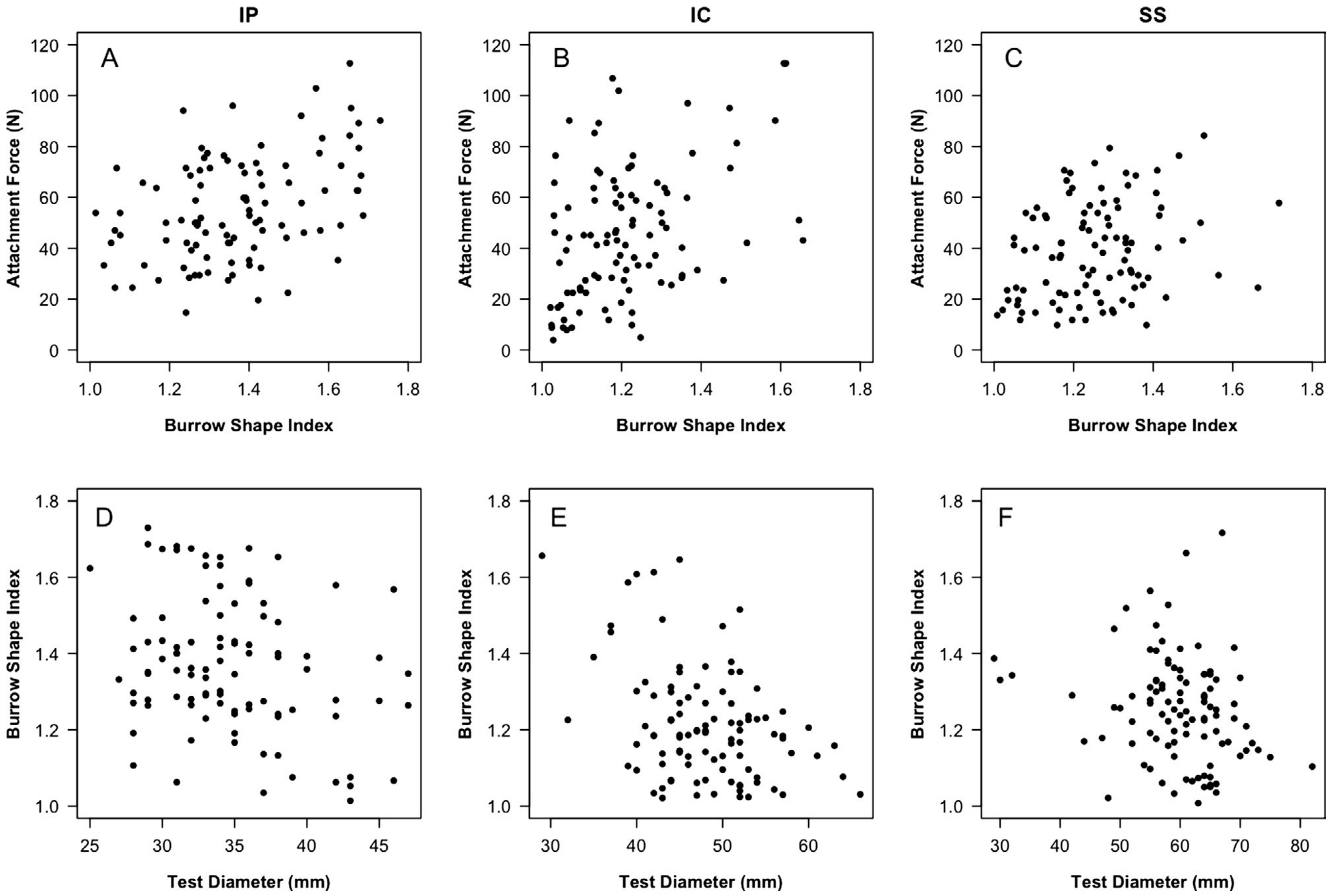

Fig. 3.4 *Paracentrotus lividus*. Bivariate scatter plots of (A–C) Attachment force (N) against Burrow Shape Index (all significant positive correlations at $p < 0.05$) and (D–F) Burrow Shape Index against test diameter (mm) (all significant negative correlations at $p < 0.05$) per habitat (IP = mid-shore intertidal pools; IC = low-shore intertidal channels and SS = shallow subtidal shores) and study site (CAB and BUR) in SW Portugal (n = 50).

BSI values were also highly variable within habitats and shores (Fig. 3.3C; Fig. 3.4D–F; Tables 3.1 and 3.2). Despite a large span of BSI values within each habitat and shore (between 1.02 and 1.88), mean BSI values measured in IP (mean ± SE: 1.31 ± 0.021 and 1.44 ± 0.021, CAB and BUR, respectively) were higher in comparison to IC (mean±SE: 1.20±0.018 and 1.23±0.021, CAB and BUR, respectively) and SS (mean±SE: 1.26±0.022 and 1.25±0.028, CAB and BUR, respectively). Again, a strong interaction effect was detected ($p < 0.01$; Table 3.1) between the two main factors, habitat and shore, on the distribution patterns of mean BSI values (SNK tests for the interaction H x S, Table 3.2). Mean BSI values were higher in intertidal rock pools (IP) of BUR than in CAB. In intertidal channels (IC) and shallow subtidal rocky habitat (SS) no differences between shores were found in mean BSI values. Significant differences were also detected in mean BSI values between habitats within each shore. In BUR, mean BSI values were higher in IP than in IC and SS. In CAB, the mean BSI values were similar between IP and SS, and higher than the ones measured in IC.

No significant correlations were found between attachment force and test diameter for urchins sampled in each of the studied habitats (IP: $r_s = 0.14$, $p = 0.17$; IC: $r_s = -0.15$, $p = 0.13$; SS: $r_s = -0.10$, $p = 0.33$; $n = 100$), refuting our hypothesis, and suggesting that factors other than size (which is related with the number and area of attachment discs) influence *P. lividus* attachment force in the field.

As hypothesized, positive significant correlations were found between *P. lividus* attachment force and Burrow Shape Index (Fig. 3.4A–C) for sea urchins sampled in each habitat (IP: $r_s = 0.36$, $p < 0.01$; IC: $r_s = 0.36$, $p < 0.001$; SS: $r_s = 0.27$, $p < 0.01$; $n = 100$), suggesting that burrowing enhances attachment force in *P. lividus,* since individuals in relatively deeper burrows require higher force to be dislodged.

Negative significant correlations (Fig. 3.4D–F) were found between BSI and test diameter within each habitat (IP: $r_s = -0.27$, $p < 0.01$; IC: $r_s = -0.30$, $p < 0.01$; SS: $r_s = -0.26$, $p < 0.01$; $n = 100$ pairs of data for each habitat), suggesting that smaller individuals occupy relatively deeper burrows while larger individuals may be found in relatively shallower burrows or outside of burrows.

ANOVA and SNK tests results (Tables 3.1 and 3.2) partially support the hypothesis that wave-exposure variation between shores and habitats influences *P. lividus* attachment force and BSI. While higher values of BSI and attachment strength were measured in intertidal pools (IP), presumably the most exposed habitat, when comparing between shores, there is no evidence that the study variables are influenced by shore exposure as expected. In fact, when significant differences were detected between shores (Table 3.2), both attachment strength and BSI, values were higher in BUR (the least exposed shore).

Table 3.1 Analysis of variance on *P. lividus* test diameter, attachment force and burrow shape index (BSI) in SW Portugal in relation to habitat (H) and shore (S). Data Transformation: Attachment force data transformed to the fourth root(x); BSI data transformed to squared root(x); Cochran's test, $p < 0.05$ (test diameter), $p > 0.05$ (attachment force and burrow shape index). n = 50. df = degrees of freedom; MS = mean square; F = test statistic. Test statistic significance levels: * ($p < 0.05$); ** ($p<0.01$); ***($p<0.001$); ns ($p > 0.05$).

ANOVA		**Test Diameter**		**Attachment Force**		**BSI**	
Source of variation	**df**	**MS**	**F**	**MS**	**F**	**MS**	**F**
H	2	15810.1	352.3 ***	1.777	16.6 ***	0.138	30.5 ***
S	1	274.6	6.1 *	1.285	12.0 ***	0.036	8.0 **
H x S	2	88.5	2.0 ns	0.599	5.6 **	0.023	5.1 **
Residual	294	44.9		0.107		0.005	

Table 3.2 SNK tests for the main terms habitat (H) and shore (S) (test diameter) and interaction term H x S (attachment force and BSI = burrow shape index). = ($p > 0.05$); > or < ($p < 0.05$). IP = intertidal rock pool; IC = low shore intertidal channel; SS = shallow subtidal rocky shore; CAB = Cabo de Sines; BUR = Praia do Burrinho.

Test Diameter	**Attachment Force**	**BSI**
H: IP < IC < SS	BUR: SS < IC < IP	BUR: SS = IC < IP
S: BUR > CAB	CAB: SS = IC < IP	CAB: IC < SS = IP
	SS: BUR = CAB	SS: BUR = CAB
	IC: BUR > CAB	IC: BUR = CAB
	IP: BUR > CAB	IP: BUR > CAB

3.5 Discussion

Rock burrowing behavior in sea urchins has been described as an adaptation for life in wave swept rocky shores (Otter 1932). In SW Portugal, coastal habitats are largely exposed to normal Atlantic swell and may be considered highly stressful environments (Instituto Hidrográfico 2006). In this region *P. lividus* is frequently found in burrows. Few studies on *P. lividus* attachment force have been conducted in the field (Santos and Flammang 2007, Gianguzza et al. 2010) and to our knowledge the attachment force of urchins in burrows has never been measured in field studies. The method used in this study allowed us to successfully detach urchins within burrows and provide a data set of field measurements regarding the rock burrowing echinoid *P. lividus* attachment force to the substratum in different habitats.

P. lividus test diameter varied among habitats: urchins were smaller in mid-shore intertidal pools and bigger in shallow subtidal shores. Such results are concordant with what has been observed in previous studies in the same region (D. Jacinto, pers. obs.).

It has been observed that under similar laboratory conditions, *P. lividus* attachment force varied with test size (Guidetti and Mori 2005), possibly due to an increase in number of tube feet with test diameter (Santos and Flammang 2007). Yet, we observed in all studied habitats that attachment force was not correlated with test size, but burrow shape index was negatively correlated with test size. The attachment force of large and small urchins is similar, but smaller animals occur in relatively deeper burrows. These results suggest that smaller individuals create or occupy relatively deeper burrows (higher BSI values) to properly attach to the substrate and resist dislodgment, while larger individuals may occur in relatively shallower burrows or even free over the substrate, as other factors like foot tenacity and the number of tube feet involved in attachment, might suffice to provide the necessary attachment force.

In all studied habitats, attachment force was positively correlated with BSI, which supports our hypothesis and indicates that burrows can enhance attachment force of *P. lividus* living in rocky shores, by allowing the use of spines as anchorage points and reducing the area exposed to drag forces. Our results partially support our hypothesis regarding attachment force and burrow shape index being influenced by local variation in wave-exposure. Higher values for both attachment strength and burrow shape index were measured in intertidal rock pools, the most wave-exposed habitat considered in this study, suggesting that urchins living in such habitat are more able to resist dislodgment by wave-induced forces since they occupy relatively deeper burrows and thus attach more firmly to the substrate. However, neither attachment force nor burrow shape index were higher in the more exposed shore (CAB). In fact, when there were differences (both variables in mid-shore intertidal pools and attachment force in low-shore intertidal channels), the opposite was observed (higher values in the less exposed shore - BUR). Other factors might explain the observed pattern in mid-shore intertidal pools. Intertidal pools of BUR are shallower than in CAB. Despite CAB being more exposed than BUR, at a smaller scale, water flow might be higher in tidepools of BUR due to local topography and tidepool characteristics. Small-scale water flow measurements should be made in order to explain burrowing behaviour variability. Another possible confounding factor between CAB and BUR is the geological formation of tidepools in both shores. CAB is made of volcanic rocks, while tide pools of BUR have been excavated in the smoother schists. Consequently, it is much easier for an urchin to excavate deeper burrows in BUR than in CAB. Mineral composition of the substrate has been shown to influence burrowing depth in *Strongylocentrotus purpuratus* (J. Hernandéz, pers. com.), another rock burrowing sea urchin, which is able to excavate deeper burrows in softer rocky shores. Another important factor that might potentially explain the observed patterns is predation pressure. As proposed by Menge and Sutherland (1987) predation pressure is expected to be higher in more benign physical environments. It is plausible to admit that predation pressure (potential predators include fishes, sea stars and birds) might be higher in BUR (the less exposed shore). If burrowing behaviour enhances urchin attachment strength preventing the success of putative predatory attacks, then the observed patterns of BSI variability between the study sites might have been shaped by local variation in predation pressure (*e.g.* sea urchins in relatively shallow burrows or outside burrows might be heavily predated in intertidal pools of the less environmental stressful shores, and as a result only urchins in relatively deeper burrows are found, hence the higher BSI values observed in BUR). Our study supports the model that burrowing behaviour enhances sea urchin attachment force, and might be an adaptive response to hydrodynamic stress as proposed by Otter (1932). However, alternative models including the influence of predation pressure in sea urchin burrowing behaviour should be properly addressed in future studies. Additional research is needed to elucidate the processes responsible for variability in attachment force and burrowing behaviour of sea urchins, considering the combined effect of exposure to hydrodynamic forces and predation pressure on burrowing behaviour, while accounting for possible confounding effects like the type of rocky substrate and characteristics of the studied populations and habitats.

3.6 Acknowledgments

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Chapter 4. Small scale patterns and processes of distribution of the stalked barnacle *Pollicipes pollicipes* at Cabo de Sines (SW Portugal)

Jacinto D., Cruz T. *in prep.* Small scale patterns and processes of distribution of the stalked barnacle *Pollicipes pollicipes* at Cabo de Sines (SW Portugal).

4.1 Abstract

Patterns and processes of distribution and abundance of the stalked barnacle *Pollicipes pollicipes* at Cabo de Sines (SW Portugal) were studied at very small scales (1-10’s m) along vertical and horizontal gradients.

Percent cover of barnacles were assessed by image analysis and varied at all spatial scales considered in this study. *P. pollicipes* percent cover varies along a wave exposure gradient (being higher on more exposed sites) and vertically along the intertidal (higher in the upper intertidal at some of the more exposed sites).

In order to explain these patterns, we investigated the variability of predation and recruitment along the same vertical and horizontal gradients.

Predation on barnacles was studied by outplanting clumps of *P. pollicipes* into different sites (exposed vs sheltered sites) and heights on the intertidal zone (low vs mid intertidal). Results suggest that predation is more intense at the sheltered sites.

Recruitment patterns were studied on artificial plates and on adult peduncles. Recruitment to artificial plates was more intense at exposed sites, while the opposite pattern was observed in adult peduncles (contrasting exposed and sheltered sites), suggesting that recruitment variability to adult peduncles along wave exposure gradients might be a density dependent process.

Both predation and recruitment processes seem to be shaping the highly variable barnacle distribution patterns at very small scales.

Understanding the maintenance of such patterns at small scales is of great importance for management strategies of an important resource such as *P. pollicipes.*

4.2 Introduction

Along its geographical range, the pedunculate barnacle *Pollicipes pollicipes* (Gmelin, 1790) occurs preferentially in wave-exposed rocky shores with intense surf action and turbulent water conditions, where it may be locally abundant forming dense aggregations in intertidal and shallow subtidal habitats (Barnes 1996). Observations carried out at different sites on wave exposed rocky shores of Portugal suggest that *P. pollicipes* distribution patterns are highly variable at smaller scales (meters apart) along both vertical and horizontal gradients (Cruz 2000, Sousa et al. 2013). This distribution is apparently common despite varying local or regional environmental conditions (rock type and structure, oceanographic conditions, amongst others). In areas where *P. pollicipes* is abundant (e.g. capes and very exposed sites along the SW coast of Portugal), its small-scale horizontal distribution is discontinuous and barnacle abundance can drop down to complete absence in just a few meters (Cruz 2000). High densities and percent cover of *P. pollicipes* are usually observed in rock walls directly facing the swell, while on the opposing walls of those same rocks barnacles are practically absent, or if present are restricted to a few, usually large individuals occupying tight crevices or rock fissures. Vertically, *P. pollicipes* abundance varies along the subtidal / intertidal gradient. Higher densities and percent cover are observed in the mid intertidal while in the low intertidal and shallow subtidal it is more common to observe sparse clumps of individuals within the biological matrix that forms the low intertidal communities (Cruz 2000, Sousa et al. 2013).

Different biological and physical processes may be responsible for shaping such spatial patterns of distribution. Barnes and Reese (1960) proposed a stimulation trigger model suggesting that the restriction of *P. polymerus* (ecologically comparable to *P. pollicipes*) to places with wave action is related to the necessity for a certain general stimulation to initiate full activity, and also to the method of macro-feeding, once this level of activity has been reached. Thus, in quiet places such as protected bays or harbours as well as in the sublittoral the necessary stimulation is absent and stalked barnacles do not occur (Barnes and Reese 1960, Barnes 1996; Borja et al. 2006). However, it is possible to observe (although in very low densities) adult *P. pollicipes* in less exposed conditions, and preliminary studies where clumps of barnacles were outplanted for several months into cages in less exposed sites where barnacles do not naturally occur, have shown that *P. pollicipes* is able to survive and thrive in such conditions (Cruz et al, unpublished data). Such observations contest the stimulation trigger model and suggest that wave exposure may be indirectly influencing *P. pollicipes* distribution patterns by affecting other ecological processes.

Both bottom-up (e.g. larval supply, settlement, growth and recruitment) and top-down (e.g. predation, human exploitation) processes may vary along physical stress gradients (e.g. wave exposure), interact and drive invertebrate population distribution variability at different spatial and temporal scales (Menge and Sutherland 1987, Rilov et al. 2004). Settlement in *Pollicipes* spp. is heavy among adults of the same species, but whether species recognition is involved in this behaviour or whether it is only the specialized requirements of the surface texture that are met most commonly by the surface of the peduncles of adults, is not known (Barnes 1996). In SW Portugal, *P. pollicipes* recruitment period extends throughout 6 months peaking in late summer (Cruz et al. 2010). Recruitment is intense and varies vertically along the intertidal. Recruitment index (IR) measured as the number of cyprids and juveniles (rosto-carinal distance, RC<6mm) per adult was significantly higher in lower intertidal levels comparing to the upper ones (Cruz 2000, Cruz et al. 2010). Higher immersion times and/or less early mortality were suggested as explanations for such variability (Cruz 2000, Cruz et al. 2010). Pineda (1994), observed recruitment in *P. polymerus* to vary between sites less than 100’s m apart. The author discusses that such patterns might result from variable larval delivery rate, concentration or diffusion of larvae by site-specific micro-hydrodynamics processes, or by strong behavioural preferences at settlement (Pineda 1994). Hoffman (1989) observed that recolonization by *P. polymerus* of denuded areas was variable at small scales, and suggested that such difference may be explained by the orientation and exposure of the substrata.

A biological process like predation may strongly influence the distribution and abundance of intertidal organisms. Predation on *Pollicipes*, as in any other intertidal organism, occurs at different life stages which in turn may affect the success and growth of their populations (Pineda 1994). Mortality and predation rates may vary at different spatial scales along vertical and horizontal gradients and may also account for the abundance patterns in *Pollicipes* populations. Several animal species (e.g. fishes, crabs, birds, worms, molluscs) have been identified as potential predators of *Pollicipes* spp. and along with human predation may play a big role in regulating barnacle abundance and distribution patterns (Barnes 1996). Mortality during the first 2-3 weeks after settlement was relatively high in *P. polymerus* spat (Hoffman 1989). Hoffman (1989) often found the nemertean *Empleconema gracile*, foraging on young animals; the worms were wrapped around small animals many of which often lacked capitula. Bernard (1988) describes that vigorous predation on *P. polymerus* by small crabs and polychetes might have led to the disappearance (within 6 weeks of settlement) of numerous small gooseneck barnacles present on adult peduncles, on solid substrate, lamelarian fronds and coralline algae in tidepools. In SW Portugal, sea breams (*Diplodus* spp.) stomach contents have often been observed filled with *P. pollicipes* (Cruz 2000). Avian predation effects on the distribution and abundance of *P. polymerus* have also been described (Meese 1993, Wootton 1997). In the Iberian Peninsula, human predation on the stalked barnacle *Pollicipes pollicipes* is intense wherever it is abundant (Cruz 2000, Molares and Freire 2003, Borja et al. 2006, Jesus 2006, Bald et al. 2006, Jacinto et al. 2010, 2011). Low intertidal stalked barnacles are subject to a greater human predation pressure due to their higher quality (e.g. tougher peduncles, bigger individuals), and higher commercial value (Jacinto et al. 2011). Such chronicle and variable harvesting pressure may account for vertical abundance variability in *P. pollicipes* intertidal populations. However, vertical abundance variability has also been observed in areas where *P. pollicipes* exploitation is prohibited (Borja et al. 2006, Sousa et al. 2013).

Based on previous observations, we tested hypotheses derived from models potentially explaining the indirect effect of wave exposure on the highly variable small-scale distribution patterns in stalked barnacle populations. We expected *P. pollicipes* abundance, predation pressure and recruitment patterns to vary along small spatial scale wave exposure gradients. We hypothesized that 1) *P. pollicipes* abundance patterns (i.e. percent cover) would be positively correlated with wave exposure measured in sites few meters apart from each other, possibly as a result of a 2) lower predation pressure and/or 3) higher recruitment rates at the most exposed sites, when conducting experiments in analogous wave exposure gradients at similar spatial scales.

4.3 Materials and methods

Study site

This study was done at Cape of Sines (SW Portugal; Fig. 4.1), a very exposed and topographically complex igneous rocky shore (mostly gabbros, diorites and syenites, but also sandstones) where stalked barnacles are abundant (Sousa et al. 2013).

Small scale distribution patterns

Percent coverage of *P. pollicipes* was studied around a small isolated rock (perimeter ~ 120m; Fig. 4.1) at two heights in the intertidal zone (Low and Mid Intertidal) by image analysis of 25x25cm photo-quadrats sampled during the summer of 2007.

Eight sites were defined at fixed angles (45º apart; N, NE, E, SE, S, SW, W, NW) with the approximate centroid of the rock area (estimated by analysis of aerial photographs). At each site, two intertidal heights were defined, based on barnacle distribution at the exposed walls: Low intertidal (where barnacle clumps occur interspersed with red algae) and Mid Intertidal (where *P. pollicipes* dominates). Height on the intertidal was kept the same between sites. Location of sites and height on the intertidal were established using a Sokkia C330 automatic level.

At each height within each site, 8 photo-quadrats (25x25cm, 1m apart) were sampled. For each photo-quadrat, percent coverage of *P. pollicipes* was estimated using the image analysis software imageJ (Version1.43; National Institutes of Health, Bethesda, MD, USA). Quadrat orientation and inclination were measured in the field using a compass and inclinometer.

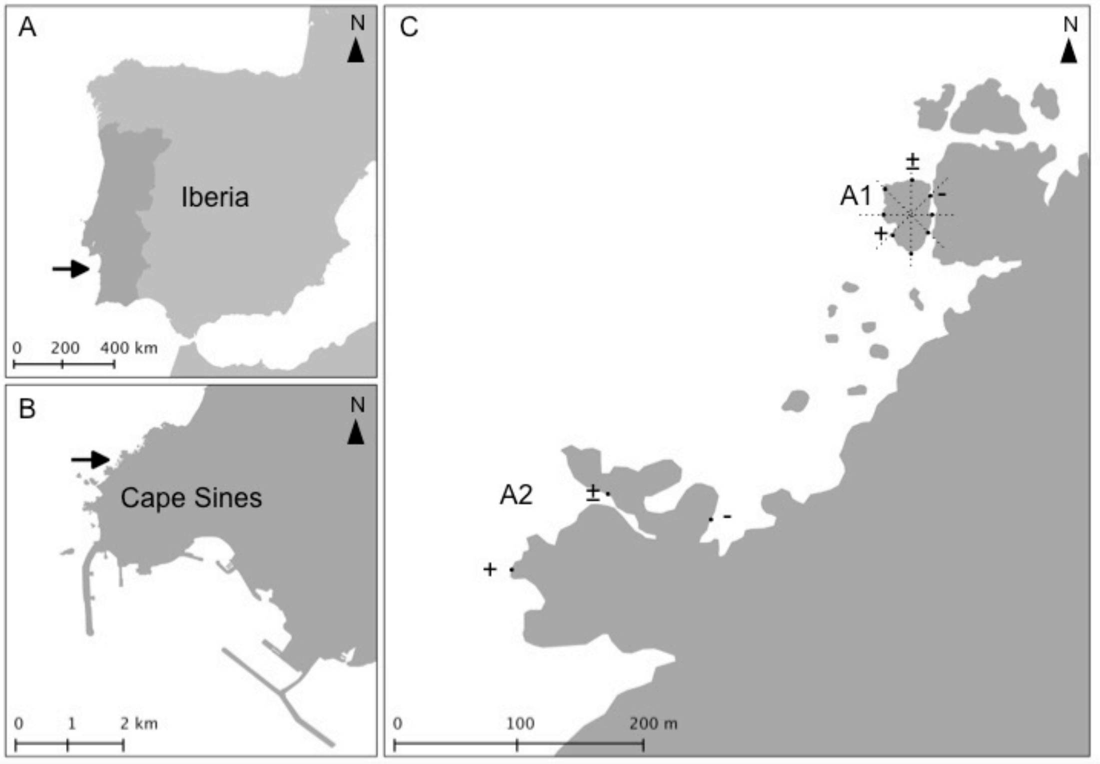

Fig. 4.1 Location of study area in the SW Portuguese coast in relation to the Iberian Peninsula (A) and Cape Sines (B). Small-scale map showing the relative position of the study areas (A1 and A2) and sites (black dots; + more exposed; ± more less exposed; - less exposed) in Cape Sines. Dotted lines depicted over area A1 (C) show the position of eight study sites defined at fixed angles (45º apart; N, NE, E, SE, S, SW, W, NW) with the approximate centroid of the rock area.

In order to contrast *P. pollicipes* percent coverage among sites and height on the shore, we performed a distance-based permutational ANOVA (Anderson 2001). A two-way design was performed, in which 'Site' was treated as a fixed factor with eight levels (N, NE, E, SE, S, SW, W, NW sites) and 'Height' as a fixed factor with two levels (Low and Mid intertidal). All analyses were based on Euclidean distances of the original raw data, with all p-values obtained using 4999 permutations of the appropriate exchangeable units (Anderson 2001). Significant terms in the full model were examined individually using appropriate *a posteriori* pairwise comparisons. The software PRIMER 6 and PERMANOVA+ was used to perform these analyses (Anderson et al. 2008).

Wave force measurement

Force transducers were built and calibrated as described by Bell and Denny (1994), and used to measure maximal wave force at the studied sites (2 transducers at the Mid intertidal and 2 at the Low intertidal) in 6 occasions during late Summer of 2007 (August 30 and 31, September 5, 12 and 29 and November 10; deployment periods of 1 day). In order to safely deploy and collect the force transducers at the study sites, we chose to consider only days of relatively calm sea. Daily maximal wave heights registered during the deployment periods by an oceanographic buoy located near the experimental site (Fig. 4.1) were between 0.54m and 1.35m. Average maximal wave force was used to compare wave exposure between sites. Due to the variability observed in maximal wave forces registered, we averaged the results obtained by the 4 dynamometers deployed at each site.

Correlation between barnacle percent coverage and wave force at the site scale were assessed using Spearman's rank correlation coefficient.

Modeling small scale variability in barnacle distribution patterns

The aim of this part of the study was to find a relationship between barnacle abundance (percent coverage) and the physical characteristics of the surrounding site (site orientation, site exposure, height in the intertidal, quadrat orientation and quadrat inclination).

Data exploration and generalized addictive models (GAM) using the Poisson distribution and log link function were performed using the R software (ww.r-project.com). Overdispersion was detected, and the standard errors were corrected using a quasi-

GAM model where the variance is given by $\varphi \times \mu$, where μ is the mean and φ the dispersion parameter. Comparisons of deviances of nested models (using *F*-test) were carried out to identify the optimal GAM model (Zuur et al. 2007). The optimal model was considered to be the most parsimonious one, including variables with significant effects and with no clear patterns in the distribution of the deviance residuals when plotted against the selected explanatory variables.

Predation

Predation pressure variability between exposed and sheltered sites was studied by outplanting chips of rock with attached barnacle clumps at both exposure conditions and observing its integrity after a few days.

In 5 occasions, we outplanted barnacle clumps taken from a nearby site with high barnacle abundance to exposed and sheltered sites (n=5-6). Chips of rock with attached barnacle clumps were carefully removed from the source area, using a hammer and chisel, and affixed to the rock with Z-Spar Splash Zone Epoxy (Kop-Coat Inc., Pittsburgh, PA).

After a few days (variable between experiments depending on wave climate; 1 to 13 days), we counted the number of rock chips left (with or without barnacles). During the experiment, several chips of rock were lost, as so these were not considered for data analysis.

Predation was considered more intense at sites were the relative number of rock chips with barnacle clumps was less by the end of the experiments.

A similar experiment was carried out during September 2008, but considering two factors: exposure (2 levels, More vs Less exposed) and intertidal height (2 levels, Low and Mid intertidal). Replicate clumps allocated to each treatment (n=5-6) were monitored from August 31st to September 8th 2007. During the monitoring period, each barnacle clump was given a survival success value according to its state: 1- clump intact, 0.5- clump with visible signs of predation (e.g. barnacles without capitulum, peduncle leftovers, visible attachment “scars” in the rock chip), 0- all barnacles predated. Mean survival rate was estimated as the sum of survival success values (within level of the considered factors) divided by the initial number of clumps.

Recruitment

Recruitment to artificial substrate

P. pollicipes recruitment variability along wave exposure gradients was studied using artificial settlement plates.

Settlement plates consisted of a 2.5x7cm rectangle of grey 3M Safety-Walk™ Resilient Medium Grade tape glued to the concavity of a 2.5cm diameter PVC semi-pipe, painted with a mixture of crushed adult barnacles and seawater (40 gl^{-1}) at least 6 h before the immersion period. Plates were attached to the substrate (concavity facing the rock surface, previously scraped of all macro-organisms) with a stainless steel screw during low tides, collected after 1 month (depending on wave conditions), and carried to the laboratory, where settled cyprids and juvenile *P. pollicipes* were identified and counted under a dissecting microscope. Settlement plates were reutilized after a cleansing bath (30min in bleach followed by 24 hours in tap water).

We followed a 4 factor experimental design, considering i) Exposure (fixed factor with 3 levels: 1, 2 and 3 along a wave force gradient; differences assessed with force transducers deployed for one day on November 27, 2007, not shown), ii) Intertidal height (fixed factor with 2 levels: Mid and Low intertidal, as previously defined), iii) Date (random factor with 3 levels: date 1, 2 and 3; during *P. pollicipes* settlement season in 2007 and 2008) and iv) Area (random factor with 2 levels: area AMA and NEG, 0.5km apart) (N=3-5 replicate settlement plates). Data was analysed by PERMANOVA (as above) and *a posteriori* pair-wise comparisons were made when appropriate.

Recruitment to adult peduncles

An experiment with caged clumps of barnacles was established in the exposed and less exposed areas of the same isolated rock used to study small-scale distribution patterns at the Cape of Sines. Before the beginning of the recruitment season (July 2007), 5 clumps of barnacles were outplanted (see method above) inside macro-predator exclusion cages (20 x 15 x 10 cm; 1cm plastic mesh size) at each site (exposed and sheltered). By October 2007, towards the end of the recruitment season, we removed the caged barnacle clumps and carried them to the laboratory, where settled cyprids and juveniles (Rostro-Carinal length, RC < 1mm) attached to adult peduncles (RC>13.5 mm) were identified and counted under a dissecting microscope. Several clumps at the most exposed site were lost due to wave climate, and the fact that different clumps had different number of adult barnacles led to a an unbalanced number of adult peduncles being analyzed between sites (n=5 adult peduncles at the exposed site; n=13 at the sheltered site). We also collected some “natural” occurring individuals (n=30) at the exposed site to compare with the experimental results. Differences between treatments were assessed by Welch’s t-test.

4.4 Results

Distribution patterns

Abundance patterns of *P. pollicipes* were highly variable at all scales considered in this study (Figs. 4.2.A and 4.2.B). Barnacle percent coverage was higher at sites facing the swell (clockwise from SW to N; maximal observed percent cover was 58.1% at a quadrat on the Mid intertidal of the N facing site), whilst at the opposing sites (NE to S) barnacles were absent or with very low percent coverage.

Variability was also detected between intertidal heights. Where barnacles are abundant, they usually have higher percent coverage at the Mid intertidal, and less percent coverage at the Low intertidal (e.g. NW and N sites).

At smaller scales (between quadrats), barnacle percent coverage is also highly variable as suggested by the range of the data shown in Fig. 4.2 (e.g. Mid intertidal at the N site, where barnacle cover varied between 0 and 58.1%.

PERMANOVA results (Table 4.1) suggest a strong interaction ($p < 0.05$) effect between the 2 main factors (Site*Height). Pairwise tests to the interaction term (Table 4.1), comparing barnacle percent coverage between intertidal heights within each site, suggest that significant differences ($p<0.05$) only occur at the NW and N sites.

On the other hand, when comparing barnacle percent coverage between sites at the low intertidal, 2 groups were determined ($p<0.5$): high percent coverage at sites SW, W and NW and low percent coverage at the remaining sites.

At the mid intertidal, 3 groups were determined: highest percent coverage (NW), high percent coverage (SW, W, and N) and low percent coverage at the remaining sites.

Fig. 4.2 *P. pollicipes* percent cover per 25x25cm quadrat, and wave force distribution around a small islet at the Cape of Sines, SW Portugal. Figure shows barnacle abundance at the A) low intertidal, B) mid Intertidal and C) maximal hydrodynamic forces measured at different sites at fixed angles (45º increments) in relation to the centroid of the study area. The summary statistics used to create the box and whiskers plots are the median of the data, the lower and upper quartiles (25% and 75%) and the minimum and maximum values.

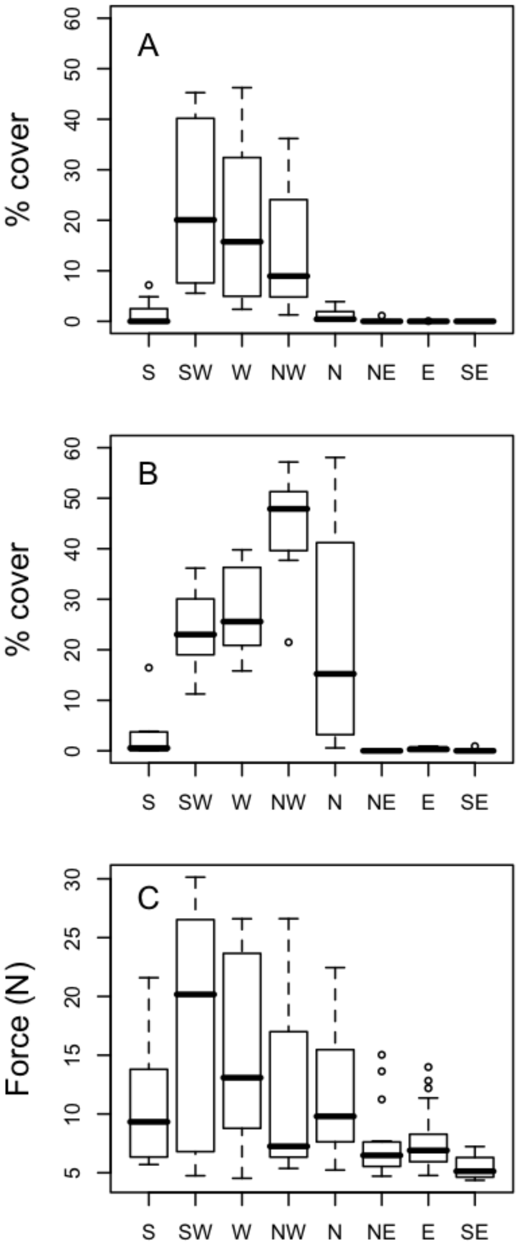

Table 4.1 A) PERMANOVA analysis of the effects of ''Height'' (HE) and ''Site'' (SI) on *Pollicipes pollicipes* percent coverage. Analyses were based on Euclidean distances of untransformed data and p-values were obtained using 4999 random permutations. N = 8 replicate photographs (percentage cover in 25x25cm photo-quadrats). Significant effects are indicated in bold ($p<0.01$). B) Pair-wise tests to the significant interaction term in the previous analyses. = ($p > 0.05$); > or < ($p < 0.05$); Mid - mid shore; Low - low shore; N, NE, E, SE, S, SW, W, NW - site orientation.

A) PERMANOVA

Source	df	MS	Pseudo-F
HE	*1*	0.19581	20.544
SI	*7*	0.2478	25.999
HE*SI	*7*	5.4582E-2	**5.7266**
Res	*112*	9.5313E-3	

B) Pair-wise tests

Low : SW = W = NW > N = NE = E = SE = S
Mid : NW > SW = W = N > NE = E = SE = S
NW, N : Low < Mid
All other sites: Low = Mid

Wave force measurement

Wave force measurements varied around the study area as shown in Fig. 4.2C. A clear pattern on wave force variability around the study area is visible, with sites from S to N (clockwise) being more exposed and subjected to higher forces than sites NE to SE (clockwise).

Mean wave force measured for the different sites are correlated with averaged (Low and Mid intertidal pooled data) *P. pollicipes* abundance (Spearman's $\rho = 0.74$; $p < 0.05$; n = 8 data pairs). Correlations are significant between wave force and barnacle abundance at both intertidal heights considered (Low intertidal: Spearman's $\rho = 0.91$; $p < 0.05$; n = 8 data pairs; Mid intertidal: Spearman's $\rho = 0.88$; $p < 0.05$; n = 8 data pairs)

Modelling small scale variability in barnacle distribution patterns

Barnacle abundance (%cover) around the study area is correlated with wave exposure, which depends on the orientation of the site to the swell.

PERMANOVA results suggest that barnacle abundance patterns vary between sites and height on the intertidal.

We modelled the response variable, barnacle percent coverage, as a function of interacting effects of two explanatory variables, one continuous and circular, site orientation (in relation to the centroid of the study area) and one nominal, intertidal level (mid and low intertidal).

The model that best explained the variability in barnacle percent coverage around the study area (74.9% of the deviance explained by this model) was a semi-parametric GAM with a Poisson distribution corrected for overdispersion, and had the form:

% cover ~ factor(intertidal level) + s(site orientation)

Numerical output for the model is shown in Table 4.2, smoothing functions and model validation plots are shown in Fig. 4.3. The effects of the individual levels of height and the smoothing terms for the effect of site angle by intertidal height were significant at $p < 0.05$.

For model validation purposes, deviance residuals were plotted against the explanatory variables (Fig. 4.3 C and D).

The variability observed between quadrats at those sites where barnacles were abundant (S, SW, W, NW, and N sites; as shown in Figs. 4.2 A and B), may be related to other explanatory variables varying at such scale. We tested for correlations between barnacle percent coverage and quadrat inclination, and results suggest a positive correlation between both variables at different intertidal heights (Low intertidal: Spearman's $\rho = 0.67$; $p < 0.05$; $n = 79$ data pairs; Mid intertidal: Spearman's $\rho = 0.41$; $p < 0.05$; $n = 79$ data pairs), suggesting that barnacle abundance increases with substrate inclination.

Table 4.2 Numerical output for a generalised additive model (GAM) using Poisson distribution corrected for overdispersion log link function (dispersion parameter. Model uses *P. pollicipes* percentage cover as response variable and intertidal level and site orientation as explanatory variables. A) Estimated parameters and B) Approximate significance of smooth terms. Bold type indicates significance (p< 0.05)

A) Parametric coefficients:

	Estimate	Std. Error	*t* -value	*p* - value
Intercept	0.6202	0.3710	1.672	0.0973
Factor(intertidal level = mid)	1.0360	0.4446	2.330	**0.0216**

B) Approximate significance of smooth terms:

	edf	F	*p* - value
s(siteAngle): Factor(intertidal level = low)	3.92	7.94	**< 0.001**
s(siteAngle): Factor(intertidal level = mid)	4.91	11.97	**< 0.001**

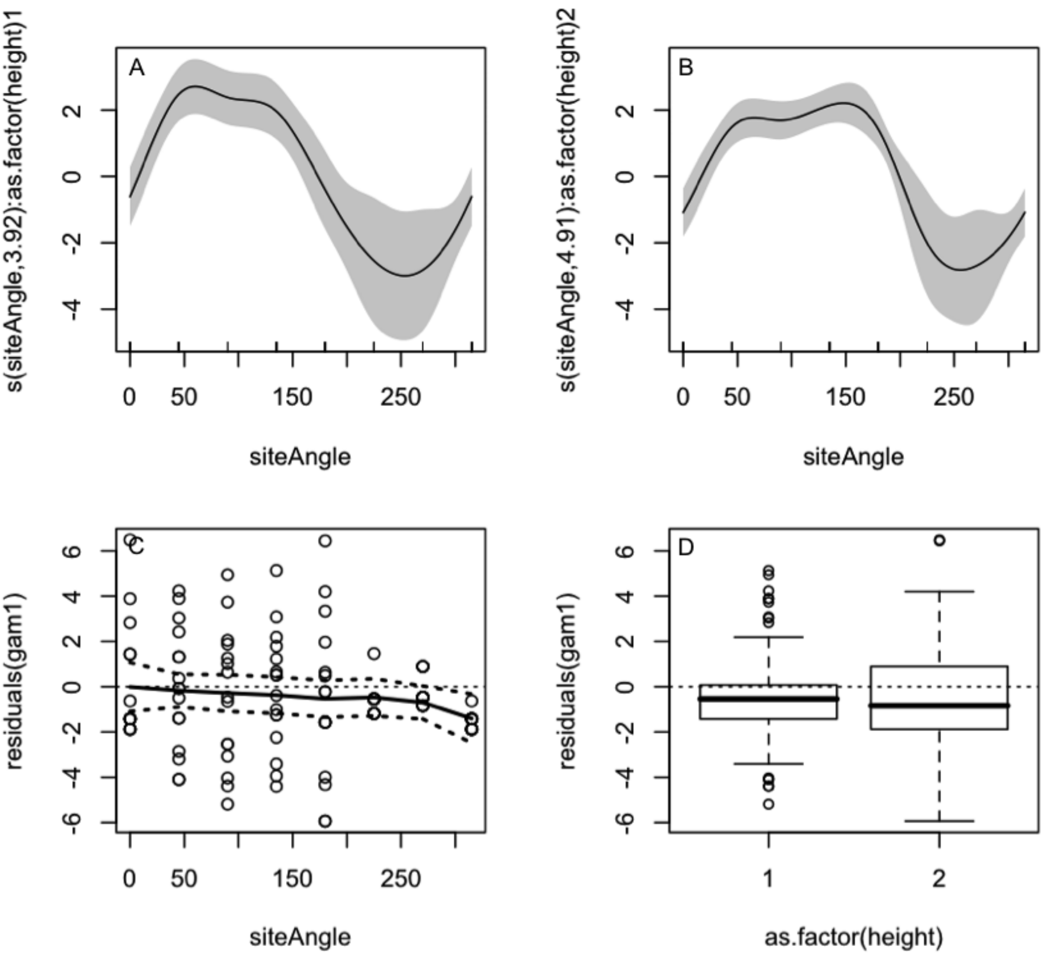

Fig. 4.3 Smoothers and deviance residuals versus explanatory variables in the GAM model with Poisson distribution (corrected for overdispersion) and Log link function. A and B: Smoothing curves for site orientation by intertidal level (Low and Mid intertidal, respectively). Vertical axis shows the contribution of the site orientation smoother to the fitted values. Solid lines are the estimated smoother, and the grey areas are 95% confidence bands. Cross-Validation was used to estimate the degrees of freedom for each smoother (edf: 3.92 and 4.91, respectively; see table 2). C: Deviance residuals versus site orientation. A LOESS curve with a span width of 0.65 and corresponding 95% confidence bands were added to aid visual interpretation. D: Deviance residuals versus intertidal level.

Predation

In all 5 of the predation experiment trials, survival was highest (80 to 100% survival rates) at the most exposed site (Fig. 4.4). At the sheltered site barnacle survival rate was always less when compared to the exposed site and varied between 0 and 65%. Results suggest that predation is more effective at the sheltered site

The 9 day experiment during which outplanted barnacle clumps to different wave exposure conditions and intertidal heights were monitored as frequently as possible, suggest that predation varies along vertical and horizontal gradients being more effective at the Low intertidal in sheltered sites (Fig. 4.5). Traces of predation were visible in clumps outplanted to the sheltered sites ~24h after the beginning of the experiment. Barnacle clumps outplanted to the low intertidal at the sheltered site were rapidly predated (after ~36 hours, all clumps were predated). At the mid intertidal of sheltered sites, barnacle clumps were gradually predated, and by the end of the experiment survival rate was 10%. At the most exposed site, survival rate was 100% for the Mid intertidal clumps, while some amount of predation was observed at the low intertidal treatment, which by the end of the experiment had a survival rate of 50%.

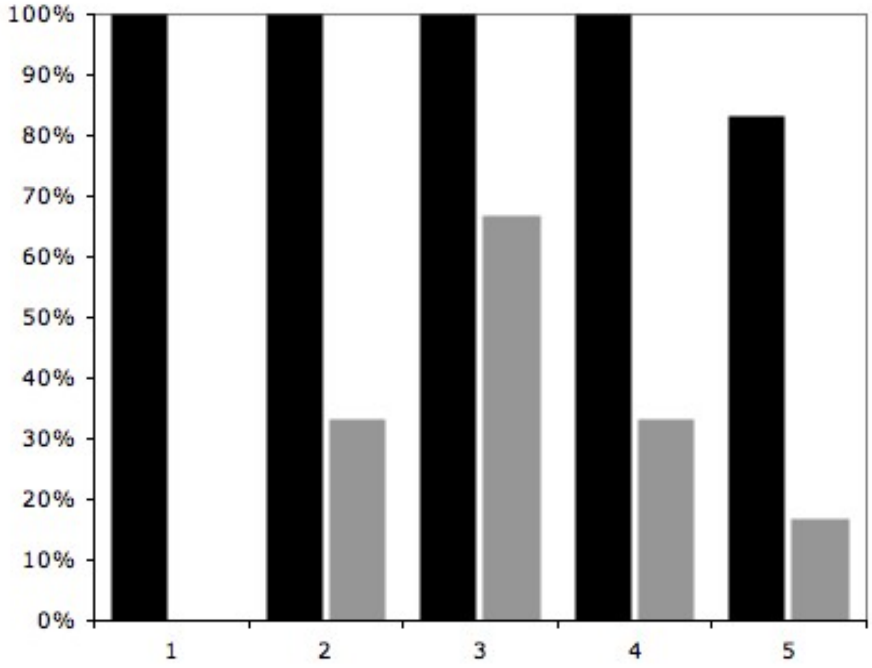

Fig. 4.4 Outplanted *P. pollicipes* clump survival rate at exposed (black bars) and sheltered (grey bars) sites in 5 different trials.

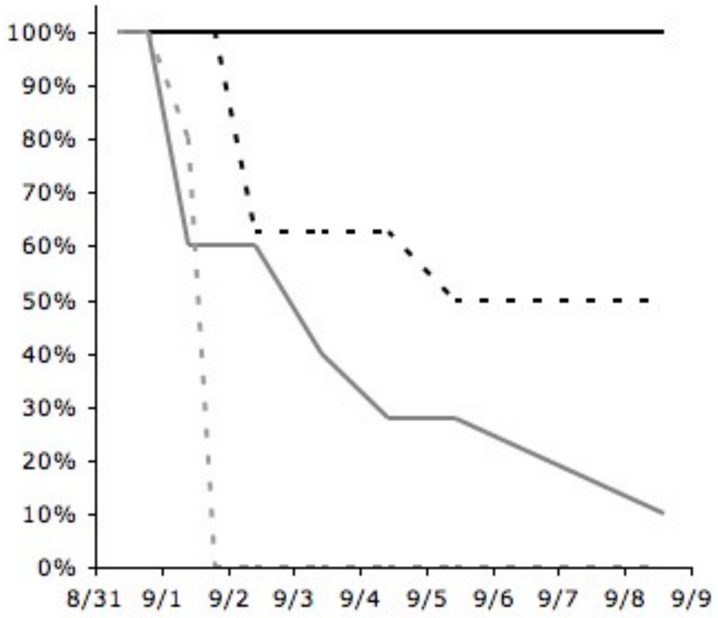

Fig. 4.5 Outplanted *P. pollicipes* clump survival rate at exposed (black) and sheltered (grey) sites, at different intertidal heights (Mid intertidal – solid lines; Low intertidal – dotted lines), at the Cape of Sines (SW, Portugal) between August 31 and September 9, 2008.

Recruitment

Artificial substrates

Mean monthly recruitment to artificial substrates was variable at all spatial scales considered (Fig. 4.6; factor Date was non-significant at $p > 0.25$, so data for that factor was pooled), and ranged from to 0 recruits (Mid intertidal on less exposed sites on both areas) to 11.15 ± 3.19 recruits (mean ± SE) per settlement plate (Mid intertidal of the most exposed site in area 1).

PERMANOVA results suggest a significant interaction effect ($p < 0.05$) between the 3 main factors considered (Exposure X Height X Area; Table 4.3).

Pairwise-tests results to the interaction term are summarized in Table 4.3. For pairs of levels of factor “Intertidal Height” within the different levels of factors “Exposure” and

"Site", show that except for the most exposed site at area 1, where recruitment was more intense at the Mid intertidal comparing to the low intertidal, no significant differences (p > 0.05) were detected between intertidal heights for each exposure level within each area.

The same tests, but for pairs of levels of factor "Exposure" within the different levels of factors "Height" and "Site", show that recruitment intensity varied along wave exposure gradients in both study areas and at both heights in a similar way. Both at the mid and low intertidal, recruitment was more intense at the most exposed sites and practically null at the less exposed sites, the only exceptions were that no significant differences (p< 0.05) were found between exposure levels 3 and 2, at the mid intertidal of area 2; and between exposure levels 2 and 1, at the Low intertidal of area 1. Recruitment was also highly variable between replicate settlement plates.

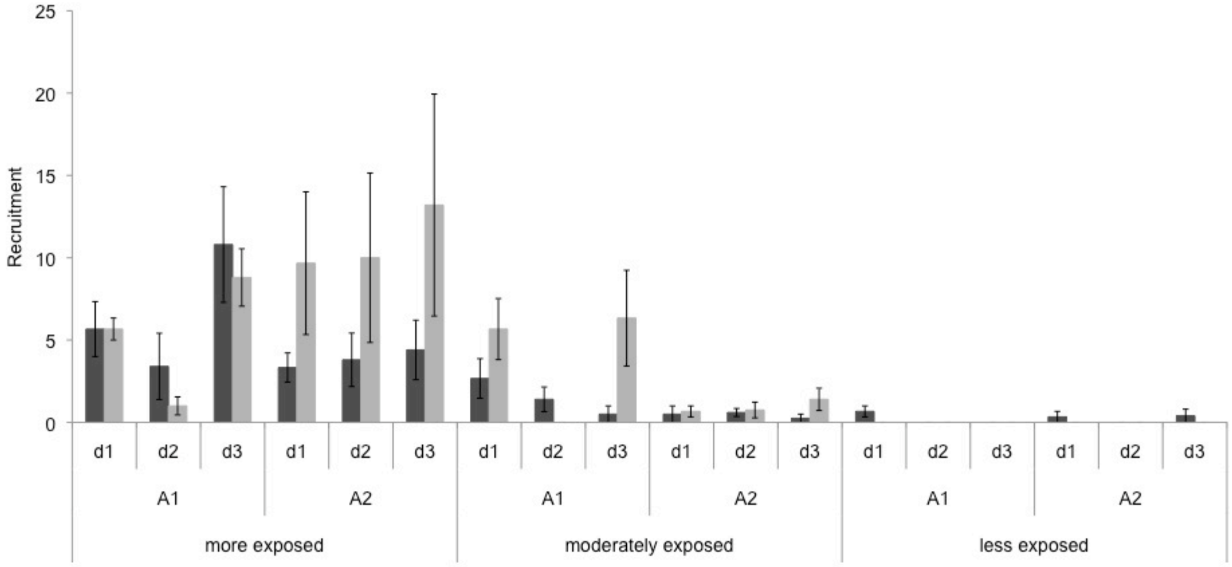

Fig. 4.6 *P. pollicipes* monthly recruitment (mean± SE) to settlement plates (N=3-5), between levels of factor exposure to wave forces (more, moderately and less exposed), study area (A1 and A2), date (d1, d2 and d3) and intertidal level (Low – dark grey bars; Mid – light grey bars).

Table 4.3: A) PERMANOVA analysis on the effects of “Exposure” (EX), ‘‘Height’’ (HE) and ‘‘Site’’ (SI) on *Pollicipes pollicipes* monthly recruitment to artificial plates. Analyses were based on Euclidean distances of untransformed data and p-values were obtained using 4999 random permutations. N = 11-13 replicate settlement plates (pooled data from 3 random dates). Significant effects are indicated in bold ($p<0.05$). B) Pair-wise tests to the significant interaction term in the previous analyses. = ($p > 0.05$); > or < ($p < 0.05$); A1 and A2, study areas.

A) PERMANOVA

Source	df	MS	Pseudo-F
EX	2	624.35	**19.591**
HE	1	58.832	1.0053
SI	1	5.4688E-3	2.834E-4
EX*HE	2	28.253	0.28746
EX*SI	2	31.87	1.6516
HE*SI	1	58.524	3.0328
EX*HE*SI	2	98.284	**5.0932**
Res	137	19.297	

B) Pair-wise tests

	A1	A2
More exposed (ME) :	Low = Mid	Low < Mid
Moderately exposed (mE) :	Low = Mid	Low = Mid
Less exposed (LE) :	Low = Mid	Low = Mid
Mid:	LE < mE = ME	LE < mE < ME
Low :	LE < mE < ME	LE = mE < ME

Adult peduncles

Recruitment patterns on adult peduncles are shown in Fig. 4.7. Mean recruitment per peduncle was 10.15 ± 4.31 (mean± SE) at the less exposed site (adult barnacles kept in cages), and 24.92 ± 4.31 (mean± SE) at the most exposed site (pooled data for caged barnacles and “natural” barnacles since no significant differences were found by t-test; p > 0.25).

The small number of adult peduncles analysed require caution when interpreting the results.

Results seem to suggest that there's a density dependent effect on *P. pollicipes* recruitment to adult barnacles. Recruitment to adult peduncles seems to be amplified in the sheltered site.

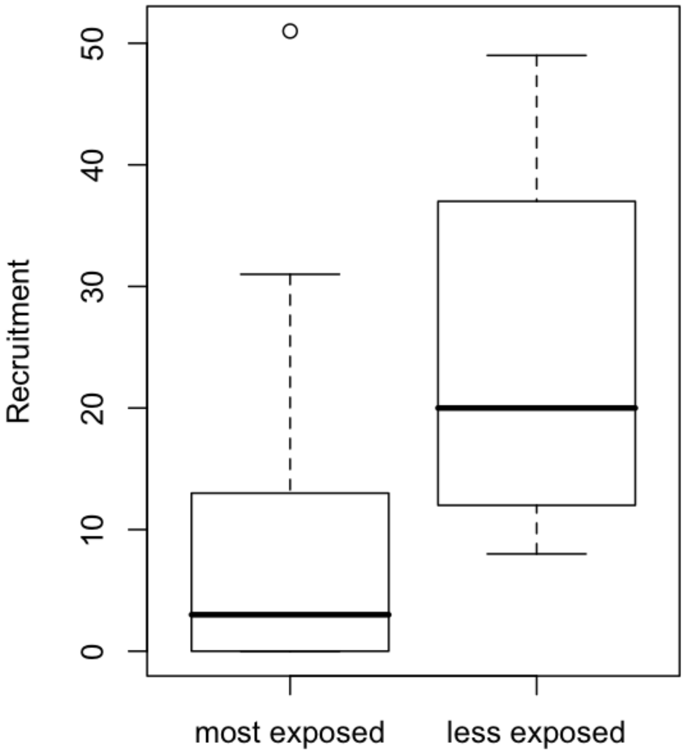

Fig. 4.7 *P. pollicipes* recruitment to adult peduncles outplanted to an exposed and a less exposed site at the Cape of Sines, Portugal. N=13.

4.5 Discussion

As hypothesized, small-scale patterns of abundance observed in this study clearly show that the stalked barnacle *P. pollicipes* abundance is related to wave exposure. Barnacles are more abundant in stretches of rocky shore directly facing the swell, and thus exposed to higher hydrodynamic forces.

Hydrodynamic forces are highly variable in space and its magnitude depends on wave climate, local topography and bathymetry, small and micro scale topography (e.g. Bell and Denny 1994, Denny et al. 2003, O'Donnell 2008). Such tight relation between *P. Pollicipes* abundance and wave exposure has been shown at larger scales (Borja et al. 2006), yet here we show how such pattern occurs at smaller scales (10's meters) and might be scale independent.

Barnes (1996) discusses how hydrodynamic forces and water flow affects the feeding behaviour of *P. polymerus*, compromising its ability to thrive in less exposed sites. Other physical factors like thermal stress could also limit *Pollicipes* spp. in such less exposed or sheltered conditions. When kept in cages in less exposed sites, barnacles where able to survive and grow for several months (this study; Cruz et al, unpublished data), so wave exposure might be shaping such patterns not by imposing direct physical limitations to the barnacles but possibly by indirectly affecting biological processes like settlement and predation.

Predation on *P. pollicipes* was more intense in less exposed sites, and at different heights on the shore (more intense in the low intertidal, compared to the mid intertidal). Such predation patterns seem to fit the model of barnacle abundance patterns being shaped by predatory effects, and may explain the spatial variability observed in barnacle populations, not only horizontally along wave exposure gradients but also vertically along the intertidal fringe.

On the other way, settlement and recruitment also varied horizontally along wave exposure gradients. Recruitment to settlement plates was more intense in exposed sites but no differences between intertidal heights were observed on most of the study sites.

Larval supply might be variable at such small scales, larval behaviour at the time of settlement might lead to such patterns (Pineda 1994), intense chemical clues from areas with higher barnacle abundance might guide larvae to these areas (Kingsford et al. 2002) and early mortality in settlers attached to artificial plates may be variable in space and related to wave exposure. On the contrary recruitment to adult peduncles (preferred settlement substrate) in less exposed sites was higher than in more exposed sites, suggesting that larval supply is not a limiting factor and that cyprids may find isolated clumps of barnacles to settle. Such observations suggest that recruitment to adult peduncles might be a density dependent process that is amplified in situations where barnacles are scarce (e.g. when isolated clumps are outplanted to less exposed sites with no tangible cover of barnacles). This relates to what was proposed by Pineda (1994) in the decreased-substrate settlement intensification hypothesis, which predicts that settlement intensification should occur at shore extremes or where available settlement area could be limiting (Bertness et al. 1992).

Both top-down (predation) and bottom-up (recruitment) processes seem to be interacting and shaping small-scale barnacle abundance patterns. We suggest that wave exposure is indirectly responsible for the observed patterns of distribution, by affecting biological processes such as settlement and predation.

Predation is more intense in less exposed sites, so it is less probable that isolated barnacles or clumps are able to survive in such situation, and when they do, they are normally restricted to tight crevices or cracks in the rocky shore. Since adult barnacle peduncles are the preferred settlement substrate for *P. pollicipes* cyprids, a negative loop is created and barnacle populations will tend to disappear from sites with higher predation rates, even if settlement is a density dependent process that is amplified in a low density population situation. This mechanism might be very important for the reestablishment of barnacle denuded sites, but is controlled by predation.

This study suggests that biological processes responsible for small-scale variability in barnacle abundance are affected by wave exposure. Since wave exposure in one site depends on its orientation to the prevailing swell, it may be used as a proxy to estimate barnacle abundance. Detailed cartography and higher resolution wave exposure models may lead to more precise estimates of barnacle abundance, potentially useful for stock evaluations of this heavily exploited resource.

Studies on *Pollicipes* cyprid distribution on the nearshore suggest that larval supply is equally intense at different sites in the SW coast of Portugal, with different wave exposure (Cape of Sines and nearshore south of the cape) (Cruz et al, unpublished data). Early mortality and predation might also be responsible for abundance patterns at larger scales (Cruz et al, unpublished data).

Our study has some limitations due to insufficient spatial replication, and such patterns and processes must be evaluated at different locations along *P. pollicipes* distribution range and at different spatial scales in order to allow generalization of the proposed model.

4.6 Acknowledgments

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Chapter 5. Predicting *Pollicipes pollicipes* (Crustacea: Cirripedia) abundance in intertidal rocky shores of SW Portugal: a multi-scale approach based on a simple fetch-based wave exposure index

Jacinto D., Cruz T. 2016. Predicting *Pollicipes pollicipes* (Crustacea: Cirripedia) abundance in intertidal rocky shores of SW Portugal: a multi-scale approach based on a simple fetch-based wave exposure index. Sci. Mar. 80(2): 229-236.

5.1 Abstract

Understanding and predicting patterns of distribution and abundance of marine resources is important for conservation and management purposes from small-scale and artisanal fisheries to industrial fisheries worldwide. The stalked barnacle *Pollicipes pollicipes* is an important shellfish resource and its distribution is tightly related to wave exposure at different spatial scales. We have modeled the abundance (percent coverage) of *P. pollicipes* as a function of a simple wave exposure index based on fetch estimates from digitized coastlines at different spatial scales. The present model accounted for 47.5% of the explained deviance, and according to the model, barnacle abundance increases non-linearly with wave exposure at both the smallest (meters) and largest spatial (kilometres) scales considered in this study. Distribution maps were predicted for the study region in SW Portugal. Our study suggests that the relationship between fetch-based exposure indices and *P. pollicipes* percent cover may be used as a simple tool able to provide stakeholders with information on barnacle distribution patterns that may lead to better assessment of harvesting grounds and dimension of exploitable areas, which may help improving management plans and support decision making regarding conservation, harvesting pressure and surveillance strategies for this highly appreciated and socio-economic important marine resource.

5.2 Introduction

Understanding and predicting patterns of distribution and abundance of marine resources is important for conservation and management purposes from small-scale and artisanal fisheries to industrial fisheries worldwide. Spatial planning and management of marine resources requires information on distribution patterns at relevant scales (Bekkby et al. 2009), which may be insufficient and/or difficult to obtain due to logistical constraints of biological sampling across large areas and/or on extreme habitats such as very exposed wave-swept rocky shores. When data is scarce, predicting patterns of distribution using relationships between species abundance and physical variables may provide the required information and assist management and conservation decision-making (Hill et al. 2010).

The role of wave exposure as a key process shaping the composition of coastal communities has long been acknowledged (Ballantine 1961, Kingsbury 1962, Lewis 1964). Wave exposure potentially affects both the organisms and their interactions, by acting directly as a mechanical stressor (McQuaid and Branch 1985, Menge and Sutherland 1987, Denny 1988), and indirectly, by affecting temperature (West and Salm 2003), sedimentation (Airoldi 2003, Schiel et al. 2006), nutrient intake (Hearn et al. 2001) and productivity (Hurd 2000), amongst other relevant ecological factors.

The stalked barnacle *Pollicipes pollicipes* is an important shellfish resource in the North East Atlantic coast from Brittany to Morocco and is intensively harvested on intertidal and shallow subtidal rocky shores (Boukaici et al. 2012, Parada et al. 2012, Sousa et al. 2013). Along its distribution range, *P. pollicipes* occurs mostly in wave exposed locations such as capes and headlands (Barnes 1996), but within such locations its abundance varies at small spatial scales (meters apart) depending on the orientation of the site to the prevailing income wave direction (Borja et al. 2006). On less exposed areas, *P. pollicipes* occurrence is rare and mainly restricted to a few individuals occupying small shelter areas like cracks and crevices. Recent observations made on the SW coast of Portugal suggest that top-down control via predation is one of the main processes shaping *P. pollicipes* abundance patterns (unbuplished results), and may vary along wave exposure gradients at a multitude of spatial and temporal scales.

Along the SW coast of Portugal, exposed rocky shores dominate the coastal landscape. Despite a few headlands, this coastline is roughly rectilinearly oriented from north to south, exposed to dominant NW and W swells (Instituto Hidrográfico 2006). At smaller spatial scales the topography of the coast often results in an intricate mesh of exposed and less exposed locations where stalked barnacle abundance is highly variable at the scale of meters.

Borja et al. (2006) have shown that the distribution, coverage, and biomass of goose barnacles in the Gaztelugatxe Marine Reserve (Basque Country, Spain), are related to the wave regime and the energy received in each coastal sector. In this coast, higher coverage and biomass of *P. pollicipes* was associated with higher energy values. The authors suggest that the numerical models used to simulate the energy produced by waves may be used as a tool in predicting potential biomass of goose barnacles along the Basque coasts.

Modeling wave energy reaching the coast often requires specialized software and elaborate datasets (e.g. detailed bathymetry, oceanographic and meteorological data) not readily available for most study regions worldwide. Existing methods range from cartographic solutions to numerical complex hydrodynamic simulations, and differ in the scale and spatial coverage of their outputs, performance and ecological relevance (Sundblad et al. 2014). Simple topographical indices, based on the openness of the coastline combined with local wind data, have been shown to be useful tools in the assessment of wave exposure variability at different spatial scales, depending on the resolution of the available cartography (Burrows et al. 2008). In recent years, several authors have developed computerized methods for deriving topographical indices of wave exposure based on digitized coastlines (Ekebom et al. 2003, Lindegarth and Gamfeldt 2005, Hill et al. 2010). Such methods often consist on the determination of the wave fetch for a particular location as the distance to the nearest coastline in angular sectors, which in combination with metrics from local wave and wind data different may be used to estimate different wave exposure indices.

The availability of satellite imagery, cartographic and GIS resources, oceanographic and meteorological data and forecast models disseminated on the Internet, provide data that researchers worldwide may access and use for research purposes at relevant spatial scales. Satellite imagery provided from online services and geographic information systems (GIS) software may be used to draw digitized coastlines of any specific location at an appropriate resolution for most coastal ecological studies. Forecasting weather services, based on data produced by meteorological and oceanographic forecast models (e.g. Global Forecast System - GFS, Weather Research and Forecasting - WRF, NOAA wave watch III model -NWW3) are found on Internet sites that provide model estimates for most coastal locations worldwide. Such data in combination with digitized coastlines may then be used in cost effective GIS projects that may provide researchers and stakeholders with the information needed according to their management goals.

We have modeled the abundance (estimated as percent coverage) of *P. pollicipes* as a function of a simple wave exposure index based on fetch estimates from digitized coastlines at different spatial resolutions and wave energy derived from oceanographic and meteorological data provided from nearby stations.

The aim of this study was to predict *P. pollicipes* abundance along a stretch of coast in SW Portugal and to discuss the potential use of such model as a simple and cost effective management tool for the stalked barnacle fishery in Portugal.

5.3 Materials and methods

Biological sampling

In the summer of 2010, *P. pollicipes* percent cover in the mid-intertidal of rocky shores along a 50 km study region in SW Portugal (a total of 94 sites 10's of meters apart, sampled in 7 areas kilometers apart, as shown in Fig. 5.1) was estimated by digital analysis of photo-quadrats (n=5) taken at each site. ImageJ software (Abràmoff et al. 2004) was used to superimpose an orthogonal 2.5 cm dotted grid on the digital images and to estimate the proportion of dots over stalked barnacles in each image.

Digital coastline

Using QGIS geographical information systems software (www.qgis.org) with OpenLayers plugin, a high resolution digital coastline of SW Portugal was created based on satellite imagery (1:2000) provided by Google. Three spatial resolutions (10 m, 100 m and 1 km cell size) were used to create grid maps of the study area from digital coastline polygons.

Fetch estimates

Using the WAVE FETCH MODEL software (Burrows et al. 2008), wave fetch was determined as the distance (m) to the nearest land cell in sixteen 22.5º angular sectors

(N, NNE, NE, …, NNW), by searching the surrounding cells up to a distance of 10 km, for every coastal cell in the grid maps (at all spatial resolutions).

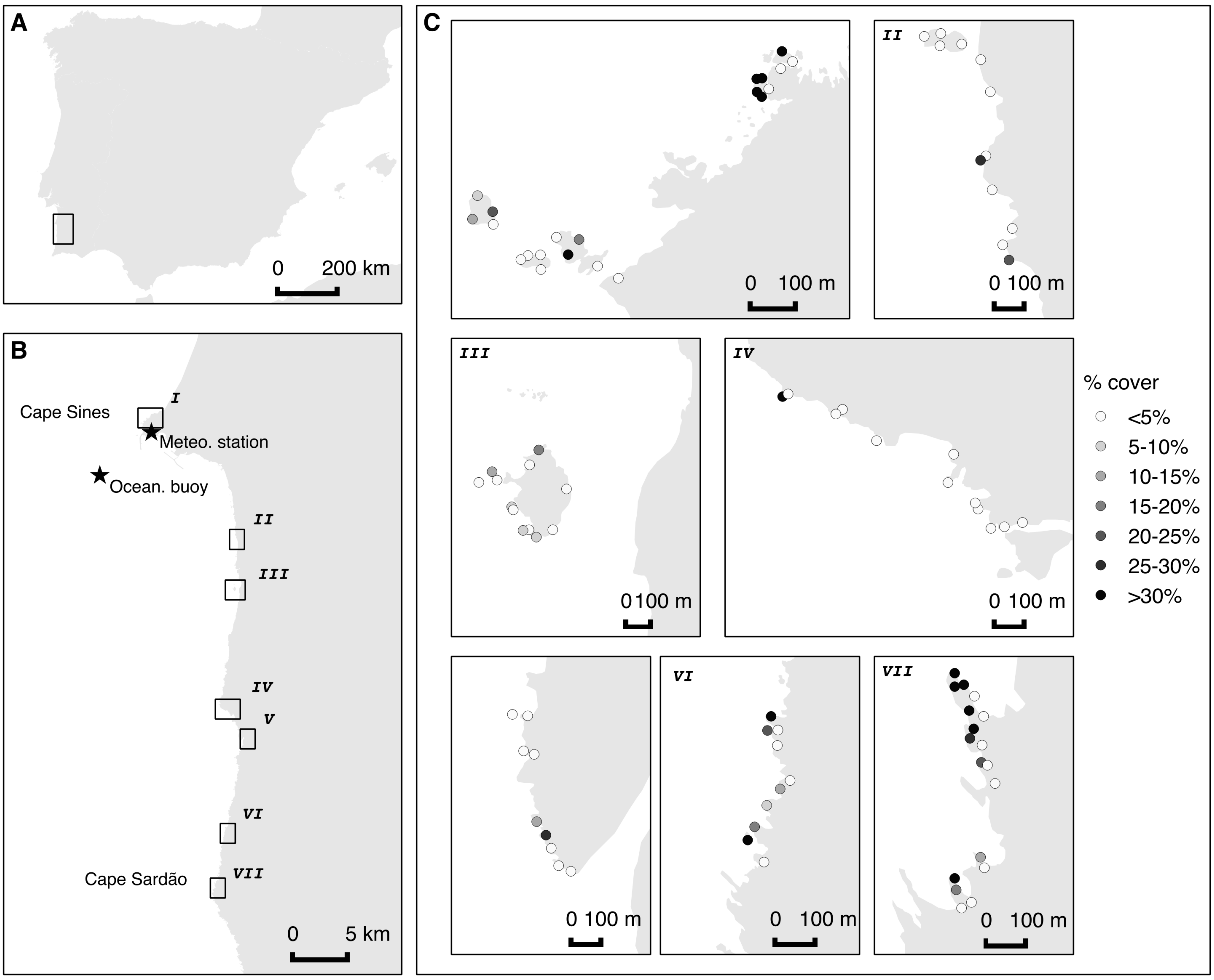

Fig. 5.1 A) Study region in relation to Iberian Peninsula and B) location of sampling areas (*I-VII*), meteorological station (Meteo. station) and oceanographic buoy (Ocean. buoy) in SW Portugal. C) Sampling sites location and mid intertidal *Pollicipes pollicipes* average percentage cover results from digital image analyses.

Wind energy

Data from a 5-year time series (2005-2010) provided by a meteo-station at a nearby location (8°52'43"W 37°57'15"N) that sampled wind direction (16 angular sections: N, NNE, NE, …, NNW) and speed (m/s) (amongst other variables) at a 10 min rate (incomplete data series with several gaps per year) was used. Wind energy for each angular sector was estimated as the product of the square of the mean wind speed and the respective mean yearly relative frequency for the available data period.

Wave energy

Data from an 5-year time series (2005-2010) provided by an oceanographic buoy at a nearby location (8°55’44”W 37°55’16”N) that sampled wave direction (16 angular sections: N, NNE, NE, …, NNW) and significant height (m) (amongst other variables) at a 10 min rate (incomplete data series with several gaps per year) was used. Wave energy for each angular sector was estimated as the product of the square of the mean significant wave height and the respective mean yearly relative frequency for the available data period.

Exposure Indices

Three exposure indices based on fetch measurements and wind and wave energy were estimated for every coastal cell in the grid maps at all spatial resolutions (10 m, 100 m and 1 km): 1) $FeEx_{sum}$ - the sum of fetch estimates for all angular sections; 2) $WaEx_{sum}$ - the sum of the product of fetch and wave energy for all angular sections and 4) $WiEx_{sum}$ - the sum of the product of fetch and wind energy for all angular sections.

Data Analysis

Using nearest point analyses in a GIS environment, each biological sampling site was assigned to the set of exposure indices from the nearest coastal cell in the grid maps at each spatial resolution considered (10 m, 100 m and 1 km).

Generalized Additive Models (GAM) were used to model the response variable, the percentage cover of *P. pollicipes,* as a function of the explanatory variables, *i.e.* each type of exposure indices ($FeEx_{sum}$, $WaEx_{sum}$ or $WiEx_{sum}$) at different spatial resolutions (10 m, 100 m and 1 km). GAMs were constructed using the Poisson distribution family (corrected for overdispersion) and the log-link function (Zuur et al. 2007). Thin plate regression spline smoothers with fixed degrees of freedom (df=6) were used for nested models comparison purposes (Wood 2006). 80% of the data was used for model building, while the remaining 20% of the data was used for model validation, which was done by comparing (paired t-test) predicted model results with data from a new set of photo-quadrats (n=18 paired data points). All analyses were done using R statistical software (www.r-project.org).

5.4 Results

Along the study region (Fig. 5.1), barnacle abundance was highly variable at all spatial scales considered (10's m to 10's km). *P. pollicipes* mean percentage cover sampled in the mid shore at the sampling sites (n=94) ranged from 0% to 64.6% (1.6% and 10.6%, median and mean, respectively). Both subsets of data used for model construction (80%) and model validation (20%) had similar distributions (Welch Two Sample t-test: t=0.099, df=25.028, p-value=0.9219).

Fetch estimates ($FeEx_{sum}$) were obtained for every coastal cell from grid maps at 1 km, 100 m and 10 m resolutions and an example of its variability in shown in Figure 5.2. $FeEx_{sum}$ varied between 35.1 km and 84.2 km for every coastal cell along the SW coast of Portugal in the 1 km grid map; 0.3 km and 132.2 km in the 100 m grid map and 0.02 km and 90.0 km in the 10 m grid map.

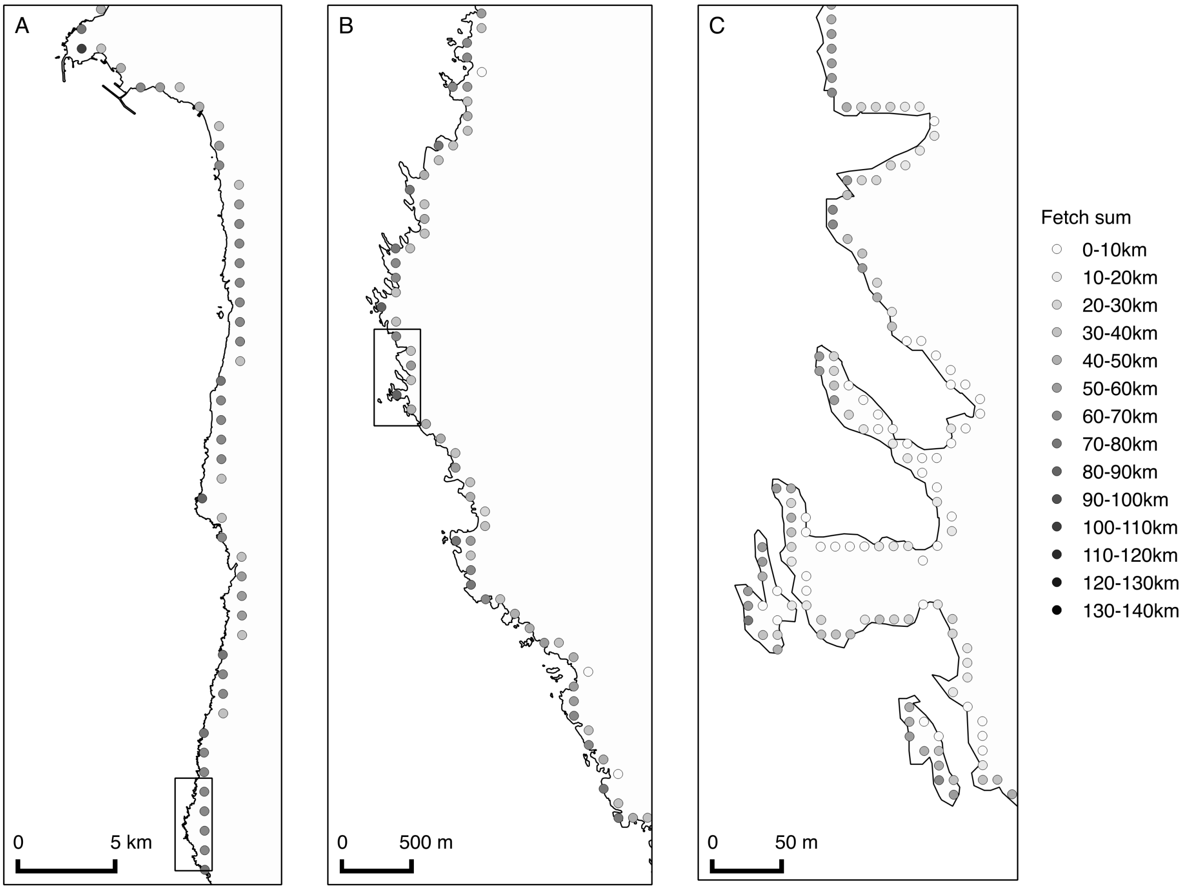

Fig. 5.2 Examples of fetch estimates ($FeEx_{sum}$) for coastal cells at different spatial scales (A, B and C represent the 1 km, 100 m and 10 m resolutions, respectively; boxes in A and B, represent the extents of B and C, respectively).

Wind and wave data collected between 2005 and 2010 from a nearby meteorological station and an oceanographic buoy are summarized in Figure 5.3, and support that the study region is mainly exposed to north-westerly winds and waves. The estimated energy per coastal sector suggests that most wind energy comes from NW-ENE but there is also a strong component of wind energy that blows the coast from SSW-S.

Wave energy, as estimated from local data, strikes the coast mainly form the WNW-NW sectors. Exposure indices based on wind and wave energy were computed for each coastal cell at all spatial resolutions considered in the present study (results not shown).

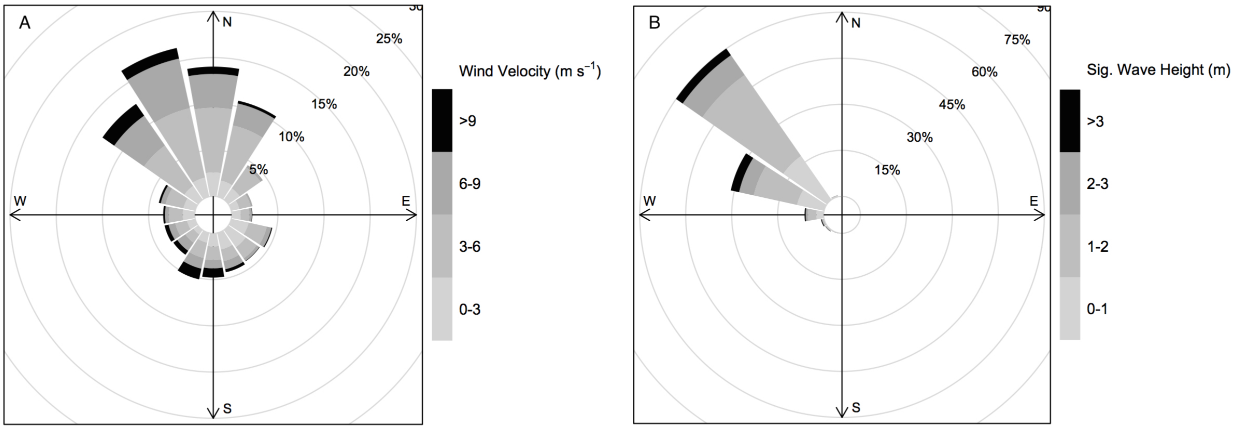

Fig. 5.3 Wind and wave patterns from data collected in a meteorological station and an oceanographic buoy between 2005 and 2010 at the Sines region (SW Portugal). A) Wind velocity (ms^{-1}) and frequency (%) by coastal sector. B) Significant wave height (m) and frequency (%)by coastal sector.

The model that best and most parsimoniously described the relationship between barnacle abundance (percentage cover) and exposure indices included F1km and F10m as selected predictive variables. The GAM model explained 47.5% of the deviance and was of the form: barnacle cover ~ 1.76 + s(F1km) + s(F10m).

Table 5.1 Numerical output for a generalised additive model (GAM) using Poisson distribution corrected for overdispersion and log-link function. Model uses *P. pollicipes* percentage cover as response variable and F1km and F10m as explanatory variables. A) Estimated parameters and B) Approximate significance of smooth terms. Bold type indicates significance (p<0.05). Deviance explained=47.5%. N=74.

A) Parametric coefficients:

	Estimate	Std. Error	*t* -value	*p* - value
Intercept	1.764	0.233	7.572	**<0.001**

B) Approximate significance of smooth terms:

	edf	F	*p* - value
s(F10m)	6	3.065	**0.0109**
s(F1km)	6	2.846	**0.0165**

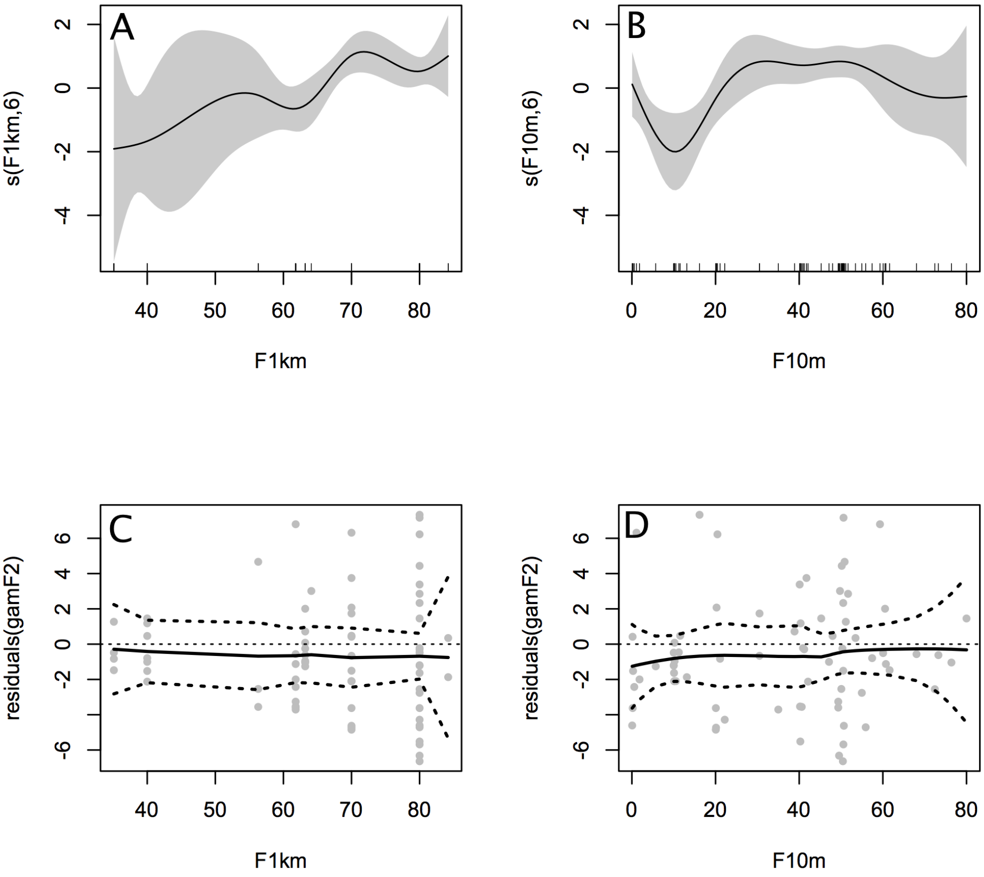

Fig. 5.4 Generalized additive model (GAM) validation plots. Smoothing functions for the explanatory variables F1km (A) and F10m (B). Standardized residuals for the optimal GAM plotted against observations for each explanatory variable (C, D), with LOESS fit predictions for mean (span 0.65; solid black line) and 95% confidence intervals (dashed black lines) superimposed on the observed data (grey dots), showing no residual patterns.

Numerical output for the model is shown in Table 5.1, smoothing functions and model validation plots are shown in Figure 5.4. The effects of the parametric intercept coefficient and the smoothing terms for both explanatory variables were significant ($p<0.05$). For model validation purposes, deviance residuals were plotted against the explanatory variables, showing no discernable patterns (Fig. 5.4 C and D). Still for model validation purposes, a paired t-test on another set of data with both observed and predicted values was performed and the result was non significant ($p=0.87$), suggesting a good agreement between observed and predicted values, thus validating the model. According to the model, barnacle abundance increases non-linearly with wave exposure at both the smallest (meters) and largest spatial (kilometres) scales considered in this study.

The use of other exposure indices, accounting for wind and wave energy, to predict barnacle abundance along the study region did not increase the power or predictive value of the model (model outputs not shown) in relation to a simpler exposure index fetch-based model. Each of the 3 models performed similarly in predicting abundance of *P. pollicipes* for a subset of data (Paired t-test: observations vs predictions; not significant; P>0.05), and no significant differences were found between the predicted abundance values based on either model (Paired t-test: not significant ; P>0.05).

Stalked barnacle distribution map based on model predictions along natural rocky coast of the study region is shown in Figure 5.5. Three areas with larger predicted barnacle abundance values (>15%) are perceivable and related with the three main headlands in the study region. A total of 54.4 km of natural rocky shores was estimated along the study region, based on the sum of coastal cells from the small-scale grid map (10 m), excluding sandy beaches and artificial coastal structures. According to the model, stalked barnacle abundances similar to what was previously reported in harvesting areas (>15% of barnacle cover in the mid shore; Sousa et al. 2013) were predicted to occur in about 17.0 km (31.3%) of rocky coast along the same region.

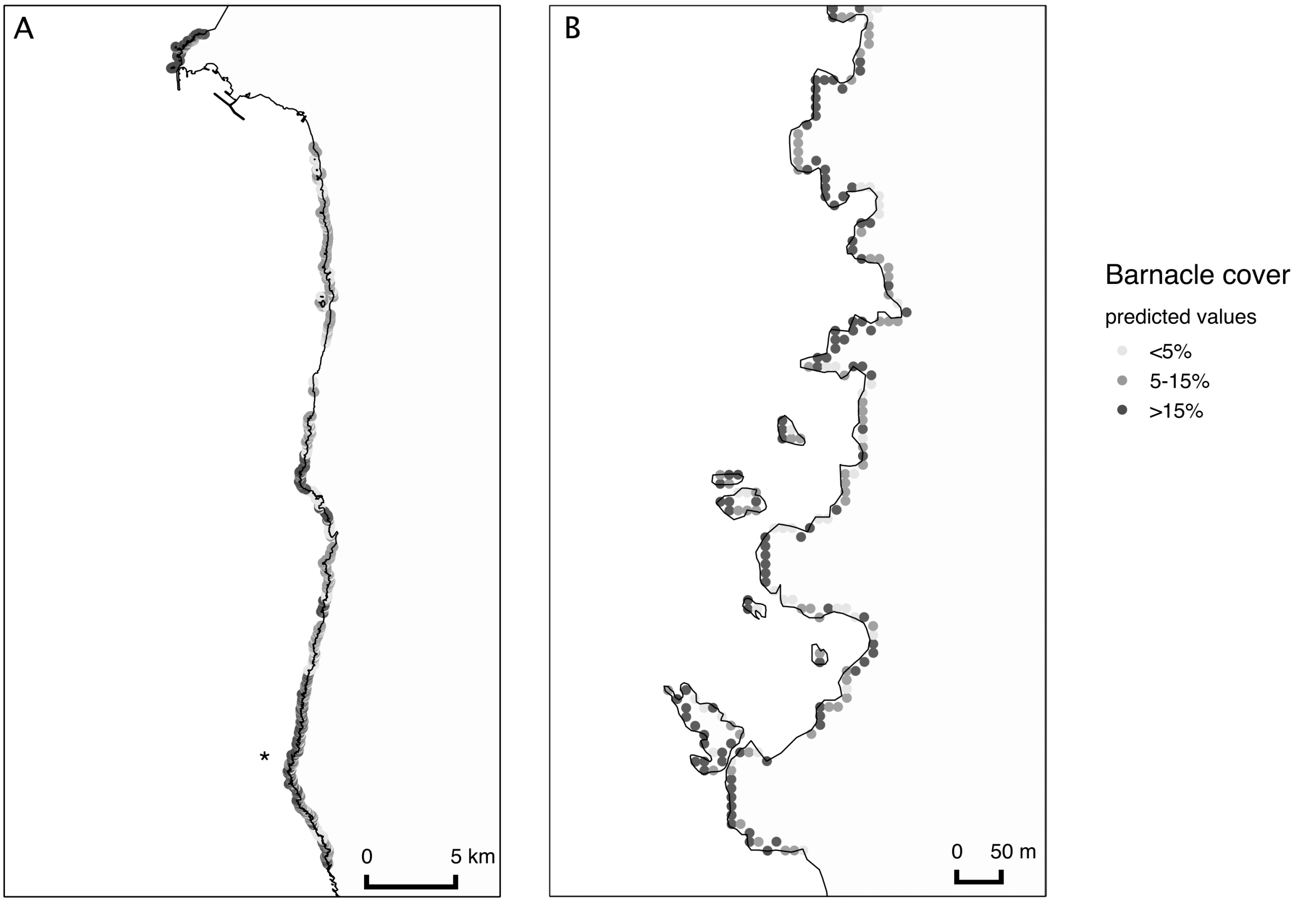

Fig. 5.5 *Pollicipes pollicipes* predicted distribution in SW Portugal natural rocky shores. A) Larger scale map reveals main areas with higher predicted barnacle cover. B) Smaller scale map (roughly centred around * in A) showing the high variability in barnacle cover associated with complex coastline configuration at smaller spatial scales.

5.5 Discussion

It has been long recognized that the distribution of the stalked barnacle *Pollicipes pollicipes* along its geographical range is associated with heavy surf and strong water flows (Barnes 1996) and previous studies have shown that wave incident energy is one of the main environmental factors determining the absence or abundance of the stalked barnacle (Borja et al. 2006), as so, the strong predictive power of wave exposure indices used in the present study is not surprising. Yet, the simple wave exposure indices, the multi-scale approach and the accessible digital tools used in the present study may provide an effective framework for barnacle distribution modeling with direct application in the stalked barnacle fishery and conservation.

In the present study we have sampled for stalked barnacles in a wide range of exposure conditions at different spatial scales along a stretch of coast in SW Portugal. Barnacle abundance data, from totally absent to high percentage covers, as observed in other studies in the same region (Sousa et al. 2013), were fed into predictive models where wave exposure indices at different spatial scales were used as explanatory variables.

Three different wave exposure indices were used: a simple fetch-based index and two others based on the previous one yet weighted with directional wind and wave data observed in the study region. Despite the added complexity, which could potentially serve the model a higher level of realism by taking into account the regional meteorological and oceanographic conditions, the wind and wave energy weighted fetch-based indices did not increase the power or predictive value of the models in relation to a simpler fetch-based model. Fetch, as a wave exposure index was a fairly good predictor of barnacle abundance in rocky shores of SW Portugal, explaining a large amount of the variation observed in *P. pollicipes* distribution patterns in the study area. Nevertheless, other factors acting on larger or smaller spatial scales, like substrate type and inclination, biological interactions, harvesting pressure and others that were not addressed in this study, are also very important in shaping barnacle distribution patterns (Sousa et al. 2013).

It should be noted that more realistic oceanographic models on marine dynamics in coastal areas based in the available offshore wave energy and including the main physical processes affecting wave energy distribution along the coast such as refraction and diffraction and also detailed bathymetry (Borja et al. 2006) could potentially lead to explain a higher proportion of the data variability that our approach could not account for.

The present study suggests that the relationship between fetch-based exposure indices and *P. pollicipes* percent cover may be used as a simple tool able to provide stakeholders with information on barnacle distribution patterns that may lead to better assessment of harvesting grounds and dimension of exploitable areas, which may help improving management plans and support decision making regarding conservation, harvesting pressure and surveillance strategies for this highly appreciated and socio-economic important marine resource. As an example, a total of 17.0 km of rocky coast with expected exploitable areas out of 54.4 km of rocky coast in the studied region was predicted according to the model. The identification of these areas could now be used for management (e.g. spatial zoning; definition of number of harvesting licenses) and surveillance purposes. This can also be useful to establish a rotational moratorium in a number of areas likely to be exploited, and may permit the maintenance of a spawning pool of larvae, which nourish and sustain the exploited areas, as suggested by Borja et al. (2000) and Bald et al. (2006).

The use of free and open source software in addition to simple biological monitoring strategies, as used in this study, provide an attainable cost effective management tool that may be implemented by stakeholders and resource users in areas where barnacle harvesting is an important socio-economic activity. Studies like this may serve to establish protected areas for conservation of certain commercially important sensitive species, such as the stalked barnacle, and to prevent the subsequent damage of the associated communities.

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Chapter 6. Growth rate variation of the stalked barnacle *Pollicipes pollicipes* (Crustacea: Cirripedia) using calcein as a chemical marker

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Jacinto D., Penteado N., Pereira D., Sousa A., Cruz T. 2015. Growth rate variation of the stalked barnacle *Pollicipes pollicipes* (Crustacea : Cirripedia) using calcein as a chemical marker. Sci Mar 79:117–123

6.1 Abstract

This study describes the use of calcein as a chemical tagging methodology to estimate growth rate variation of the stalked barnacle *Pollicipes pollicipes,* an ecologically important intertidal species and economic resource, in SW Portugal. Calcein tagging had a high success rate (94%) in marking both juvenile and adult barnacles for a period of 2.5 months, providing a valuable method for obtaining reliable data in growth studies of *P. pollicipes.* Growth rate decreased with barnacle size and was highly variable amongst individuals, particularly in smaller barnacles. No effect of shore level on barnacle growth was detected. Growth rates were higher in smaller juvenile barnacles, peaking at a 1.1-mm monthly increment in rostro-carinal length (RC) for individuals with RC=5 mm, and decreased with barnacle size (monthly growth rates of 0.5 mm for adult barnacles with RC~12.5 mm). Growth rates observed in adults with commercial interest (RC≥18 mm) was <0.25 mm per month. The advantages of tagging *P. pollicipes* with calcein were the possibility of mass marking individual barnacles of different size cohorts within a short period (less than 1 day of manipulation); and reduced time of fieldwork, which is very important because this species inhabits very exposed rocky shores.

6.2 Introduction

Pollicipes pollicipes is a common barnacle species on very exposed intertidal rocky shores from Brittany to Senegal, where it forms dense aggregates of individuals (Barnes 1996). It is a very important economic resource in Spain and Portugal, and is heavily exploited (Molares and Freire 2003, Jacinto et al. 2010). Despite its economic and ecological importance, several aspects of its biology and ecology are still poorly understood, most likely as a result of the difficulty associated with conducting ecological studies in the extreme exposed habitats where this species occurs (Barnes 1996, Cruz 2000, Cruz et al. 2010), and the difficulty of studying growth in pedunculate barnacles.

Growth studies of *Pollicipes* spp. are mostly based on population size structure analyses and estimates of growth rates of a few aged or un-aged individuals that are marked in the field (e.g. Page 1986, Phillips 2005, Cruz et al. 2010). Marking techniques of individuals in these studies include mapping individuals in relation to marks made in adjacent substrata, or using marks such as insect tags glued to the capitulum plates of suitably-sized individuals. However, these techniques are difficult to apply and maintain on very exposed shores where time to work is limited. Consequently, their success might be low and small animals may be very difficult to mark.

In stalked barnacles, peduncular growth mostly occurs in a narrow zone between the capitulum and the peduncle (Chaffee and Lewis 1988), and on the capitulum, primary plates (scuta, terga and carina) grow in thickness and area by laminar and marginal accretion over the whole inner surface from an underlying epidermis where the plates are embedded (Anderson 1994, Barnes 1996). The most widely used variable for growth and size structure studies in *Pollicipes* spp. is the maximum rostro-carinal length (RC) (Phillips 2005, Cruz et al. 2010, Boukaici et al. 2012), although other variables such as total length (Hoffman 1984), capitulum height (Page 1986, Cardoso and Yule

1995) and capitulum base diameter (Parada et al. 2012) have also been used.

Growth rates in *Pollicipes* spp. are highly variable in space and time (reviewed by Barnes 1996). Factors affecting barnacle growth may include immersion time and food availability, light, season, water temperature, microhabitat and population density. Previous observations of *P. pollicipes* growth patterns on the SW coast of Portugal (Cruz 1993, 2000, Cruz et al. 2010) suggest that growth rates vary with barnacle size, season and intertidal level. Cruz (2000) observed that on average un-aged juveniles (RC<10 mm) grew by 0.47 mm RC per month (winter, spring and summer), while un-aged adults (RC>10 mm) grew by 0.11 mm RC per month in summer and 0.47 mm RC per month in winter and spring. When monitoring the size of 11 individual barnacles that have recruited onto a cleared surface, Cruz et al. (2010) estimated an average growth of 1.3 mm RC per month during their first year. Size structure analyses of *P. pollicipes* attached directly to primary substratum and/or to the base of conspecifics evidenced that barnacles at the low tide level reached a higher maximum size, possibly indicating that growth at this tidal level was higher than at the high shore (Cruz et al. 2010).

The use of chemical tagging as a mass marking technique may provide new insights in growth rate variation in *P. pollicipes*, as a large number of individuals from different cohorts and habitats may be individually and simultaneously tagged and studied with relative ease and safety. Calcein ($C_{30}H_{26}N_2O_{13}$) is a fluorochrome that binds to calcium and becomes incorporated in calcified structures of growing animals when exposed to this chemical agent, and has been successfully used in ecological studies of several marine species (Moran 2000). Immersion in a calcein solution leaves a fluorescent mark in calcified structures, which is visible under epifluorescent microscopy and has been used to identify marked individuals and to estimate growth rates in several organisms, including fishes (Wilson et al. 1987, Leips et al. 2001, Frenkel et al. 2002), ascidians

(Lambert and Lambert 1996), molluscs (Kaehler and McQuaid 1999, Moran 2000, Van der Geest et al. 2011), echinoderms (Russell and Urbaniak 2004, Ebert et al. 2008) and crustaceans (Helms 2004, Kilada et al. 2012).

Chemical tagging in stalked barnacles was first described by Helms (2004), who has successfully used calcein marks to study recruitment and juvenile growth patterns in *Pollicipes polymerus.* The fluorescent mark visible on the carbonate plates of the capitulum was used to distinguish old recruits (marked individuals) from the new ones (not marked) and also to estimate growth rates during the study period by measuring the marginal increment of the capitular plates after the calcein treatment.

In this study, we have adapted the calcein tagging methodology proposed by Helms (2004) in order to mark multiple juvenile and adult barnacles overnight, an important requirement in such extreme environments with small temporal windows of appropriate oceanographic conditions for fieldwork, and to estimate growth rate variation with intertidal vertical level and size of the stalked barnacle *Pollicipes pollicipes* in SW Portugal. We expected growth rates to decrease with barnacle size and vary with intertidal level (larger growth rates in low shore barnacles).

6.3 Materials and methods

Study area

This study was carried out between July and September 2012 at Cabo Sardão (SW Portugal; 37°36’25”N, 8°49’02”W), a very exposed headland of metamorphic schists, where *P. pollicipes* is abundant and also heavily exploited. As in other areas along the Portuguese coast, *P. pollicipes* abundance varies along the shallow subtidal/intertidal gradient (Sousa et al. 2013). Higher densities, percentage cover and biomass are observed on the mid-shore than on the low shore, yet low shore barnacles have a higher proportion of adults with moderate and high commercial value, while juveniles are relatively more abundant on the mid-shore (Sousa et al. 2013). For the purposes of the present study, two vertical levels were considered: 1) high, corresponding to the middle/upper intertidal level of *P. pollicipes* local distribution and 2) low, corresponding to the lower intertidal level of its local distribution.

Animal collection and chemical marking

On the 20th June 2012, during the morning low tide, several chips of rock with attached barnacle clumps (containing barnacles of different sizes) were detached from the intertidal substrate (in both the low and high vertical levels; n=10 and n=16, respectively) using a hammer and chisel, and transported to the laboratory in buckets. Barnacle clumps were immersed in containers of 10-15 L of filtered seawater at 200 mg calcein/L and were continuously aerated with diffusive air stones for 20-22 hours. Barnacles were not fed during the marking period. Calcein solutions were made from a stock of 6.25 g/L of calcein in distilled water and buffered to *ca*. pH 6 with the addition of sodium bicarbonate to increase solubility (Wilson et al. 1987, Helms 2004). The next day, during the morning low tide, the barnacle clumps were transported back to the same shore area and fixed randomly to the rock with Z-Spar Splash Zone Epoxy (Kop-Coat Inc., Pittsburgh, PA), at the vertical level where they had been collected. On the 9th September 2012, after a growth period in the field of 76 days, experimental clumps present at the study site were collected from the shore (3 from each intertidal level; the remaining barnacle clumps were kept in the intertidal area for further studies, or were either lost or had no barnacles attached at the time of collection), brought to the laboratory and frozen until further analysis. Chemical marking success was measured by dividing the number of individuals with fluorescent marks and the total number of animals retrieved from the field.

Laboratorial analyses

All barnacles (RC>1 mm) present in each clump were unfrozen, detached, counted, measured with callipers (RC, precision=0.1 mm) and immersed in commercial bleach (Sodium Hypochlorite 3.5%) for 1 to 60 minutes, depending on barnacle size, in order to dissolve the soft tissues and separate the capitular plates from the rest of the body. The timing of the bleach bath was the minimum required to remove soft tissues, separate capitular plates and expose the edges and calcein marks under magnification. Initially, all smaller individuals (approx. RC<5) were immersed for 1 minute. After a first visual inspection, if necessary, additional immersion time (in 1-min blocks) was added. Larger individuals (approx. RC 5-15 mm) were initially immersed in bleach for 5 min, and if necessary, additional immersion time (in 5-min blocks) was added. Very large individuals (approx. RC>15) were initially immersed in bleach for 10 min and, if necessary, additional immersion time (in 10-min blocks) was added. Adult individuals required more time to prepare the plates for observation: more time immersed in bleach (up to 20 hours in a few cases); and if needed some abrasion with sandpaper or scrapper to remove calcareous algae and other organisms attached to the plates. The scutum and tergum of the capitular plates (both pairs; Fig. 6.1) were observed and photographed at an appropriate magnification (7-20×) under an epifluorescent dissecting microscope (Leica M165FC with a UV light source and GFP3 filter) equipped with a digital camera (Leica DF 295) connected to a computer. The entire initial capitular plates and/or its edges, if stained, fluoresced under appropriate light, while the new material did not. By measuring the amount of new material added to the capitular plates it was possible to estimate individual growth in *Pollicipes polymerus* (Helms 2004). Whenever calcein marks were identified, the following variables were measured with a digital image analysis software (Leica Application Suite v3.8) in each plate: the maximum length of each plate (scutum or tergum: sc_f or tg_f, respectively; Fig. 6.1) and the maximum length of the original plate at the time of marking (whose edges are detectable by the fluorescent calcein mark; scutum or tergum: sc_i or tg_i, respectively, Fig. 6.1).

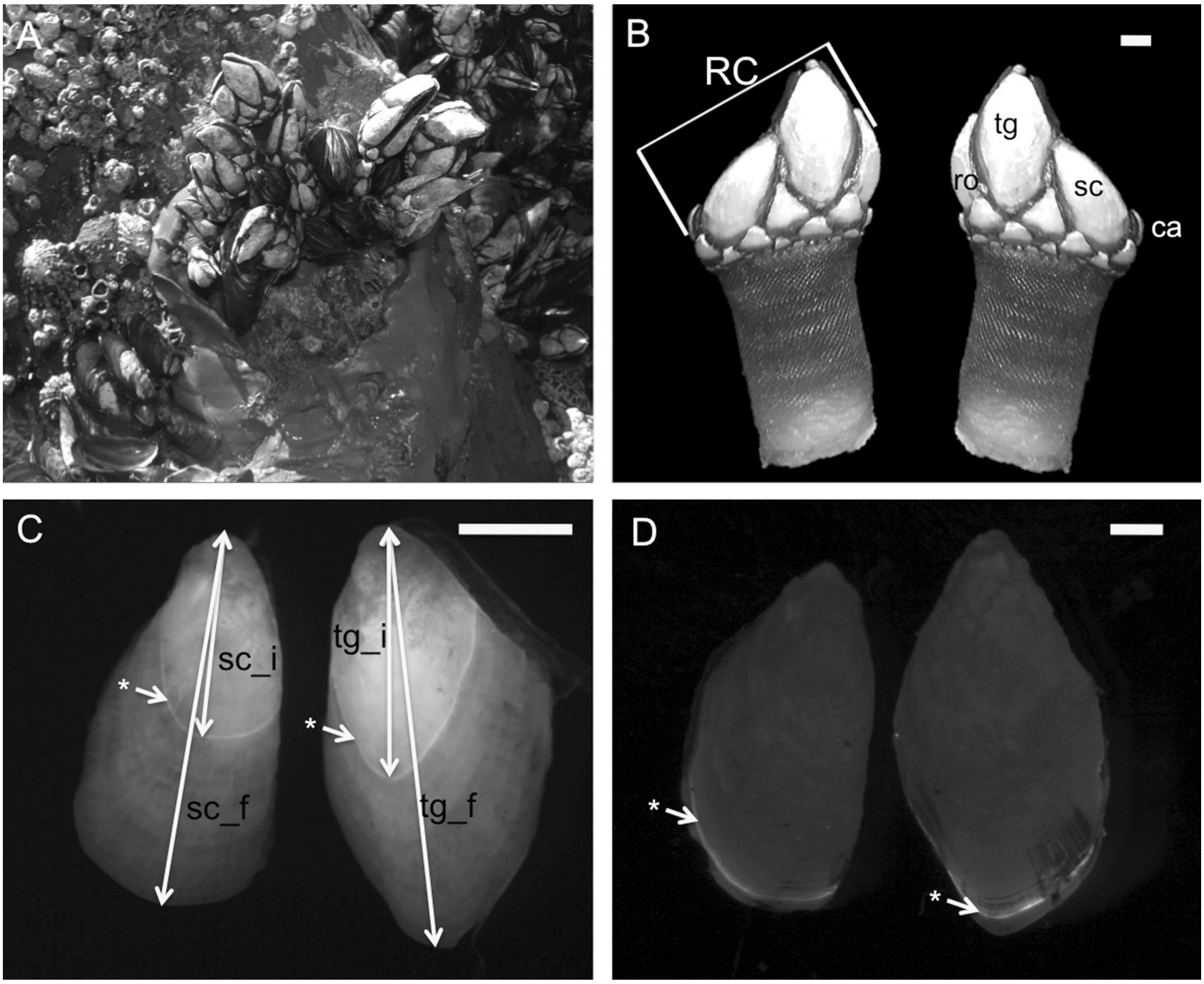

Fig. 6.1 A, a clump of calcein marked barnacles fixed to the rocky substrate with marine epoxy; B, *P. pollicipes* lateral view, identifying capitular plate position (sc, scutum; tg, tergum; ro, rostrum; ca, carina) and measured variable (RC, maximum rostro-carinal length); C, left side scutum and tergum plates from a marked juvenile barnacle (* indicates the edge of the calcein mark in the initial capitular plates), arrows show measured variables (sc_i, initial scutum length: sc_f, final scutum length; tg_i, initial tergum length; tg_f, final tergum length) for each capitular plate; D, left side scutum and tergum plates from a marked adult *P. pollicipes* showing the calcein marks visible only at the edge of the initial capitular plate area (*). 1-mm scale bars are shown in images B, C and D.

Data analyses

Exploratory analyses included multiple pair-wise scatterplots and Pearson product-moment correlation tests to detect relationships and collinearity between measured variables. All maximum capitular plate lengths (sc_f and tg_f from both sides) were highly and positively correlated among each other and with RC ($r>0.98$, $p<0.01$ for all paired comparisons, $215 \leq n \leq 259$ pairs of observations), suggesting that either plate could be used to study barnacle growth. All estimates of growth used in the present study were based on measurements made on the right scutum of each marked barnacle, which was the subset of data containing the fewest missing values (4.6%). A total of 269 barnacle growth measurements (corresponding to 127 and 142 individuals from the low and high intertidal, respectively) were used.

In order to estimate growth in a unit that may be easily used for comparisons with other barnacle growth studies, a linear model describing the relationship between RC and the maximum right scutum length (sc) was applied ($n=259$ paired data). Model coefficients ($RC=1.66*sc$; see results) were then used to convert all measurements made in the right scutal plates (sc_f and sc_i) into RC units (RC_f and RC_i, respectively). Growth rate (dRC) was based on estimated RC values (dRC = RC_f – RC_i).

Monthly growth rate (dRC30) was estimated by dividing the growth rate (dRC) by the growing period in the field (2.53 months), assuming linear growth during that period.

In order to describe growth rate variation of *P. pollicipes* with size and tidal level, the response variable (monthly growth rate, dRC30) was modelled as a function of the explanatory variables, estimated barnacle initial size (RC_i, ranging from 0.14 mm to 20.95 mm) and vertical level (low and high intertidal), using generalized additive models (Zuur et al. 2007). This model was used because exploration of data procedures revealed potential violation of homogeneity and non-linearity (Zuur et al. 2007). Comparisons of deviances of nested models (using an F-test) and graphical analysis of residuals distribution were carried out to identify and validate the optimal model (Zuur et al. 2007). Data exploration and analysis were performed using R software (www.r-project.org).

6.4 Results

Fluorescent marking success rate

Fluorescent marks were observed in 282 barnacles (size range: 1.5 mm to 19.9 mm of RC) out of a total of 300 individuals that were collected in the field. Most non-marked individuals were juvenile barnacles (n=16, RC<7.8 mm) that may have settled after chemical marking, although we also observed two non-marked adult barnacles (RC>12.5 mm), probably already present in the clump before marking. Such observations suggest a 94% success rate for the calcein tagging method in both juvenile and adult stalked barnacles (RC<20 mm) for a period of approximately 2.5 months. Calcein marks were easily observed under epifluorescence, particularly in smaller individuals (Fig. 6.1). Not all of the four capitular plates observed per individual had visible or distinguishable fluorescent marks and some plates were broken or incomplete (tergum 13%; scutum 5%), which made them unsuitable for analysis.

Relationship between maximum capitular plate length and maximum rostro-carinal length

Maximum capitular (tergum and scutum) plate length measurements were highly and positively correlated among each other and with RC (Pearson's product-moment correlation, $r>0.98$ and significant at $p<0.01$ for all paired comparisons, $215 \leq n \leq 259$ pairs of observations), suggesting that either capitular plate (sc or tg from the right or left side) may be used to estimate RC.

The most parsimonious and significant linear model for describing the relationship between the RC length and scutum length (sc) had the form RC=1.66*sc (standard error for regression coefficient, se=0.008; adjusted $R^2=0.99$; n=259 paired data; $p<0.001$, Fig. 6.2). All measurements made in the scutal plates (sc_f and sc_i) were converted to RC units (RC_f and RC_i, respectively) using the above-mentioned formula.

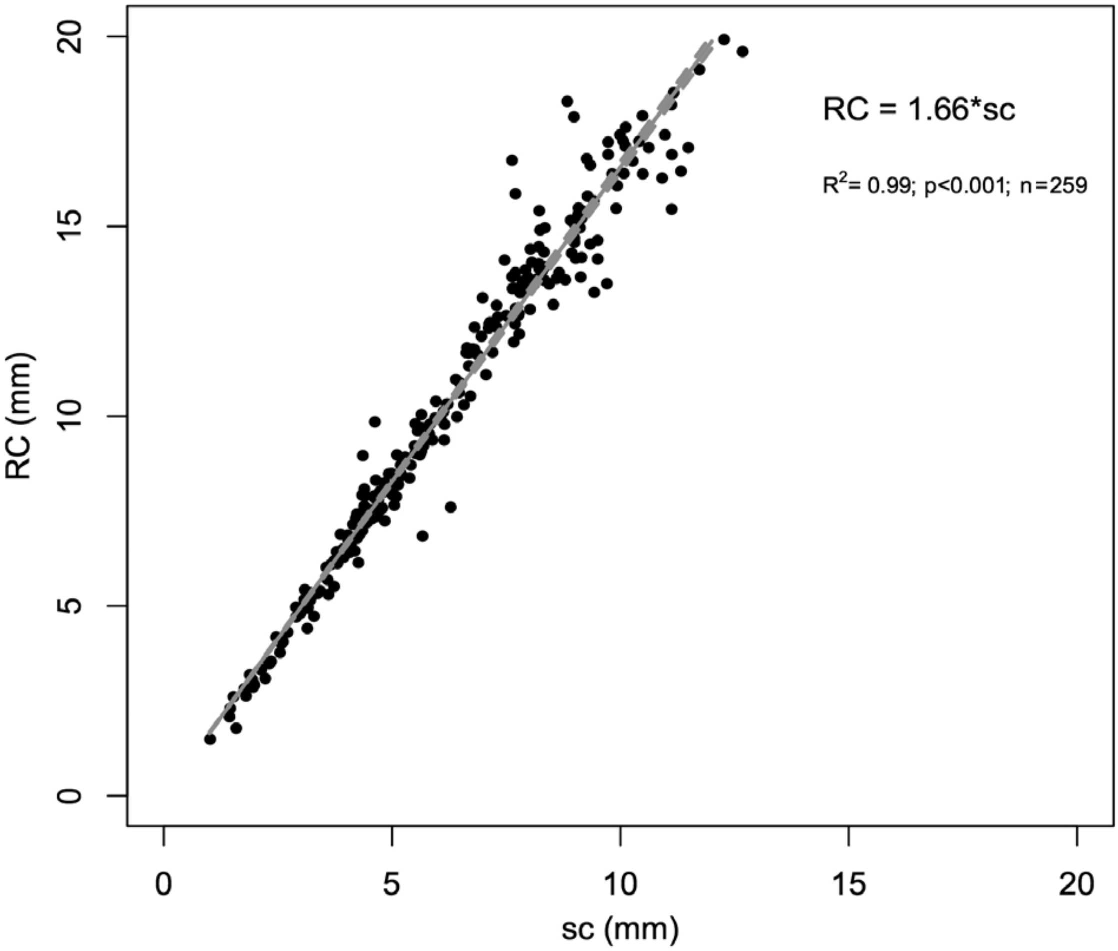

Fig. 6.2 Relationship between maximum rostro-carinal length (RC) and maximum scutum length (sc) in *P. pollicipes* from Cabo Sardão (Portugal). Linear regression model prediction for the mean response value (solid grey line) is shown superimposed on the observed data (black dots).

Growth rate variation

Monthly growth (dRC30) was highly variable (0.00 to 1.95 mm) between individuals (Fig. 6.3). The most parsimonious generalized additive model explaining growth variation (deviance explained, DE=47.5%; n=269; p<0.001) included barnacle initial size (estimated RC_i used as proxy for barnacle initial size) as the only explanatory variable, and had the form dRC30=0.75+s(RC_i), where s(RC_i) is the smoothing function for the covariate (Fig. 6.4). No residual patterns were observed and the model was validated (Fig. 6.4B).

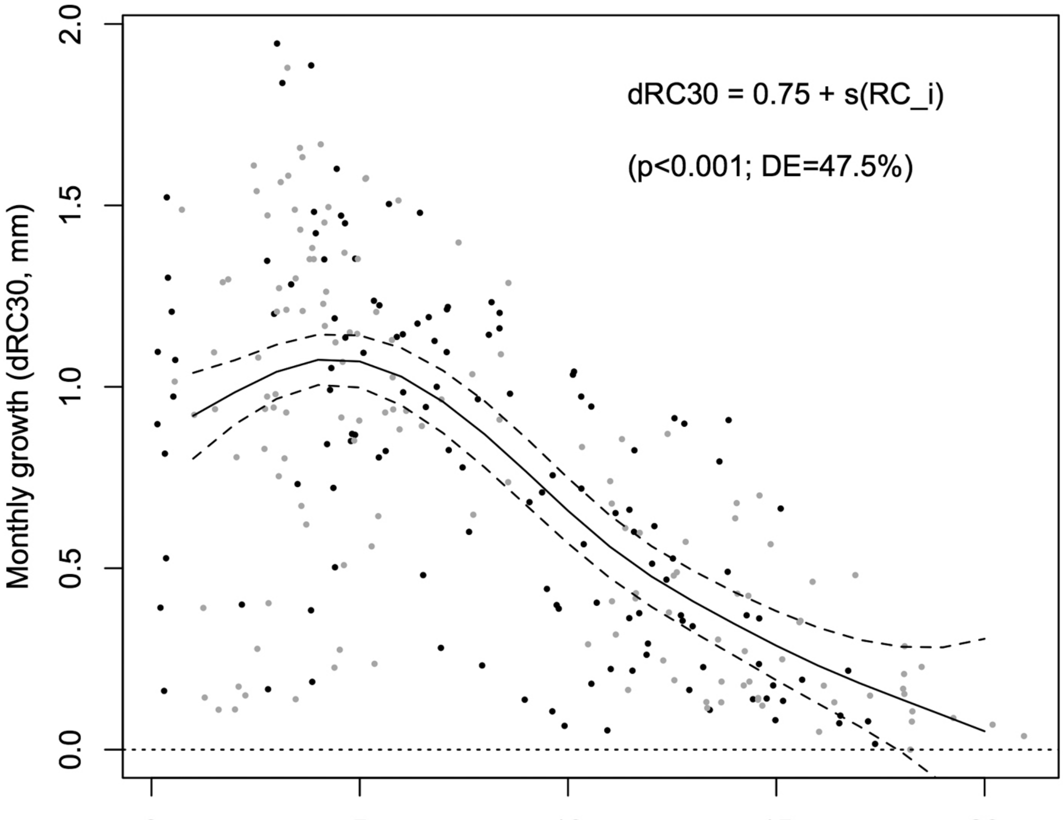

Fig. 6.3 *P. pollicipes* monthly growth (dRC30) variation with initial barnacle size (RC_i). Generalized Additive Model (GAM) predictions for mean (solid black line) and 95% confidence intervals (dashed black lines) are superimposed on the observed data for low- and high-shore levels (black and grey dots, respectively).

Growth rate in *P. pollicipes* decreased with barnacle size and was highly variable among individuals, particularly in smaller barnacles (Fig. 6.3). Average growth rates were higher in smaller juvenile barnacles (peaking at 1.1 mm per month for 5 mm RC individuals), and gradually decreased with barnacle size (monthly growth rates of 0.5 mm for adult barnacles with RC~12.5 mm). Average growth rates observed in larger adults (RC≥18 mm) of commercial interest was <0.25 mm per month.

As expected, barnacle growth decreased with initial barnacle size but, contrary to our hypothesis, no effect of shore level on barnacle growth was detected (non-significant effect for factor Shore Level (p>0.9) in the GAM model that included both explanatory variables, RC_i and Shore Level).

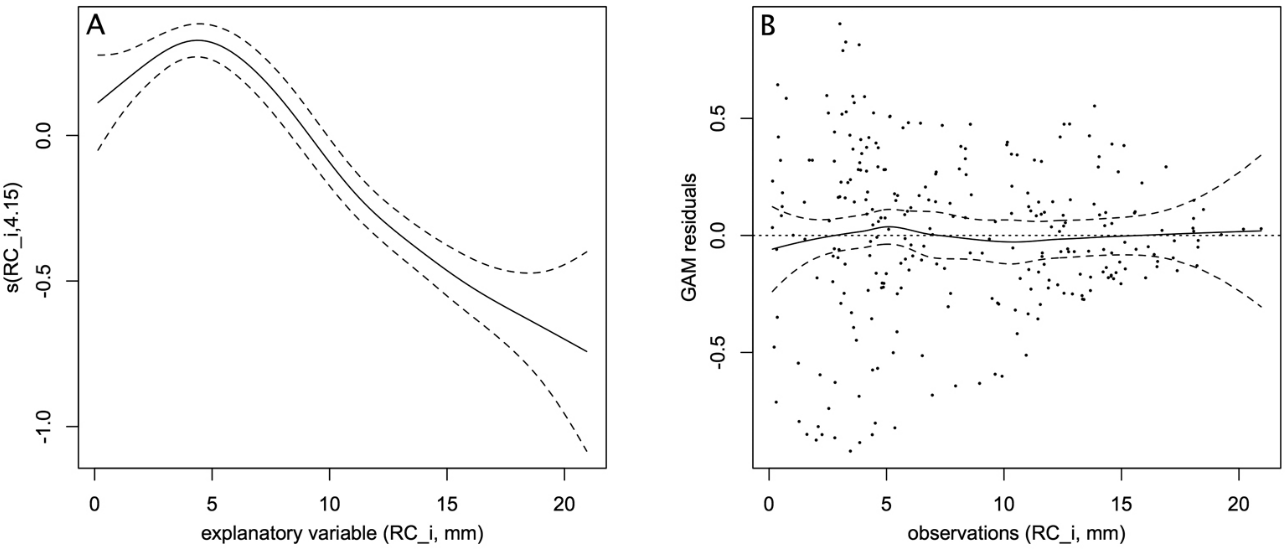

Fig. 6.4 Generalized additive model (GAM) validation plots. A) smoothing function; B) standardized residuals for the optimal GAM plotted against observations. Loess fit predictions for mean (span 0.65; solid black line) and 95% confidence intervals (dashed black lines) are superimposed on the observed data (black dots), showing no residual patterns.

6.5 Discussion

In this study we used a calcein solution (200 mg/L) to simultaneously mark overnight (20 h) several *P. pollicipes* clumps of individuals with different sizes (<20 mm RC) collected in the field at different tidal levels (high and low shore) on a shore of SW Portugal. Chemical tagging with calcein had a high success rate (94%) in marking both juvenile and adult barnacles for a period of at least 2.5 months, and can be considered a valuable method for easily obtaining reliable data in growth studies of *P. pollicipes*, an important marine resource, allowing its use in management models of barnacle fisheries such as the one proposed by Bald et al. (2006).

Our observations suggest a rapid increase in size during the first year, with most individuals reaching maturity (RC≥12.5 mm; Cruz and Araújo 1999) within one year. Growth rates varied with barnacle size, as has been previously observed (Cruz 1993, 2000, Cruz et al. 2010). An average non-linear trend in growth rate with size was observed. Mean growth rates were higher in juvenile barnacles (peaking at 1.1 mm per month for individuals with RC~5 mm), and gradually decreasing with barnacle size (monthly growth rates of 0.5 mm for adult barnacles with RC~12.5 mm). Mean monthly growth rates observed in larger adults of higher commercial interest (>18mm RC) was <0.25 mm.

Mean growth rate estimates presented here for the stalked barnacle *P. pollicipes* at C. Sardão (SW Portugal) are comparable to previous results of studies carried out in the same region (Cruz 1993, 2000, Cruz et al. 2010). When monitoring the size of 11 individual barnacles that had recruited onto a cleared surface, Cruz et al (2010) estimated an average growth of 1.3 mm RC per month during their first year of life. The higher growth rates observed by Cruz et al. (2010) on juvenile barnacles may have been due to a lower intra- and interspecific competition pressure upon the animals that have settled on a cleared surface than upon animals that live and grow within an aggregate, such as the ones measured in the present study and by Cruz (1993, 2000). When mapping and measuring un-aged individuals within barnacle clumps, Cruz (2000), reanalysing data presented by Cruz (1993), estimated that on average, juvenile barnacles (RC<10 mm) grew 0.47 mm RC per month (winter to summer), while adults (RC>10 mm) grew 0.11 mm RC per month in the summer and 0.47 mm RC per month in winter and spring. The growth rates presented here for juvenile barnacles are larger, in general, than had been previously reported. This might be due to a measurement of larger juveniles in Cruz (2000) than in the present study, as measurements done in Cruz (2000) were made in the field with callipers. The growth rates reported by Cruz (2000) for adult barnacles during the summer period are within the range of what we observed in the present study.

Growth rates were highly variable between individuals but, contrary to our expectations, were not higher in the low shore, as suggested by the size structure analyses of *P. pollicipes* carried out by Cruz et al. (2010). The study may not have been long enough to provide the expected variation. As only one site was considered, more studies and data including more sites are needed to ensure proper spatial replication. However, the manipulation procedure used in the method might disrupt the natural situation under which these barnacles normally live. Unlike low-shore populations, mid- to higher-shore *P. pollicipes* populations tend to aggregate in dense stands of barnacles, which the collection-marking-outplanting procedure might disrupt, possibly affecting the intra- and interspecific competition pressure under which these mid- to high- shore individuals live and masking any putative variation in growth rates related to vertical level in intertidal habitats.

Though most variation in the stalked barnacle growth rate seems to be related to barnacle size, highly variable growth rates were observed between similar sized individuals, particularly in juvenile barnacles. This might be related to growth patterns associated with natural occurring phenomena that barnacle populations are subjected to, such as barnacle clump density, position within the clump and microhabitat. Helms (2004) studied juvenile growth in *P. polymerus* and compared growth rates from juvenile *P. polymerus* in different clump sizes and position within the clump. Growth rates were higher in peripheral barnacles and in barnacles occurring in smaller-sized clumps, possibly due to intraspecific competition for space and food between juveniles and adults within the barnacle aggregate (Helms 2004). More observations at suitable spatial and temporal scales and including ecologically relevant covariates are needed to explain the high intraspecific variability in growth rate observed in the present study.

Chemical marking techniques with calcein have a great potential in stalked barnacle growth studies, mainly because they allow mass marking of individual barnacles of different size cohorts within a short period (less than 1 day of manipulation) and involve less fieldwork time, which is a major advantage because this species lives on extremely exposed rocky shores. However, one major caveat of this method is that it does not allow individual growth to be followed over time, as animals must be sacrificed for data acquisition. Further adaptations to this method may partially overcome this issue, for example by tagging the same animals at different time intervals using calcein, which fluoresces with a different colour. The use of calcein as a chemical marker may lead to further experiments on this important resource and make it possible to test hypotheses addressing spatial and temporal variability in growth patterns at a multitude of scales.

6.6 Acknowledgments

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Chapter 7. The state of the fishery, conservation and management of the stalked barnacle *Pollicipes pollicipes* in Portugal

Cruz T., Jacinto D., Sousa A., Penteado N., Pereira D., Fernandes J.N., Silva T., Castro J.J. 2015. The state of the fishery, conservation and management of the stalked barnacle *Pollicipes pollicipes* in Portugal. Mar. Environ. Res. 112: 73–80.

7.1 Abstract

The stalked barnacle *Pollicipes pollicipes* is the most important intertidal economical resource in Portugal. The assessment of the state of the fishery, conservation and management of *P. pollicipes* in Portugal was made for the first time in three regions with different regulations regarding this fishery: two marine protected areas ("Reserva Natural das Berlengas", RNB; and "Parque Natural do Sudoeste Alentejano e Costa Vicentina", PNSACV); and the Center coast. Different approaches (independent observations, inquiries, logbooks) and sources of data (past and recent) were used. An overall negative tendency of the state of the fishery and conservation of this resource was observed in all regions, with the exception of the stable tendency detected in PNSACV when using the inquiries approach. A weak management was considered to be in practice at Center and at PNSACV, while an acceptable management was inferred for RNB. We recommend a change into a co-management system that should be tested in pilot regions as RNB and/or PNSACV.

7.2 Introduction

Barnacles are key organisms on rocky shores. Additionally, a few species of shallow water barnacles are exploited being important economic resources, namely the giant barnacle *Austromegabalanus psittacus* in Chile, the acorn barnacle *Megabalanus azoricus* in Azores (Portugal) and the stalked barnacles of the genus *Pollicipes* (López et al. 2010). All four species of *Pollicipes* are edible (*Pollicipes polymerus* - eastern North Pacific, *Pollicipes elegans* - eastern Central and South Pacific, *Pollicipes caboverdensis* - Cape Verde islands, and *Pollicipes pollicipes* - eastern North Atlantic) (Barnes 1996, Fernandes et al. 2010), but *P. pollicipes* can be considered the most important intertidal economical resource on rocky shores of North Spain and continental Portugal (e.g. Molares and Freire 2003, Sousa et al. 2013). In Portugal and Spain, its commercial value can range from 20 to 200 euros per kg in restaurants.

P. pollicipes inhabits very exposed rocky shores and its exploitation can be very dangerous. This species is a simultaneous hermaphrodite, cross-fertilizes, incubates and develops eggs within the mantle cavity until larval hatching, and has a larval phase composed of six naupliar stages and a last stage, the cyprid, that settles and methamorphoses into a juvenile. Recruitment is very high on conspecifics (Cruz et al. 2010b), which turns the exploitation of this species potentially detrimental for juveniles.

Management of *P. pollicipes* fishery can be divided into bottom-up approaches as the co-management systems in practice in Galicia (Molares and Freire 2003) and in Asturias (Rivera et al. 2014), Spain, and into top-down regional (Basque Country, Spain; Borja et al. 2006) and national (Portugal; Sousa et al. 2013) systems. In Galicia and Asturias, fishermen and regional authorities participate in management and data gathering processes (co-management), and there are official and representative data about this fishery (Macho et al. 2013, Molares and Freire 2003, Rivera et al. 2014). In

Portugal, the National Administration centralizes the management, most of the catches is sold directly to intermediaries or to the final consumer without regulation and, consequently, fishery data are lacking or do not estimate the real pressure upon this resource.

In Portugal, specific regulations on the fishery of *P. pollicipes* are recent (mostly from 2000), different along the Portuguese coast and have frequently changed (see revision of the specific regulations in Sousa et al. 2013). Four regions with different regulations can be identified: the nature reserve “Reserva Natural das Berlengas” (RNB) and the marine park “Parque Marinho Prof. Luiz Saldanha” (PMLS) (both in central Portugal); the natural park located in SW Portugal (“Parque Natural do Sudoeste Alentejano e Costa Vicentina”, PNSACV) including the Cape of Sines; and the rest of the coast where a national regulation is applied (Sousa et al. 2013). RNB is where regulation for professional barnacle harvesting has first begun in Portugal (1989, modified in 2000 and 2011), and it includes spatial and temporal closures, a limited number of harvest licenses, size and bag limits, and catch reporting in logbooks (Jacinto et al. 2010, 2011). In RNB, recreational harvesting of *P. pollicipes* is not allowed. In PMLS, barnacle harvesting has been forbidden since 2005. In PNSACV, professional barnacle harvesting is subject to specific regulations since 2006 (changed in 2008 and 2011), while recreational fishery of barnacles in PNSACV has specific regulations since 2009 (changed in 2011), both including spatial and temporal closures, size and bag limits (Sousa et al. 2013). In the rest of the coast, a general legislation for professional harvesting (since 2000, reviewed in 2006 and 2010) and a general legislation for recreational fishery (since 2006, reviewed in 2009 and 2014) were implemented and both include size and bag limits, and temporal closures. The definition of each management rule is not similar in all regions (see Sousa et al. 2013).

The main goal of this study was the evaluation of the state of the fishery, management and conservation of *P. pollicipes* in Portugal considering three regions with different regulations regarding the fishery of this species: RNB; PNSACV including the region of the Cape of Sines; and the coast from Cape Carvoeiro to Cape Raso (Center) as representative of the national regulation (Fig. 7.1).

This evaluation was made using recent original and published data (Sousa et al. 2013), and data from past studies (Cruz et al. 2008, Jesus 2004).

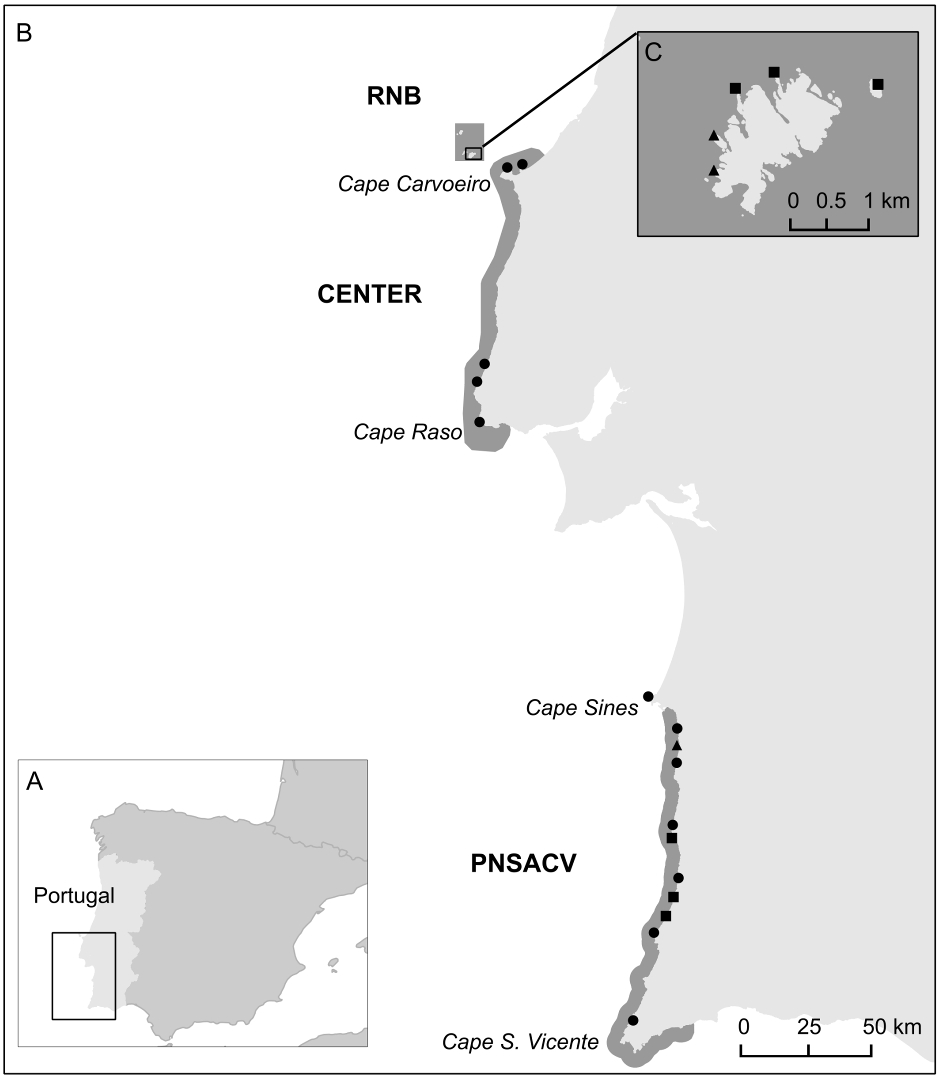

Fig. 7.1 Map of the study location within the Iberian Peninsula (A), study regions (B) (RNB with a more detailed map (C), Center and PNSACV) and sampling sites (●- Professional and recreational harvesting allowed (ProRec); ■ - Professional harvesting allowed (Pro); ▲ – No-take areas (No)).

7.3 Materials and methods

Study regions

Three different regions were evaluated: RNB; Center; and PNSACV including the Cape of Sines (Fig. 7.1). These regions correspond to different regulations regarding the fishery of *P. pollicipes* (Sousa et al. 2013). In each region, there are different types of areas: in RNB there are no-take areas (No) and areas where only professional harvesting is allowed (Pro); in PNSACV there are no-take areas, areas where recreational and professional harvesting are both allowed (Pro-Rec), and areas where only professional harvesting is allowed; and in all coast of Center, recreational and professional harvesting is allowed.

The state of the fishery and conservation

The state of the fishery and conservation of *P. pollicipes* was defined for each region and by using different approaches/data summarized in Table 7.1.

Table 7.1 Approaches, time of data acquisition and respective references used for accessing the state of the fishery and management of *Pollicipes pollicipes* in three Portuguese regions.

	RNB	Center	PNSACV
Independent observations on biomass and size	2006 and 2011	2011	2011
Logbook data	2000 to 2012	No available data	2007 to 2012
Inquiries to fishermen	2005 and 2013	2013	2002 and 2013
References	[a], Cruz et al. (2008), Jacinto et al. (2010), Jacinto et al. (2011), Sousa et al. (2013)	[a], Sousa et al. (2013)	[a], Jesus (2004), Sousa et al. (2013)

[a] original data, present study.

Independent observations on biomass and size

In each region and type of area, a different number of sites was considered (total of 24 sites) (Fig. 7.1), and in each site, two vertical levels were defined: mid and low shore (see more details in Sousa et al., 2013). Low shore barnacles are more exploited by fishermen than mid shore barnacles (personal observations). In each site and vertical level, biomass and size (rostro-carinal length, RC) of *P. pollicipes* were estimated in 2011, by destructive sampling of 15 X 15 cm quadrats (n = 3) according to the method described in Sousa et al. (2013). In RNB, biomass and size data were also estimated in 2006 using the same method (Cruz et al. 2008). Average (±standard error) of biomass (kg/m^2) and percentage of biomass size classes (RC, mm: <12.5, 12.5 - 20, 20 - 25, >25, for comparison in RNB between 2006 and 2011; <17.5, 17.5 - 20, 20 - 22.5, >22.5, for comparisons among regions in 2011) were calculated per site in each type of area and region, for both 2006 and 2011 for RNB, and for 2011 in Center and PNSACV.

Statistical comparisons of biomass (total and per size class) between 2006 and 2011 in RNB were done separately for each vertical level using permutational multivariate analysis of variance, PERMANOVA (Anderson 2001), including three factors: years (fixed factor with two levels, 2006 and 2011); type of area (fixed factor with two levels, RNB Pro and RNB No, see Fig. 7.1); and site (random factor nested in type of area, with two/three levels in the low shore and two levels in the high shore). Sample size is three (the three quadrats).

Analysis of biomass (total) was based on Euclidean distances of untransformed data. Unrestricted permutation of raw data and Type III sums of squares were applied (Anderson et al. 2008). We used PERMANOVA to analyse univariate data, due to an unbalanced design resultant of the different number of sites per exploitation regime in the low shore.

Size structure of biomass was analysed by PERMANOVA (Anderson 2001), based on a matrix of Manhattan distances (Legendre and Legendre 1998), calculated from a multivariate data matrix of the relative biomass of barnacles in each size class per replicate.

In all analyses, homogeneity of dispersion was tested using the PERMDISP routine (Anderson 2006). When appropriate, pair-wise a posteriori comparisons were conducted. The software PRIMER 6 & PERMANOVA+ (www.primer-e.com, Anderson et al. 2008) was used to perform all statistical procedures.

Statistical comparisons among regions and type of area in 2011 were performed by Sousa et al. (2013).

In RNB, the state of the fishery and conservation of *P. pollicipes* was considered as having: a negative tendency if the biomass and the relative frequency of size classes of big animals (RC > 20 mm) have decreased in 2011; a positive tendency if those values were higher in 2011; and a stable tendency if the values were similar or contradictory. In Center and PNSACV, as there are no past studies, this state was defined by comparing the biomass and size data obtained in these regions with the values observed at RNB (based in Sousa et al. 2013).

Logbook data

In RNB and PNSACV, professional fishermen must report their catches in logbooks, and the renewal of their individual annual licenses depends on the delivery of individual logbooks. Logbook data are available from 2000 in RNB, and from 2007 in PNSACV, and were analyzed for both regions until 2012. Total number of licenses in RNB got a maximum of 58 in 2001 (55 in 2000; Jacinto et al. 2010), and gradually decreased (~50 from 2002 to 2004; 45 from 2005 to 2008; and 40 from 2009 to present), while in PNSACV is 80 since 2006. For each region, we have compiled data on number of harvesting days and harvested amounts per day that were declared by each fisherman in each year.

Logbook data from RNB on exploitation made during 2005 and 2006 have been validated in a previous study (Jacinto et al. 2010) and were considered representative of the harvesting effort in this region. Consequently, we have calculated the annual variation of the amount harvested in each year, of the number of harvesting days (effort unit-UE), and of the amount caught per harvesting day (CPUE) for the period from 2000 to 2012. These results have been partially presented (2000 - 2006) in a previous study (Jacinto et al. 2010).

Logbook data from PNSACV were compared with answers given by professional harvesters to inquiry (see next section), namely on the number of harvesting days per year, and the average amounts collected by day. Logbook data were considered to underestimate the real effort in this region, and data will not be presented.

The state of the fishery and conservation of *P. pollicipes* at RNB was considered to show a negative tendency if CPUE data decreased with time, a positive tendency if CPUE data increased with time, and a stable tendency if there was no consistent pattern of CPUE with time.

Inquiries to fishermen

Professional fishermen of these three regions were individually and directly interviewed during 2013. The number of inquiries performed in each region was: 32 in RNB (total number of licenses is 40); 49 in PNSACV (total number of licenses is 80); and 26 at Center (there are no individual specific licenses for harvesting *P. pollicipes* at Center, a maximum number of general harvesting licenses was established from 2010). We have asked them about their temporal (last 5 years) perception on the evolution of the amount and size of *P. pollicipes* (see additional online information). Similar questions were performed in past studies in RNB (2005, n = 38; Cruz et al. 2008), and in PNSACV (2002; n = 67; Jesus 2004).

The state of the fishery and conservation was defined as showing a negative, positive or stable tendency if the perception of the fishermen on the temporal evolution of the resource was negative, positive or stable, respectively. In RNB and PNSACV, the answers to the same questions made in previous studies were also used to reinforce the definition of the state.

The state of the management

The state of the management of *P. pollicipes* was assessed for each region and by performing inquiries to professional fishermen from these three regions in 2013, as described above.

In these inquiries, a set of closed questions (see additional online information) was used to gather the opinions of fishermen about each management rule in practice in each region (temporal closures, bag and size limits, number of licenses, protected areas), namely their agreement and sense of fulfilment. Additionally, specific questions were done in order to know if they belong to a fishery association, and to get their opinion on surveillance and on co-management.

The state of the management was defined as weak, acceptable or good. A good management state was defined if the majority of the following conditions was satisfied: the majority of the fishermen agreed with all rules; the perception of compliance with all rules by the majority of fishermen was positive; most of the fishermen belonged to a fishery association; and their perception of surveillance was positive. An acceptable stable management state was decided if half of these conditions was satisfied, while a weak management was inferred if most of these conditions was not satisfied.

7.4 Results

The state of the fishery and conservation

The nature reserve of Berlengas (RNB)

The majority of the fishermen considered that the amount of barnacles has decreased in the previous five years both in 2005 and 2013 and this negative tendency was stronger in 2013 (Fig. 7.2A). In relation to size, in 2005, the large majority of the fishermen answered that size did not change in the last five years, while have considered that barnacles became smaller from 2009 to 2013 (Fig. 7.2B).

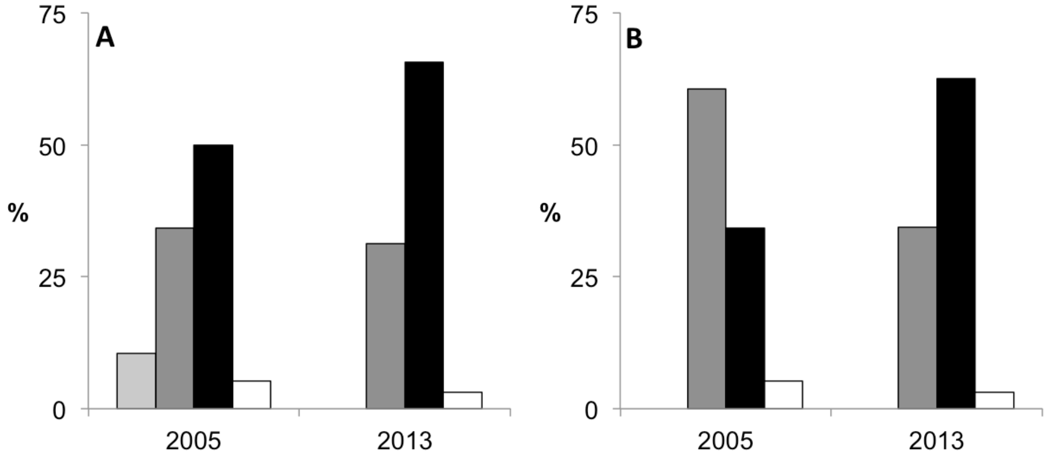

Fig. 7.2 Percentage of professional fishermen from RNB that answered that the amount (A) and size (B) of *P. pollicipes* in this region has increased (░), did not change (▒), and has decreased (■) in the last 5 years, when interviewed in 2005 and 2013 (n=38 in 2005; n=32 in 2013). (□)- did not answer.

The temporal patterns of the fishery of *P. pollicipes* in RNB (2000 - 2012) revealed from logbook information were that: the average amount of barnacles collected in RNB per year was 14 tones; while the annual effort by fisherman (UE divided by number of licenses) was 22 days; the amount collected by fisherman and day (CPUE) was in average 14.2 kg; but CPUE has decreased during this period, notably since 2007 when a major break was detected (Fig. 7.3).

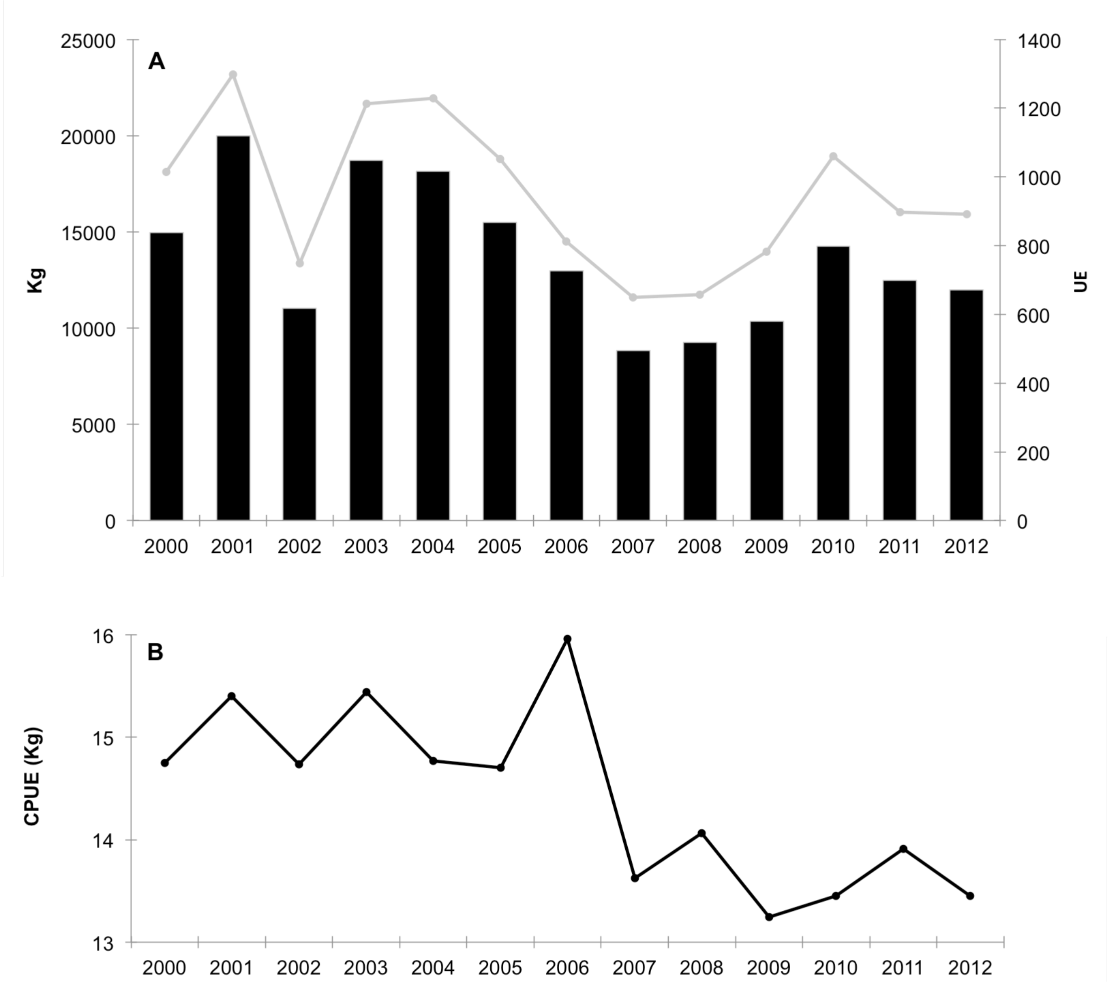

Fig. 7.3. Annual variation, 2000–2012, of the A) total harvest of *P. pollicipes* (kg; black bars, primary axis) and harvesting effort (unit effort (UE): fishermen x day; grey line, secondary axis), and B) CPUE - catch per unit effort (kg), at RNB, based on logbook information. Number of annual licenses varied from 40 to 58.

The average biomass of *P. pollicipes* at the high shore of RNB sites was in average 5.6 kg/m^2 in 2006 and 6.3 kg/m^2 in 2011 and there was not a consistent temporal pattern of increase or decrease of biomass for each sampled site (interaction between factors year and site (type of area) was significant, $P < 0.05$, but with no consistent pattern between years in the different sites, PERMANOVA and pair-wise tests results). Contrarily, on the more exploited low shore, the average biomass was in general lower in 2011 (1.6 kg/m^2 in 2006 and 0.7 kg/m^2 in 2011) and presented a significant ($P < 0.05$) pattern of decrease in the majority of sites (interaction between factors year and site (type of area) was significant, $P < 0.05$, PERMANOVA and pair-wise tests results) (Fig.

7.4A and D).

In relation to size, there were not significant differences between years in the high shore (factor year and interactions with factor year were not significant, P > 0.05, PERMANOVA results), while significant differences between 2006 and 2011 were found in two sites in the low shore (interaction between factor year and site (type of area) was significant, P < 0.05, PERMANOVA and pair-wise tests results), where big and very big barnacles were relatively less abundant in 2011 (Fig. 7.4B and C-high shore; Fig. 7.4E and F-low shore).

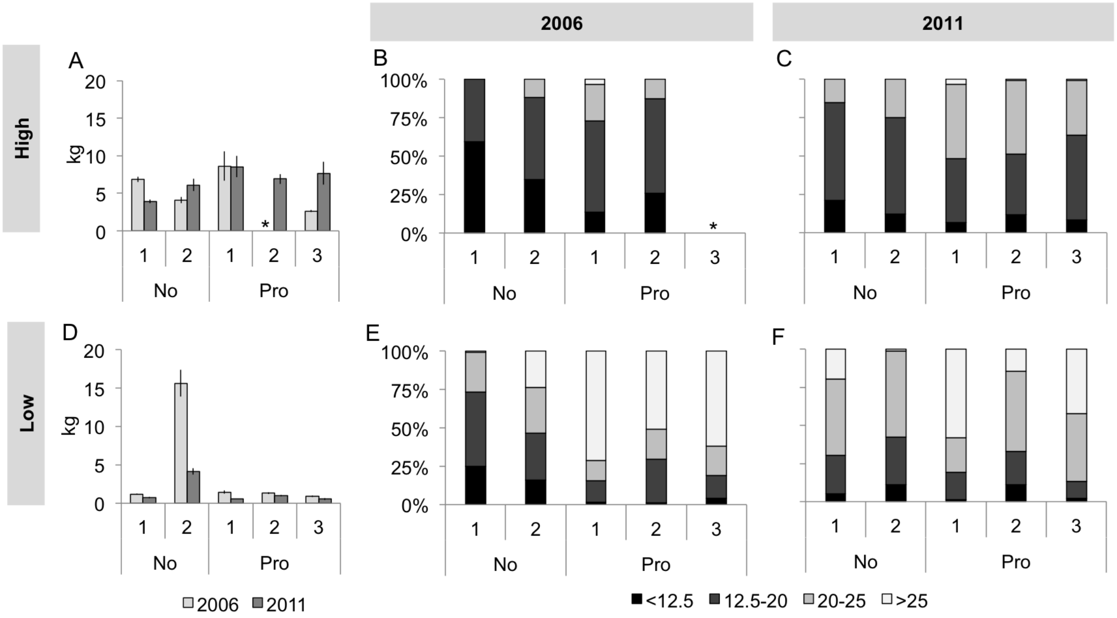

Fig. 7.4 *Pollicipes pollicipes* biomass (kg/m^2) in 2006 and 2011 at the high (A) and low (D) shore of No and Pro sites at RNB (mean ± standard error) and relative biomass per size class (RC, mm) and site, at the high (B, C) and low (E, F) shore assessed in 2006 (B, E) and 2011 (C, F) at RNB. Results partially presented (2011 data) in Sousa et al. (2013). *- no data. Pro- Professional harvesting allowed. No- No-take areas.

The coast from Cape Carvoeiro to Cape Raso (Center)

The large majority of the fishermen from the Center considered that the amount of barnacles has decreased from 2009 to 2013, and that barnacles have become smaller during this period (Fig. 7.5).

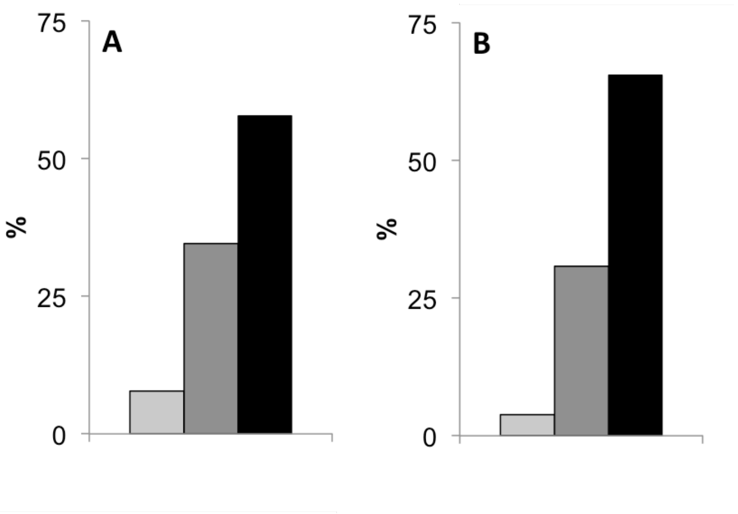

Fig. 7.5 Percentage of professional fishermen from Center that answered that the amount (A) and size (B) of *P. pollicipes* in this region has increased (▫), did not change (▪), and has decreased (■) in the last 5 years. Inquiries performed in 2013 (n=26).

In the Center region there is no logbook information and there are no known studies made before 2011 to assess biomass and size of barnacles. Comparing the biomass and size values obtained in 2011 between the Center and RNB (where past data are available) we have observed that: biomass in the mid and low shore was lower at Center (mid-2.4 kg/m^2; low-1.2 kg/m^2) than at RNB (mid- 6.3 kg/m^2; low-1.6 kg/m^2 (Fig. 6A and C); and the frequency of big and very big animals (RC > 20 mm) is the lowest at Center, both in the low and mid shore (Fig. 7.6B and D). Statistical analyses between these regions performed in Sousa et al. (2013) confirmed these patterns.

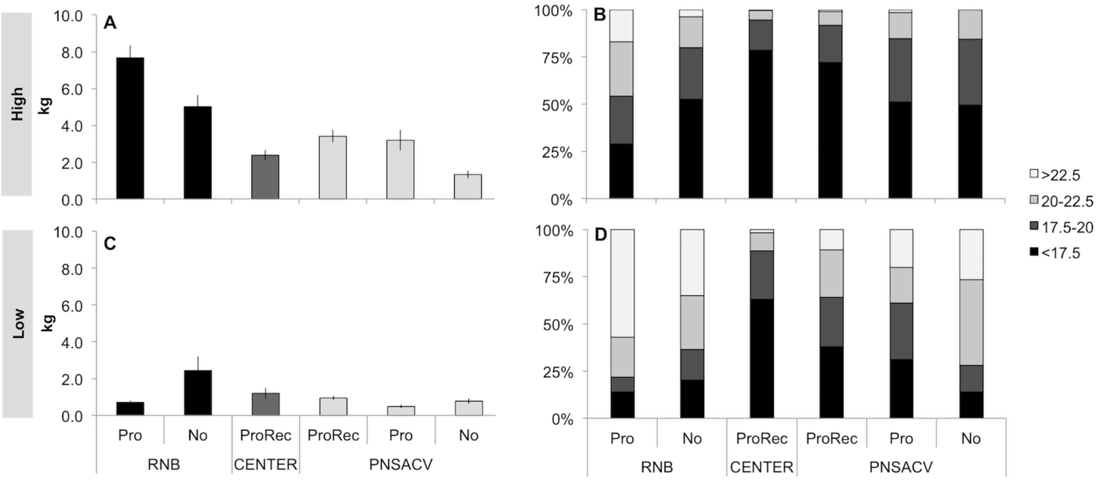

Fig. 7.6 *Pollicipes pollicipes* biomass (kg/m^2) in 2011 at the high (A) and low (C) shore of different types of areas sampled at RNB, Center and PNSACV (mean ± standard error) and relative biomass per size class (RC, mm) and type of area, at the high (B) and low (D) shore in 2011 at the same regions. Results previously published and adapted from Sousa et al. (2013). ProRec- Professional and recreational harvesting allowed. Pro- Professional harvesting allowed. No- No-take areas.

The natural park of the SW coast (PNSACV)

The majority of the fishermen considered that the amount of barnacles at PNSACV did not change in the previous five years when inquired in 2002 and in 2013 (Fig. 7.7A). In relation to size, in 2002, the large majority of the fishermen answered that size became smaller in the previous five years, while the majority have considered that barnacles were similar in size from 2009 to 2013 (Fig. 7.7B).

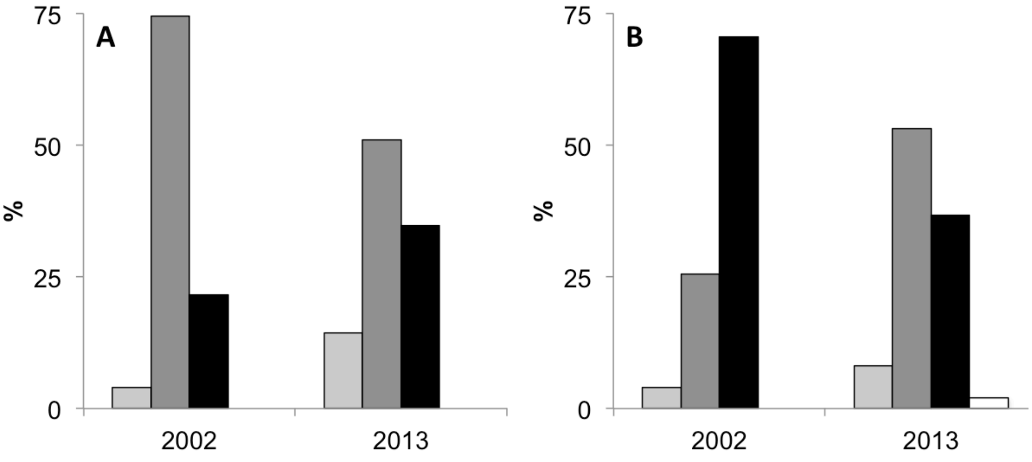

Fig. 7.7 Percentage of professional fishermen from PNSACV that answered that the amount (A) and size (B) of *P. pollicipes* in this region has increased (░), did not change (▒), and has decreased (■) in the last 5 years. Inquiries performed in 2013 (n=49). (□)- did not answer.

Although there are logbook data available from 2007 to 2012 at PNSACV, we did not considered the information reliable, namely when we have compared the total number of harvest days per year declared in the logbooks by the fishermen (average of 24 days) and the results from the inquiries, as more than 90% of the fishermen estimated in the inquiries that exploited barnacles in more than 30 days per year. However, the amount of barnacles harvested in each day (CPUE) was similar when both approaches were considered, as an average of 8.4 kg was declared in the logbooks and an average of 8.7 kg was answered in the inquiries.

As in the Center, there are no known past and comparable studies with estimates of biomass and size at PNSACV sites, so comparisons with the average values obtained in RNB were done and revealed the following patterns: biomass in the mid and low shore was lower at PNSACV (mid-2.6 kg/m^2; low-0.7 kg/m^2) than at RNB (mid-6.3 kg/m^2; low-1.6 kg/m^2 (Fig. 7.6A and C); and the frequency of big and very big animals (RC > 20 mm) is lower at PNSACV than at RNB, both in the low and mid shore (Fig. 7.6B and D). Statistical analyses between these regions performed in Sousa et al. (2013) confirmed these patterns.

The state of the management

Most of the fishermen at RNB and at Center have agreed with each of the management rules that are in practice in each region (temporal closure in general, bag limit, size limit). On the contrary, the agreement is in general lower in PNSACV, where there are specific rules that the majority of the fishermen did not agree, namely the specific period of temporal closure (15th September to 15th December) that according to them should be shorter, the number of licenses that they would like to be higher and the existence of no-take areas (Table 7.2).

The sense of fulfilment of each of these rules was different among regions and among rules (Table 7.2). The rule that fishermen considered that is less fulfilled is the bag limit (by collecting more than the bag limit), namely at RNB and Center, and the rule that was more complied was the seasonal temporal closure (Table 7.2). In all regions, the sense of nonfulfillment was higher than compliance (compliance<45%, Table 2). In RNB and PNSACV, there was no rule that most of the fishermen has considered as not being fulfilled a lot of times, while in Center, 65% of the fishermen considered that bag limit was not fulfilled a lot of times (Table 7.2).

Table 7.2 Professional fishermen opinion on current management rules regarding the fishery of the stalked barnacle *Pollicipes pollicipes* in Portugal and their perception on the compliance with each rule. RNB (n=32); Center (n=26; *n=7); PNSACV (n=49). Agreement of temporal closure was asked in general (first value) and in relation to the specific period in practice in each region (second value). Data for the category "Nonfulfillment-some times" are not shown but can be obtained by difference.

	RNB			CENTER			PNSACV		
	Agree	Nonfulfillment		Agree	Nonfulfillment		Agree	Nonfulfillment	
		absent or rare	a lot of times		absent or rare	a lot of times		absent or rare	a lot of times
Maximum nr of licenses	88%	-	-	-	-	-	43%	-	-
Seasonal temporal closure	100/66%	38%	6%	85/58%	35%	23%	82/20%	35%	18%
Size limit	78%	28%	28%	86%*	31%*	38%*	55%	31%	14%
Bag limit	88%	25%	31%	69%	19%	65%	76%	44%	15%
Protected areas	56%	34%	25%	-	-	-	13%	31%	13%

The answers to the inquiries have also shown that while all fishermen at RNB belong to a fishery association, this did not happen in Center (90% do not belong) and PNSACV (60% do not belong). When inquired about the surveillance in each region, in all regions, surveillance was considered as not existing or not sufficient for the large majority of the fishermen (84% in RNB; 85% in Center; and 76% in PNSACV).

Additionally, the large majority of fishermen knew the concept of co-management in RNB and PNSACV (97% in RNB and 92% in PNSACV) but did not know this concept at Center (54%). In RNB and PNSACV, the large majority of the fishermen agreed with the implementation of co-management in the respective region (81% in RNB and 71% in PNSACV), as well as in the Center (69%), after explaining the meaning of co-management.

The state of the fishery, conservation and management

The state of the fishery, conservation and management of *P. pollicipes* in the three regions is summarized in Table 7.3. An overall negative tendency of the state of the fishery and conservation of this resource was observed in all regions and by using different methods, with the exception of the stable tendency in PNSACV that was decided based on the inquiries approach.

In relation to management, the conditions that were satisfied (value 1) in each region and by the order presented in the methods section were: 1-0-0-1 in RNB; 1-0-0-0 in Center; 0-0-0-0 in PNSACV. So, while a weak management was considered to be in practice at Center and at PNSACV, an acceptable management was inferred for RNB (Table 7.3).

Table 7.3 The state of the fishery, conservation and management of *Pollicipes pollicipes* at three regions of the Portuguese coast (RNB, Center and PNSACV) based on three approaches and data (see table 1).

		RNB	CENTER	PNSACV
The state of the fishery and conservation	Biomass and size	Negative tendency	Negative tendency	Negative tendency
	Log-book data	Negative tendency	No data	Data not representative
	Inquiries to fishermen	Negative tendency	Negative tendency	Stable
The state of the management	Inquiries to fishermen	Acceptable	Weak	Weak

7.5 Discussion

The assessment of the state of the fishery, conservation and management of the stalked barnacle *P. pollicipes* in Portugal was made for the first time. Although this species can be considered the most important intertidal economical resource in continental Portugal, official data on this fishery are not representative of the real pressure upon *P. pollicipes*. Additionally, monitoring the abundance of this species is difficult due to the very harsh environments it inhabits. Here we have combined different approaches and sources of data, as well as recent and past information, to build up and define these states.

Three Portuguese regions (RNB, Center and PNSACV) were chosen for this assessment, as these regions have different regulations regarding the exploitation of *P. pollicipes*, as well as different times of implementation (see revision in Sousa et al. 2013). An overall negative tendency of the state of the fishery and conservation of this resource was observed in all regions and by using different methods, with the exception of a stable tendency in PNSACV that was decided based on the inquiries approach. In relation to management, while a weak management was considered to be in practice at

Center and at PNSACV, an acceptable management was inferred for RNB.

Several aspects might potentially explain the more pessimist state defined for the Center region: it is not part of a marine protected area; there are less management rules in practice; there are no specific licenses for exploiting barnacles in this area and a maximum number of general licenses for harvesting marine animals was only recently (2010) defined (maximum number of licenses per port authority must not be higher than 10% in relation to the number of licenses of 2009); it is closer to big cities (e.g. Lisbon, Cascais); and so, the total number of fishermen (professional and recreational) might be higher than in the more regulated areas of RNB and PNSACV. In opposition, a more benign scenario was achieved in RNB that is a marine protected area where recreational harvesting is not allowed and that was the first area in Portugal to have management rules for *P. pollicipes* exploitation. Besides, RNB is a group of islands and, consequently, rocky shores are more inaccessible, and exploitation of barnacles must be done by using boats, which can contribute to a lower harvesting pressure by illegal fishermen.

However, the overall situation was considered to be negative independently of the region. In fact, in all regions the large majority of the professional fishermen (>65%, data not shown) considered that there was an excessive harvesting of *P. pollicipes* conducted by recreational harvesters and by illegal and legal professional harvesters.

In the Portuguese coast, recreational fishing is traditional, can be regular and intense in some regions and involves several activities (shore and boat angling, shellfish and bait collection and freediving fishing), including *P. pollicipes* collection on intertidal and shallow subtidal rocky shores (Castro and Cruz 2009, Veiga et al. 2010). From 2007 to 2013, the mean annual number of marine recreational fishing licenses in continental Portugal was ca. 174835, most of them (77.6%) allowing *P. pollicipes* collection with several restrictions (data from the website of the national marine fishery administration-DGRM).

Professional fishermen considered that the first-sale price of *P. pollicipes* has decreased during 2009 - 2013 (>60%, data not shown) due to the excessive exploitation and to the general economic crisis that is affecting Portugal in recent years. In a debate about the fishery and management of *P. pollicipes* in Portugal promoted by us in December 2013 with different stakeholders (e.g. national fishery administration-DGRM; national conservation institute-ICNF; associations of fishermen), and where about one hundred of fishermen (mostly professionals) were present, the highlights were: excessive exploitation; insufficient surveillance; lack of association and union among the fishermen.

In this debate, besides discussing the actual problems of the top-down system of management of *P. pollicipes* in practice in Portugal, the changing to a co-management/bottom-up system was also discussed. In fact, when inquired, the large majority of professional harvesters in RNB and PNSACV knew the concept of co-management (>90%), and agreed (>70%) with their implementation. This knowledge has been acquired in recent years, namely by visits to Galicia organized by local Portuguese associations and by participation in the "*Pollicipes* International Conference" (Cruz et al. 2010a). As in Galicia, the Portuguese fishermen are aware that the co-management system of *P. pollicipes* can add commercial value to the resource by a more effective and profitable distribution and commercialization. Additionally, a better surveillance might also derive from a co-management system where surveillance might also be achieved with the enrolment of the fishermen.

The co-management system implemented in Galicia inverted the overexploitation of *P. pollicipes* that happened before the 1990s (Molares and Freire 2003). In Galicia, fisheries associations and the fisheries administration share the management of this resource based on territorial use-rights for fishing (TURFs) (Molares and Freire 2003). Recently, it has been analysed the role of the “Bare-foot Fisheries Advisors” (BFAs) (equivalent to technical assistance) in the Galician TURFs (Macho et al. 2013). The BFAs have been considered a key actor in management and governance of small-scale fisheries, as they help providing good quality fisheries data and act as an essential link between different stakeholders (fishermen, managers, scientists) (Macho et al. 2013).

We strongly recommend a change into a co-management system of the fishery of *P. pollicipes* in Portugal. The state of the resource has presented a negative tendency and a weak/stable management was described in the present study. Good practices of co-governance systems should be imported and adapted to Portugal. Two regions could act as pilot regions for testing this change: PNSACV (as recommended by Castro and Cruz 2009) and RNB. In these regions, many fishermen are members of fishermen associations, know the concept and agree with its implementation. Besides, debates and meetings with the national administration have given the impression that managers are also receptive to a change of paradigm. The next step is to join the willingness for a change demonstrated from both of these stakeholders, and to work on the legal and practical aspects of the implementation of a co-management system in pilot regions.

7.6 Acknowledgments

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Chapter 8. Conclusion and final remarks

The present thesis contributes to the knowledge of temperate rocky shore ecology, with direct implications in the management and conservation of important local marine resources. Patterns and processes of abundance and distribution of the sea urchin *Paracentrotus lividus* and of the stalked barnacle *Pollicipes pollicipes*, have been studied. Both species live at the very edge of both subtidal and intertidal habitats of wave-swept rocky shores Southern Europe and are important local marine resources.

The results presented provide insights into the relative importance of wave exposure as a key factor affecting, at different spatial scales, two species with distinct natural histories: the sessile filter feeding stalked barnacle *Pollicipes pollicipes* and the mobile keystone herbivore sea urchin *Paracentrotus lividus*. Studies on the distribution and abundance patterns of these common marine species at different spatial scales, at both local and regional scales, led to discussions on how wave exposure may set the framework under which important biological and ecological processes vary and influence species biology and interactions.

The work carried out and presented in chapters 2 and 3 of the present thesis, is the result of intensive underwater observations and fieldwork carried out along rocky shores of southern Europe at a wide range of spatial scales. These studies, of descriptive hypotheses-testing nature, explored how variations in hydrodynamic conditions may affect patterns of density, size structure and microhabitat usage of the sea urchin *Paracentrotus lividus* populations. Observations carried out at a wide range of spatial scales (10's of meters to 1000's of kilometres) and in contrasting oceanic climates (Atlantic and Mediterranean), suggest that that the effects of hydrodynamic forces on sea urchin populations are context dependent and vary according to background oceanic climate. *P. lividus* ecological patterns varied between regions but wave exposure and depth gradients effects on urchin density, size structure and microhabitat usage, were not consistent within each region. Most of the variation in urchin abundance occurred at small spatial scales (metres) suggesting that habitat complexity, i.e. the presence of cracks and crevices, is an important determinant of patterns of distribution of this species. Despite regional variation in abundance, no effect of wave exposure was found within each region. In NW Italy a higher proportion of medium-sized individuals was found, while in SW Portugal, smaller individuals represented a large proportion of the populations. Size structure also varied between exposed and sheltered habitats in SW Portugal, suggesting that the proportion of individuals from different size cohorts may vary along wave-exposure gradients as a result of direct or indirect effects of hydrodynamic forces. In SW Portugal, most urchins occurred in burrows, while in NW Italy, urchins were mainly observed in crevices. These results suggest that creating/occupying burrows might be an adaptive response to oceanic climate that allows sea urchins to better withstand stressful hydrodynamic conditions and, therefore, are more common on exposed Atlantic coasts. Results were consistent with the hypotheses of variable population size structure and microhabitat usage along wave exposure gradients, as a result of direct mechanical stress on sea urchins and/or indirectly by affecting the predation pressure upon sea urchins.

Sea urchin attachment forces while in burrows were measured to study its relationship with burrow shape, urchin size and habitat (Chapter 3). Results support the model that burrowing behaviour enhances sea urchin attachment force, by allowing the use of spines as anchorage points and reducing the area exposed to drag forces and might be an adaptive response to hydrodynamic stress as proposed by Otter (1932). However, alternative models including the influence of predation pressure in sea urchin burrowing behaviour should be properly addressed in future studies. The main findings of chapters 2 and 3 constitute an important contribution to the knowledge of the ecology of the sea urchin *P. lividus* and set the baseline for the reasoning behind the work carried out by

Mamede (2014) on the ecological relationships between sea urchins, their predators and prey in SW Portugal.

The work described in chapter 4 combined both descriptive and manipulative experimental approaches to study the influence of wave exposure on the small-scale patterns of distribution and of important life-history processes, such as recruitment and predation, affecting the stalked barnacle *Pollicipes pollicipes* on rocky intertidal habitats. A tight relation between *P. pollicipes* abundance and wave exposure has been previously observed at larger scales (Borja et al. 2006), yet here it was described a similar pattern occurring at smaller scales (10's meters) and discussed the putative respective processes leading to such patterns. The experimental approach used to study small-scale variation in both top-down (predation) and bottom-up (recruitment) processes suggest that wave exposure might be shaping stalked barnacle distribution patterns not by imposing direct physical of physiological limitations to the organisms but possibly by indirectly affecting biological processes like settlement and predation which vary at very small spatial scales. Results from this study are important contributions to the conceptual rational on the ecology of stalked barnacles, namely on the drivers of spatial variation in abundance.

The strong correlation found at different spatial scales between wave exposure and stalked barnacle abundance and distribution patterns in this and previous studies (Barnes 1996, Borja et al. 2006) as well as its social and economic importance along the Portuguese coast led to the development of the study presented in chapter 5. Spatial planning and management of marine resources requires information on distribution patterns at relevant scales (Bekkby et al. 2009), which may be insufficient and/or difficult to obtain due to logistical constraints of biological sampling across large areas and/or on extreme habitats such as very exposed wave-swept rocky shores.

Predicting patterns of distribution using relationships between species abundance and physical variables may provide the required information and assist management and conservation decision-making (Hill et al. 2010). The results presented in chapter 5, suggest that the positive relationship between simple fetch-based exposure indices and *P. pollicipes* percent cover may be used as a simple tool able to provide stakeholders with information on barnacle distribution patterns that may lead to better assessment of harvesting grounds and dimension of exploitable areas, which may help improving management plans and support decision making regarding conservation, harvesting pressure and surveillance strategies for this highly appreciated and socio-economic important marine resource. Similar models might be applied to other areas along *P. pollicipes* distribution range with direct implications on resource management and conservation.

Alongside with spatial planning, population dynamic models that include several social and biological parameters such as the one proposed by Bald et al. (2006) may be essential tools for conservation and management purposes regarding the stalked barnacle fishery. Such models make important assumptions that could be subject to improvement, namely regarding growth estimates of stalked barnacles in relation to the environment (Bald et al. 2006). In order to improve method feasibility and accuracy of growth estimates, in the work described in chapter 6, chemical marking techniques with calcein were used to study growth variation in the stalked barnacle *P. pollicipes*. This study combined field manipulative experiments with an intensive laboratorial component. The results discussed in chapter 6 suggest that calcein marking techniques have a great potential in stalked barnacle growth studies, mainly because they allow mass marking of individual barnacles of different size cohorts within a short period (less than 1 day of manipulation) and involve less fieldwork time, which is a major advantage because this species lives on extremely exposed rocky shores. Results suggest that barnacle growth rates are highly variable between individuals and mostly driven by barnacle size. Mean growth rates were higher in juvenile barnacles (peaking at 1.1 mm per month for individuals with RC~5 mm), and gradually decreased with barnacle size (monthly growth rates of 0.5 mm for adult barnacles with RC~12.5 mm). Mean monthly growth rates observed in larger adults of higher commercial interest (>18mm RC) was <0.25 mm. Contrary to our expectations, growth rates were not higher in the low shore, as suggested by previous studies (Cruz et al. 2010). This novel method is a critical contribution to bioecological studies of *P. pollicipes,* which has led (e.g. Mateus 2015) or may lead to further experiments on this important resource and make it possible to test hypotheses addressing spatial and temporal variability in growth patterns at a multitude of scales, providing reliable estimates of growth to further improve population dynamic models applied to resource management and conservation programs.

Chapter 7 provides for the first time an assessment of the state of the fishery, conservation and management of the stalked barnacle in Portugal, combining different approaches and sources of data, including independent field observations and inquires made to professional harvestmen, from both recent and past studies. This review focused on three regions in Portugal with different regulations regarding the exploitation of *P. pollicipes* (two marine reserves with specific regulations and a stretch of coast under the national regulation for barnacle harvesting), as well as different times of implementation (Sousa et al. 2013). According to the data attained, the state of the resource has presented a negative tendency and a weak or stable management was described for the Portuguese coast. A discussion on the problems of the top-down system of management of *P. pollicipes* in practice in Portugal is presented and alternatively a change into a co-management/bottom-up system of the fishery of *P. pollicipes* is recommended, following the examples and good practices of co-governance observed in other regions where this model has been in practice for several years now (ex. Galicia and Asturias, Spain) and has been fostering good results regarding ecological and economical sustainability of marine resources (Molares and Freire 2003, Rivera et al. 2014).

It is generally acknowledged that the fisheries are complex structures coupling human (i.e. social, economic, and political components) and ecological systems (Liu et al. 2007). Social and ecological processes interact at different spatial and temporal scales, and it is essential to match both systems to improve responsible planning and management of natural resources and ecosystem services for human well being and sustainability. Conservation and management of marine resources demand a wealth of information on population dynamics, stock status, harvesting activities and socioeconomic subjects. There is still a long way ahead regarding conservation and management of sea urchin *P. lividus* and stalked barnacle *P. pollicipes* populations along the Portuguese coast. Both species are important marine resources and exploited along Portuguese the coast, specially the stalked barnacle *P. pollicipes* which is a highly appreciated shellfish in both local and foreign markets, and thus is highly exploited by professional harvestmen, recreational fishermen and poachers. The same is true for the sea urchin *P. lividus*, yet to a lesser extent, although a renown interest on its gonads in local markets is uprising and new economic channels to foreign markets are being trailed (Andrew and Agatsuma 2002, Bertocci et al. 2014), which may lead in a near future to an important commercial activity concerning sea urchin harvesting in Portugal. For that, and also due to their ecological importance as key species in coastal marine communities, fisheries related bioeconomic and bioecological studies should be continued preferably as part as long term and large scale monitoring plans to evaluate the status of the standing stock, temporal and spatial patterns of resource abundance and harvesting activities and its impact on local biodiversity and community ecology.

Unfortunately, in what concerns artisanal fisheries like the stalked barnacle or the sea urchin fisheries in Portugal, there are no monitoring plans encouraged by the national administration and most research is punctual, project based and mostly driven by the interest in ecological studies of different research teams. Efforts should be made in order to accommodate research interests to bioeconomical and conservation needs as part of marine resource management plans. Regarding research in small artisanal fisheries as the ones addressed here, one of the solutions could follow the general lines of co-management and the role of trained technical assistants working together with fisher's guilds and responsible for the provision of good quality fisheries data (spatially and temporally), not only from sales data but also from catches, field surveys, and fishers' knowledge (Macho et al. 2013).

Along with fisheries driven research, fundamental biological and ecological studies should be addressed. Many aspect of the natural history of both stalked barnacles and sea urchins along the Portuguese coast are still insufficiently studied, namely settlement and recruitment spatial and temporal variability, population connectivity, growth patterns and density dependent effects on local assemblages. Also, the fear of an increasing number of overexploited and collapsed stocks over time due to inadequate management or high market demand impossible to fulfil with wild stocks, may lead to new approaches and opportunities regarding aquaculture of stalked barnacle and sea urchins in Portugal as an alternative to supply the market and for re-stocking programs (Silva 2012, Franco 2014).

Portugal and its people have a long and well-recognized historical relationship with the ocean and the services it provides. Coastal and maritime activities have traditionally been important to the country's economy and to the historical, social and cultural identity of the Portuguese, and like many other coastal areas elsewhere on the globe, there is a significant concentration of population (~75%) on its coastal strip (Carneiro 2007). Although the Portuguese coast is highly productive and biodiverse (Lima et al. 2007), its biological communities are under a wide array of stressors of both natural and human-related sources. Now, more than ever, under a globally changing and population-increasing world, the sustainability and resilience of marine resources depend on our approaches to their management and conservation, which should be active and data-driven societal responses to real pressures.

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