UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

CENTRE FOR BIOLOGICAL SCIENCES

THE INFLUENCE OF ENVIRONMENT AND EXPLOITATION ON SEX CHANGE IN LIMPETS

by

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ABSTRACT

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Patellid limpets are harvested around the world and many stocks are currently overexploited. This study investigated the influence of environment and harvesting upon the biology of target species (*Patella* spp.) and upon the macro-community structure of non-target species.

A broad-scale survey of *Patella* spp. across the British Isles and Portugal revealed that non-exploited *Patella vulgata* populations from England had traits indirectly associated with protandry. Females predominated in larger size classes; cumulative frequency distributions of males and females were different; sex ratios were biased towards the first sex and smallest sizes of males were smaller than the smallest sizes of females. In Portugal, *P. vulgata* populations did not show these patterns, suggesting that protandry was not occurring in those *P. vulgata* populations, although alternative explanations include the influence of low recruitment leading to fewer small males. In the England *Patella depressa* appeared to be gonochorist with a sex ratio of 1:1. In Portugal, however, *P. depressa* also showed some patterns indicating the possibility of slight protandry.

In a manipulative experiment to simulate size-selective harvesting of limpets by humans, changes in sex ratio of *P. vulgata* in the south-west of England were monitored over an 18-month period of repeated removal of bigger limpets. There was strong evidence that the size at sex change decreased in response to the exploitation treatment, emphasizing its plasticity. Sex change occurred at a bigger size than expected from the overlap in male and female size classes. The limpet exploitation led to establishment of *Fucus* spp. At the end of the experiment, control plots had lower percentage cover of *Fucus* spp. than both low- and high-exploitation plots. Univariate and multivariate analyses confirmed that the communities on the two shores responded differently to the same source of disturbance.

A comparative survey of populations across a gradient of exploitation in the Canary Islands indicated that the abundance of *Patella aspera* decreased from 1992 to 1999, while for *Patella candei crenata* no differences were detected, suggesting that *P. aspera* was under a higher harvest pressure during that period. The observed differences in catches between islands in 1999 indicated that overexploitation of limpet stocks at that time was not yet evident.

The results of the field surveys and the manipulative experiment are discussed with respect to the role of ecological experimentation and aquaculture in resource management, and suggestions are made for key issues in future research and conservation.

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Declaration of Authorship

I, Carla Débora Gonçalves Borges declare that the thesis entitled 'Influence of environment and exploitation on sex change in limpets' and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;

- no part of the thesis has previously been submitted for a degree or any other qualification at this University or any other institution;

- where I have consulted the published work of others, this is always clearly attributed;

- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

- I have acknowledged all main sources of help;

- the thesis is based on work done by myself, and jointly with others. Patrick Doncaster and Steve Hawkins advised on issue-conception in Chapter 1 to 6; Moira Maclean contributed to fieldwork and limpet dissections in the south of England in Chapter 2; Tasman Crowe contributed to the experimental design and ANOVA analysis (model 1) in Chapter 3, multivariate analysis in Chapter 4 and issue conception of Chapter 5; Manuel Machado contributed to fieldwork in Chapter 5 and presented some data on his University of Southampton Masters dissertation;

- none of this work has been published before submission.

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Acknowledgements

CHAPTER 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

The phenomenon of sex change, or sequential hermaphroditism, has been a subject of recurrent interest in biology (Ghiselin 1969; Charnov 1982, 1993; Hardy 2002; Espinosa et al. 2009a; Rivera-Ingraham et al. 2011; Fenberg and Roy 2012). In molluscs it has been reported among gastropods and bivalves, being more common in prosobranchs (Heller 1993). Limpets are no exception and it is found in the genera *Patella, Cymbula and Lottia* (Orton 1920, 1928, 1946; Dodd 1956; Orton et al. 1956; Bacci 1952, 1965, 1975; Montalenti 1958; Orton and Southward 1961; Frenkiel 1975; Branch 1974a; Lindberg and Wright 1985; Wright 1989; Creese et al. 1990; Espinosa et al 2009a; Rivera-Ingraham et al. 2011; Fenberg and Roy 2012). Many of these molluscs are consumed by humans and the selective removal of the bigger limpets by harvesters seriously distorts sex ratios, compromising the reproductive output of populations (Branch 1975; Duran and Oliva 1987; Hockey et.al. 1988; Espinosa et al 2009b; Fenberg et al. 2010; Rivera-Ingraham et al. 2011; Fenberg et and Roy 2012). Since limpets are keystone grazers, the reduction of their numbers on the shore modifies its macrobiotic community structure (Jones 1948; Southward 1964; Hawkins 1981a; Hawkins and Hartnoll 1983; Jenkins et al. 2005, 2008; Coleman et al. 2006). Therefore the understanding of sex change mechanisms (genetically *versus* environmental determination and modulation) and their consequences would allow the implementation of better resource management measures. These practices are essential for the conservation of rocky shores around the world.

The overall aim of the work for my thesis was to assess the effects of human exploitation of protandric patellid limpets on their population ecology and upon the

composition of the rocky shore community (non-target species). *Patella vulgata* was used as a model species to measure population parameters and investigate changes in sex change patterns due to exploitation. Work was also undertaken on exploited populations in the Macaronesian islands. This chapter begins by reviewing the *status quo* of the exploitation and conservation of intertidal organisms and communities. I then consider the evolution of hermaphroditism among animals, before discussing the sex change strategies; concluding by focusing on the mechanisms of sex determination and change in molluscs. Detailed objectives of the study are set out in section 1.6.

1.2. EXPLOITATION AND CONSERVATION OF INTERTIDAL ORGANISMS

Since the beginning of civilization, food resources of coastal areas have been exploited by humans (Thompson et al. 2002). The exploitation of coastal resources results from the increasing human occupation of those areas since prehistoric times (McKusic and Warren 1959; Meighan 1959; Waechter 1964; Roubet 1969; Voight 1973; Roche and Texier 1976; Volman 1978; Buchanan et al. 1978, 1984; Schweitzer 1979; Yesner 1984; Buchanan 1988; Cunliffe and Hawkins 1988; Stiner 1994; Yates 1998; Henshilwood 2001; Halketta et al. 2008). Observations have been made on prehistoric middens across the world where reduction in size of marine molluscs and/or a change in species representation through a shell midden sequence were taken as an indicator of intensification in exploitation (see Yesner 1984; Jerardino et al. 1992; Milner et al. 2007). Evidence from stratified middens of Quoygrew (Scotland) showed a trend towards the intensification of marine resource use from 10th to the 13th centuries due to limpet reduction in size through time. Age data demonstrated a lowering of average age, suggesting intensification in gathering rather than environmental influences (Milner et al. 2007). Both vertebrates and invertebrates have been harvested in the intertidal zone for human consumption all around the world (e.g. see Moreno 1984; Lasiak 1991, 1992; Eekhout et al. 1992; Kyle et al. 1997). Limpets are no exception and many of the stocks are now overexploited (e.g. see Raffaelli and Hawkins 1996; Navarro et al 2005; Ramirez et al. 2009; OSPAR Comission 2010). Since these are keystone grazers (Jones 1948;

Southward 1964; Hawkins 1981a; Hawkins and Hartnoll 1983; Jenkins et al. 2005; Coleman et al. 2006) that regulate community structure on rocky shores through their grazing activity, a drastic reduction of their numbers is likely to have wider effects upon the whole community (e.g. see Siegfried et al. 1985; Hockey and Bosman 1986; Moreno et al. 1986; Duran and Oliva 1987).

The advance of the industrial revolution at the beginning of last century increased the pressure on coastal zones (see Thompson et al. 2002 for a review). Since then, besides being prone to increasing exploitation rates, many coastal areas have been destroyed to give space for urban development. As a result of human activities both inland and in the coastal zone itself, marine ecosystems and resources are rapidly deteriorating in many parts of the world (Thompson et al. 2002; Branch et al. 2008). The coastal zone also receives pollution, both by direct discharge and via river systems. It is affected by changes in salinity and sedimentation regimes as a result of the damming of rivers and siltation due to deforestation and other land use changes (see Thompson et al. 2002 for details). Together this anthropogenic disturbance weakens ecosystem resilience and often causes irreparable damage.

Shellfish have been heavily exploited in many parts of both the developed and developing world (Raffaelli and Hawkins 1996). In the third world, subsistence collecting of shellfish has been an important source of protein (see Branch 1975; Siegfried et al. 1985; Duran and Oliva 1987; Hockey et al. 1988; Lasiak 1992; Kyle et al. 1997) but as populations increase, pressure on resources has also increased and many stocks have been at risk, particularly in islands such as the Azores and the Canaries where space is limited (see Núñez et al. 2003; Navarro et al. 2005; Ramirez et al. 2009). Moreover, in many countries shellfish collecting has switched from a subsistence activity to a highly profitable commercial operation purveying luxury gourmet items for the menu (Raffaelli and Hawkins 1996).

Only during the last decades of the 20th century, humans came to be considered as a key component in the structure and the dynamics of rocky intertidal and subtidal communities, with attempts being made to quantify effects of human exploitation upon targeted species and the shore community (e.g. Moreno et al. 1984; Siegfried et al. 1985; Hockey and Bosman 1986; Moreno et al. 1986; Duran and Oliva 1987). Much of the research was prompted by a pronounced decline in many stocks (see Kay et al. 1982; Bustamante and Castilla 1987). Reasons for these declines centred chiefly around changes, both qualitative and quantitative, in the patterns of

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exploitation and were the result of, among others factors, the rapid growth of human populations in coastal areas, the replacement of subsistence by commercial exploitation, and technological advances both in methods of collection, processing, storage and transportation (Eekhout at al. 1992; Santos et al. 1995). Many species of algae (e.g. *Durvillaea antarctica, Iridaea boryana, Porphyra columbia* and *Gelidium* spp.) and invertebrates (e.g. the mussel *Perna perna,* the gastropod welk *Concholepas concholepas,* and limpets of the genera *Fissurella* spp.*, Patella* spp.*, Cymbula* sp. and *Lottia* spp.) have been taken both for local consumption and export (Paine 1994). In addition to direct collection for food, seaweeds are also harvested to make a variety of products such as agar, carrageenans (see Armisen and Galatas 1987; Stanley 1987) and alginates. The latter end up in a variety of cosmetics, pharmaceuticals and processed foods such beer, ice cream, face cream and shampoos (Raffaelli and Hawkins 1996). Shells of many marine invertebrates are also collected for ornamental purposes (Wells 1981; Wood and Wells 1988; Thompson et al. 2002) and many intertidal animals are used as bait in addition to their use as a food resource (Hockey and Bosman 1986; Eekhout et al 1992; Kyle et al. 1997).

1.2.1.   Community consequences of harvesting

   Most effects of exploitation on populations of intertidal invertebrates have been addressed by comparing sites with and without exploitation, before and after human exclusion. One of the most revealing "human exclusion" experiments was initiated in 1982 at Las Cruces (Chile) by J. C. Castilla and his co-workers where access was restricted by a chain link-fence. Duran and Castilla (1989) concluded that their study illustrated a clear case of "cascade effects" (Paine 1980) due to release from press-perturbation (*sensu* Bender et al. 1984). They observed that exclusion of a top predator (humans) in rocky intertidal resulted in the increase of a keystone predator (*C. concholepas)*, and two species of keystone grazers (the limpets *Fissurela crassa* and *Fissurela limbata*). In turn, the increase of *C. concholepas* reduced the cover of the mussel *Perumytilus purpuratus*, a dominant space competitor, favouring settlement of macroalgae in newly available primary space. This state was transient, since macroalgae were subsequently eliminated from the system, most probably due

to the grazing action of limpets. After five years, the community at the non-harvested area was dominated by barnacles.

Mobile grazing herbivores are important target species for human exploitation in many systems (Kingsford et al. 1991 and references therein) and limpets have being harvested commercially, (for both local consumption and export) worldwide. Marine herbivores have often been shown to control the distribution and abundance of their algal food (Jones 1948; Southward 1964; Southward and Southward 1978; Underwood 1979; Branch 1981; Hawkins 1981a, b; Lubchenco and Gaines 1981; Gaines and Lubchenco 1982; Hawkins and Hartnoll 1983; Hawkins et al. 1992; Raffaelli and Hawkins 1996; Jenkins et al. 2005; Coleman et al. 2006). Herbivores in turn can be regulated by predators such that their potential effects on algae are not always realised (Dayton 1975; Breen and Mann 1976; Simenstad et al. 1978; Silva et al. 2008, 2010).

The influence of people on the intertidal community of Transkei in South Africa where traditionally people have supplemented their diet with marine invertebrates was investigated by Hockey and Bosman (1986). Comparisons between exploited and protected sites were made in terms of zonation, density, cover (of algae and colonial invertebrates), invertebrate size distributions, species and community structure. The most intensive intertidal exploitation was upon the limpets *Patella* spp. (now including *Cymbula* spp. and *Scutellastra* spp.) and and *Cellana capensis*, which accounted for 79.1 % of all intertidal species in middens (Hockey and Bosman 1986). The relative abundance of algae and sessile inedible invertebrates was greater and modal and mean sizes of exploited species were smaller, at exploited sites than at protected sites. Indeed, comparative analysis of size-frequency distributions of limpets (*Crassostrea cuccullata, Patella* (now *Scutellastra) granularis* and *Cellana capensis*) from the protected sites revealed a decrease in abundance of bigger sizes due to selective predation (Hockey and Bosman 1986).

Hockey and Bosman (1986) concluded that disturbance through selective predation by humans increases species richness, in line with the "intermediate disturbance hypothesis" (Caswell 1978), and also leads to a convergence of community characteristics, whilst unexploited communities have more divergent characteristics. Nevertheless, maintenance of high diversity through biological disturbance in the form of human predation may be undesirable due to the resulting dominance of inedible species and small individuals of targeted species. Progressive

size reduction leads to intensified collecting pressure on the remaining individuals that, coupled with spatial encroachment of inedible species, inevitably leads to the degradation of the intertidal zone communities as a food resource. Therefore, a management plan was suggested, centred on a rotational "cropping approach" (Hockey and Bosman 1986). Moreno et al. (1984) demonstrated in a study of the impact of human predation on intertidal invertebrates in southern Chile that reductions in size and density of key grazing gastropods, the limpets *Fissurella* spp., leads to an increase of cover of macroalgae; mainly the red algae *I. boryana*, which is exported to Japan.

An holistic approach was adopted by Eekhout et al. (1992) to the exploitation of limpets (*Patella* (now *Cymbula*) *granatina* and *Patella* (now *Scutellastra) argenvillei*). They combined measurement of population parameters in undisturbed and experimentally harvested populations, assessment of the effects of exploitation on the community as a whole and development of quantitative population and economic models, in a way to provide a sound basis for the rational management of living intertidal resources. Empirically derived quotas and minimum size limits for both species were suggested as chief mechanisms for regulating a potentially economically viable and ecologically sustainable fishery. In this study the authors also observed that limpet removal resulted in a decrease in the cover of encrusting coralline algae and an increase in the cover of fleshy macroalgae. Similar results after flood-induced mass mortalities of limpets stocks are documented in Branch et al. (1990). Such an increase in macroalgae cover after natural or experimental removal of grazing herbivores is well documented (Jones 1948; Southward 1964; Hawkins 1981b; Hawkins and Hartnoll 1983 for a review; Raffaelli and Hawkins 1996; Jenkins et al. 2005; Coleman et al. 2006). In addition several lines of research elsewhere have shown that some encrusting coralline species may depend on the activities of grazers to prevent overgrowth and smothering by other organisms (Steneck 1982, 1991).

Advances in studying human effects on intertidal communities included adopting more objective methods (Pombo and Escofet 1996). When studying the effect of exploitation on the limpet *Lottia gigantea,* these authors ranked the intertidal gathering using various criteria: the pattern of visiting by gatherers to the shore, several features of the sites explaining degrees of exploitation (topography, size of settlements or fishing communities, distance from mainland or major human

settlements, habits of consumption of intertidal species by local people), existence of length protective management programmes (Pombo and Escofet 1996). Ecological implications of exploitation were explored through the conceptual model proposed by Catterall and Poiner (1987) for assessing the impact of traditional shell gathering on intertidal molluscs. The model uses life history and habitat information to predict the extent to which a given intertidal shellfish population would be either susceptible to depletion or resilient, according to size at maturity, intertidal burying, adjacent subtidal populations, benthic mobility and pelagic larvae.

Several studies have reported a reduction of the size range of populations, decreased abundance and decreased mean size of several limpet species in harvested versus non-harvested sites, or along gradients of human activity (Branch 1975; Moreno et al. 1984; Oliva and Castilla 1986). Pombo and Escofet (1996) also verified that mean size of specimens in older middens was significantly larger than in a recent midden. Maximum and mean size in artisanal catches of *L. gigantea* also decreased along the exploitation gradient and the difference between mean size in the catches and in the intertidal population became smaller as exploitation increased.

1.2.2. Conservation and management measures

Managing coastal living resources can be a difficult task since most of the catches are not included in official fisheries statistics. Management and understanding the population and community consequences of overexploitation need a broad-based approach (Eekhout et al. 1992). This must encompass studies of the foraging behaviour of people, the density and distribution of collectors, the population biology of target species, including life history characteristics, and the role these species play in the community. Such an approach would facilitate understanding the role of people as top predators in many coastal systems and give insights allowing innovative management measures. These could include closed seasons during which all collecting is banned allowing reproduction to occur combined with rotational or proportional harvesting. Meteorological features such as the occurrence of severe winter storms, at least in the case of lower shore species, might considerably curtail any exploitation activities providing a natural closed season that should not be ignored (see Eekhout et al. 1992).

The existence of a pelagic larval stage and the associated capability for dispersal may not be important in the persistence of populations if the refuges for the species are few or far from the sites of intense harvesting. Relatively isolated islands, like the Azores (Santos et al 1995; Hawkins et al. 2000) and Hawaii (Christopher et al. 2007), seem particular prone to recruitment over-fishing because much of the reproductive output is probably dispersed from the islands (Raffaelli and Hawkins 1996). On the other hand, long-distance recruitment, when present, may mask a declining reproductive fitness of a population if relative abundance alone is used as an index of status (Hockey et al. 1988). Whatever the management programme adopted it must ensure that maturity can be attained before individuals are noticeable or valuable to harvesters and enter the fishery, in order not to compromise population output and continuity. This is particularly important for protandric (sequential hermaphrodites) species, such as limpets of the genera *Patella*, *Cymbula* and *Lottia.* In such species, individuals start as males, switching latter in life to female. Hence, heavy exploitation of those populations leads to fewer females in the population as larger limpets (predominantly females) are selectively removed. Moreover, under certain situations smaller individuals are probably been removed before they have time to change sex. All this seriously distorts sex ratios and reduces the output of eggs by the population, compromising its structure and reproductive output. In these species (more than in others) catch strategies should include a minimum and maximum size in order to guarantee the sustainable use of the resource. However species with proven sex change plasticity would allow catch strategies to be more flexible (see Rivera-Ingraham et al. 2011).

1.2.3. Effects of human exploitation upon gonochoric and sequential hermaphroditic prey - the role of marine protected areas

The main effects of human foraging activities upon target populations have been examined by comparing exploited and non-exploited populations from designated Marine Protected Areas (MPAs) or areas with restricted access. Lasiak (1993) described the temporal and spatial variations in the density, size composition and growth of exploited and non-exploited populations of the intertidal limpet *Cellana capensis* from the Transkei between 1989 and 1991. The timing and intensity of

recruitment varied between shores and amongst sites within shore, and recruitment success was relatively poor, not even being recorded at some of the protected sites until July 1991 (Lasiak 1993). Juvenile and adult limpets were both significantly more abundant at the exploited than at the protected sites, although limpets at the exploited sites tended to be much smaller than those found at the protected sites (Lasiak 1993). Lasiak (1993) suggested that the gradual decline in density of limpets observed at the protected sites reflected poor recruitment as well as senescence of older individuals. The absence of significant differences in growth rate amongst shores suggested that differences in the intensity of competitive interactions may have been offset by the higher microalgal production at the exploited shore and that upshore migration of larger individuals may counter the intense competition experienced at mid-tidal levels on the exploited shore (Lasiak 1993). Differences in the rate of recruitment could also account for some of the observed differences in population characteristics (Lasiak 1993).

Kido and Murray (2003) studied *L. gigantea* population structures, growth rates, and gonadal production at eight southern California sites, four of which were longstanding MPAs. Greater mean sizes and higher frequencies of larger individuals occurred at sites with lowest human visitation (Kido and Murray 2003). Mean shell lengths of *L. gigantea* were negatively correlated with the number of visitors and collectors. The presence of collectors, small mean shell size, and the absence of large limpets indicated that *L. gigantea* populations were affected by humans at the sites studied (Kido and Murray 2003). Kido and Murray (2003) stated that lower densities and higher frequencies of larger limpets were found in a subpopulation living on open-rock surfaces compared with a subpopulation occupying smaller, patchy habitats within mussel beds. Limpets in patchy habitats grew more slowly and produced less gonadal mass than limpets inhabiting open rocks, despite the availability of more microalgal food (Kido and Murray 2003). They concluded that effective MPAs may take decades to reverse the effects of human impacts on *L. gigantea* populations in southern California. This was because of the age of larger limpets and the potential for collectors to remove larger individuals and shift populations towards smaller size structures,

Being aware of the influence of spatial variation in the outcome of reproduction, competition and predation, and likely effects on the viability of populations in MPAs, Lasiak (2006) established a nested sampling design. This was used to assess

differences in density and biomass of seven species of patellid limpets at the scales of shores, sites and plots inside and outside a marine protected area (MPA) on the southeast coast of South Africa. At the scale of plots, significant variation was more common inside than outside the MPA, which was possibly related to differences in the general pattern of space occupancy and quality of habitats available to limpets inside and outside the MPA (Lasiak 2006). Nevertheless, significant variation at the scale of sites was rare; this suggested that either the processes contributing to variability at this scale counteract each other or that the sites were similar in terms of habitats and ecological processes (Lasiak 2006). At the scale of shores, significant variation was more common in densities than in biomass, but both occurred with equal frequency inside and outside the MPA, which was probably driven by a combination of recruitment and/or mortality (Lasiak 2006).

Branch and Odendaal (2003) compared populations of the protandric limpet, *Cymbula oculus*, between two South African Marine Protected Areas, Dwesa and Tsitsikamma, and four exploited sites, and between sites exposed to or sheltered from strong wave action. *C. oculus* is particularly vulnerable to harvesting (Lasiak 1991; Branch and Odendaal 2003). It has no subtidal refuge, and because it is a broadcast spawner, there is the possibility of Allee effects, such as depression of fertilisation success as population density drops (Quinn et al. 1993). In particular, it is a protandric hermaphrodite (Branch 1974a), like several other limpets of the genera *Patella* and *Lottia* (Bacci 1947; Orton et al. 1956; Wright and Lindberg 1982; Creese et al. 1990). In common with fish that change sex (e.g. Buxton 1993; Harmelin et al. 1995), this carries the risk that one sex will be selectively harvested because it is larger, thus distorting sex ratios (Branch and Odendaal 2003). It has a dispersive larval stage and questions therefore exist about its capacity to export larvae to adjacent areas (Branch and Odendaal 2003). Limpets are strongly influenced by intensity of wave action, some thriving and others suffering from high wave action (e.g. Denny et al. 1985; Bustamante et al. 1995; in Branch and Odendaal 2003). *C. oculus* is known to be relatively mobile and to have low tenacity, both features suggesting it will be more successful in areas with relatively low wave action (Branch and Marsh 1978 in Branch and Odendaal 2003). Compared with harvested sites, limpets in MPAs were 30–50% larger, adult densities 75% greater and biomass 30–90% greater (Branch and Odendaal (2003). Growth rate and age-at-maturity were unaffected by harvesting, nevertheless inside MPAs survivorship was

10-fold higher; reproductive output a staggering 80-fold higher; and the female: male ratio was 0.58:1, but 0.11:1 at harvested sites (Branch and Odendaal 2003). Conversely, recruitment was three times higher in harvested than protected areas, and inversely correlated with adult density (Branch and Odendaal 2003). Limpets at sheltered sites were 65% larger, biomass 80% greater, female proportions 40% higher, survivorship 25% greater and growth 33% greater, leading Branch and Odendaal (2003) to conclude that wave action had negative effects of similar magnitude to harvesting. Hence, it was not surprising that recruitment was 45% greater at wave-sheltered sites. The authors stated that outside MPAs harvesting was decimating *C. oculus* populations and that the effects reported were detected only inside the MPAs, being masked by harvesting elsewhere. Branch and Odendaal (2003) concluded that the impacts of harvesting and wave action could never have been detected without the existence of MPAs, emphasizing their importance for base-line studies as well as for protection tools used for coastal management.

Biogeographic patterns could be used to site marine reserves with a middle/edge arrangement of 'biodiversity reserves', linked to biogeographical regions (Hockey and Branch 1994). Such reserves would achieve conservation of both representativeness and high diversity areas that could be complemented by a second tier of reserves with the specific purpose of improving local yields of exploited species (Hockey and Branch 1994). Whatever the methodology used to assign MPAs for conservation of limpet stocks, it must ensure that the spatial structure and hydrodynamics are favourable for the species present. Therefore different characteristics of MPAs will apply to protect species with different biological traits and life-histories.

1.3. THE EVOLUTION OF HERMAPHRODITISM AMONG ANIMALS

The necessity to reproduce at all costs should favour the development of selfing wherever and whenever the environment is such that the transfer of gametes between individuals is hindered. It appears that selfing has originated convergently in adaptation to special conditions, for it is often facultative. Even though selfing is considered quite detrimental on the whole, it is more advantageous to self-fertilize than not to reproduce at all (Darwin 1876). Hermaphroditism might predispose an

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organism to selfing, but this does not tell us why the sexes became united in the first place.

Ghiselin (1969) proposed three models to explain the origin of different types of hermaphroditism in animals:

(1)The low density model invokes the advantage of being able to mate with any other member of the species where the probability of encountering a suitable mate is low (such as low mobility caused by sessility, or low population density). It predicts the frequent occurrence of hermaphroditism and other sexual arrangements (as self-fertilization and close association between members of the two sexes) among sessile organisms, deep-sea animals and parasites. Besides those, other adaptations could evolve in response to the same selective influence like parthenogenesis (vegetative propagation) and apomixis (asexual reproduction, usually applied to plants without meiosis) (Ghiselin 1969). This model does not always suffice to account for many instances of hermaphroditism and is not workable for sequential hermaphroditism, such as protandry or protogyny.

(2)The size advantage model is the only one that applies to animals exhibiting sequential hermaphroditism in populations not subjected to chronic or periodic low densities. It explains sequential hermaphroditism as occurring where an individual reproduces most efficiently as a member of one sex when small or young, but as a member of other sex when it gets older or larger (Ghiselin 1969). Indeed, when the reproductive functions of one sex are better discharged by a small animal, or those of the other sex by a larger one, if as it grows it assumes the sex advantageous to its current size then its reproductive potential increases. Eggs are more expensive to produce than sperm and the larger the female, the greater the number of eggs that could be produced. In benthic marine invertebrates with a free-swimming larval stage, an individual that happened to reach, without much delay, a suitable spot for post-larval development, could grow rapidly and be ready to function as a female, with greater reproductive potential than later, smaller arrivals. An individual delayed in finding a suitable locality could still reproduce when small, but could do so better if male than female (Ghiselin 1969). This model predicts protandry where young stages must hunt for a suitable environment and protogyny when there is a sexual selection for largest males or when males care for the young (Ghiselin 1969).

(3)The gene dispersal model is based upon the idea that individuals may be adapted to the genetic environment (the population genetic structure), as affected by

the prevailing conditions of gene flow. In the inbreeding version it is hypothesised that where mobility is restricted, sibling crosses become more frequent. Protandry and protogyny would help to prevent inbreeding by self-fertilization, although a similar phenomenon as merogony (in which an individual has offspring all of the same sex) would have the same effect. The sampling-error version of this model is based upon the fact that genetic environment is also influenced by genetic drift and analogous phenomena, such as numerical excess of one sex, in small populations (Wright 1938). Hermaphroditism is conceived as an effective way of minimize those deleterious consequences. As both inbreeding and sampling error have as consequence the reduction on genetic variability, hermaphroditism would compensate for both of these effects of limited gene dispersal (Ghiselin 1969).

As basis to his comparative study, when testing the proposed models, Ghiselin (1969) used examples available on the literature. The possible pathways for hermaphroditism were prompted based in the advantages that would accrue to the individual rather than to the population. Conditions were reviewed in which there had been a shift in habitat or way of life (as elimination of larval stage in some gastropods) possibly correlated with the evolution of the hermaphroditic stage (Ghiselin 1969). A version of Bock's principle of multiple pathways of adaptation (Bock 1959) was invoked, as it states that when a group of organisms adapts to a particular selective influence, it may do so by adopting divergent mechanisms which deal with the same functional problem. Thus, for example, where the low density model should be expected to apply, as in parasites and sessile organisms, simultaneous hermaphroditism and phenomena of the dwarf-male type should be seen, although other factors (e.g. inbreeding and merogony) might interfere to prevent the evolution of either or both.

With his systematic review Ghiselin (1969) found evidence for his proposed models in several taxa. The sexual biology and way of life of the Ctenophora were strictly consistent with the low density model since all are hermaphrodites either simultaneous or protandric and able to store sperm (which has much the same functional effect). As they are pelagic or benthic with little mobility as adults, but invariably have some mobile period in their life cycle no appliance was found of the gene dispersal model (Ghiselin 1969). The low density model was also applied to the Trematoda, which are simultaneous hermaphrodites, except for two families with males and females living in close association (Ghiselin 1969). Protandry observed in

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Polychaeta was explained by the gene dispersal model, while in the Gasteropoda, where it is quite frequent among various unrelated groups of intertidal limpets, it was suggested to occur in line with the size advantage model (Coe 1938a, 1953; Bacci 1955, 1965; Hoagland 1975; Wright and Lindberg 1982; Wright 1989; Collin 1995; Warner at al. 1996; Hobday and Riser 1998).

1.3.1. The adaptive significance of sequential hermaphroditism in animals

Before Ghiselin's (1969) size-advantage model was proposed, virtually all explanations of the role of sequential hermaphroditism in the life of a species suggested benefits that might accrue to the population rather than to the individual (see Nikolski 1963; Atz 1964; Noe 1969). Nevertheless, the high overall zygote production which may occur under conditions of hermaphroditism should be viewed as an advantage rather than a positive adaptation (Warner 1975). So, explaining the evolution of hermaphroditism must be done on the basis of the advantage it confers to the individual. The adaptive value of traits should not be viewed as being "good for the population", but "for good of the individual", or more precisely "for the good of the gene" (Krebs and Davies 1993).

With the advent of Ghiselin's size advantage model as explaining sequential hermaphroditism, different authors attempted to explain its evolution by other mechanisms. Warner (1975) investigated how spawning behaviour, age structure and the schedule of female fecundity interact to produce selection for sequential hermaphroditism. Examples taken from the literature were used to model the theoretically optimum schedules for sex change that included shrimps and teleost fishes, and results were applied also to other animal groups (Warner 1975). It was proposed that selective pressure for sequential hermaphroditism could exist if spending part of the mature life span as a male and part as a female yields a higher life time expected reproductive potential than that for non-hermaphrodites. Warner (1975) suggested that protandry may be selected for in populations when individuals mate randomly, as long as female fecundity increases with age for at least the first few years of the mature life span. The pressure would be stronger as faster as fecundity increases with age, being in concordance with Ghiselin's size advantage

model. Selection for protogyny was suggested when inexperience, male dominance, mate selection or territoriality leads to a differential in male expected fecundities in succeeding ages, or when female fecundity decreases with age. Mate selection was pointed out as the strongest pressure for protogyny under conditions of low mortality and constant or decreasing age-specific female fecundities. In most of the studied cases biased sex ratios were found. Such deviations from the expected 1:1 ratio (Fisher 1930; Leigh 1970) are due to the fact that selection in sequentially hermaphroditic populations does not equalize parental expenditure on the offspring of the two sexes, nor does it ensure equal expected lifetime reproductive potentials for males and females (Warner 1975). Rather than overall sex ratios, the important parameter in sequentially hermaphroditic populations is the size- or age- specific sex ratio pattern (Wenner 1972; Warner 1975). Warner (1975) suggested that in populations where there is some mating success of young males, male fecundity at early ages may still be high enough to minimize selection pressure for changing sex (for some examples see Gadgil 1972; Warner et al. 1975; Warner and Hoffman 1980). In some species of fish (e.g. *Thalassoma bifasciatum* and *Sparisoma rubripinne*) there is male dimorphism: some members of a population are primary males with one type of mating system, while others are protogynous males, with different behaviour (Warner 1975; Warner et al. 1975).

Since animals react more to size than to age of their conspecifics in mating interactions, growth rate of the individual was considered an important parameter to consider in sequential hermaphroditism (Warner 1975). This idea was developed by Iwasa (1991) in one of his proposed alternative models for evolution of sex change. Using dynamic programming, he showed that the evolutionary stable strategy under an enhanced mortality or reduced growth rate of reproductively active individuals may include an extended non-reproductive period intervening between male and female activities. According to Iwasa (1991) evidence for sex changers who have an extended non-reproductive period may be implicit as the "early sex changer" reported for several coral fishes (see Moyer and Zaiser 1984; Hoffman et al. 1985; Aldenhoven 1986). Hoffman et al. (1985), for example, stated that non-reproductive individuals enjoy the benefit of faster growth by spending more time per day in feeding than reproductively active individuals. When analysing the cost of reproduction either mortality or growth rate differed between sexes. Natural selection may favour a sexual strategy in which each individual takes the less costly sex (with

a lower mortality or higher growth rate) first, and then turns to the more costly sex, even with the size advantage of fertility equal between sexes. Although the two alternative mechanisms to Ghiselin's size advantage model (the mortality-advantage model and the growth-rate-advantage model) had been previously proposed as favouring sex change evolution (e.g. Charnov 1982), Iwasa's innovation was in confirming them by modelling it in protogynous populations.

1.4. SEX DETERMINATION AND SEX CHANGE IN ANIMALS

Sex change, or sequential hermaphroditism, is phylogenetically widespread but uncommon in both the plant and animal kingdoms (reviewed in Policansky 1982). There is evidence of sex change in most groups of invertebrates (see Ghiselin 1987) indicating independent lines of evolution. In molluscs it has been reported among gastropods and bivalves being more common among prosobranchs (Heller 1993). In molluscs, despite its theoretical advantages over gonochorism, it is not common (Heller 1993) and when it occurs is almost exclusively protandric (except in the bivalve superfamily Galeomatacea where some cases of protogyny are mentioned, see Oldfield 1961; Fretter and Graham 1964). Alternating sexuality was early reported in many oyster species, where individuals change sex back and forth several times (Coe 1943; Sastry 1979; Mackie 1984).

Nevertheless the animal kingdom has examples of a diversity of sex-changing strategies to increase reproductive success: some individuals change sex early, others change sex late, some individuals change sex more than once, and others do not change sex at all (Munday et al. 2006). Understanding the scale at which individuals gather information on reproductive value is crucial to the understanding of the selective advantage of sex change, since variation in the timing of sex change among species, populations and individuals can often be traced back to differences in the structure of the local mating group (Munday et al. 2006).

1.4.1. Sex change strategies in animals

In this section I present the different sex change strategies in animals focusing later on the most revealing work on sequential hermaphroditism in molluscs that will be reviewed with emphasis on the gastropod families of the Calyptraeidae, Patellidae and Lottidae. The final discussion will provide a synthesis of the proximal (ecological) and ultimate (evolutionary) questions raised by such studies.

The size advantage model predicts that sex change will occur earlier in populations with slower growth rates and/or higher mortality rates (Warner 1988; Munday et al. 2006). This pattern has been documented in a range of protogynous fishes (Cowen 1990; Buxton 1993; Gust 2004) and protandric shrimp and fish species (Taylor et al. 2000; Charnov and Hannah 2002). The mean age at sex change and demographic rates co-vary in genetically connected populations of fishes (Gust et al. 2002; Gust 2004) and shrimp (Charnov and Hannah 2002), indicating that the timing of sex change in these populations is a flexible response by individuals to local conditions. This conclusion is supported by the relatively rapid changes (within a few years and less than one generation) in the age or size at sex change in some fish populations following a change in the mortality rate (Hawkins and Roberts 2003; Munday et al. 2006). Nevertheless, evidence has been found to suggest that other species have a relatively fixed size at sex change. In at least one protandric shrimp (Bergström 1997), one protandric limpet (Branch and Odendaal 2003) and some large protogynous fishes (Petersen and Warner 2002) there was no variation in size at sex change despite considerable short-term variation in age structure and mortality rates within and between populations (Munday et al. 2006).

In haremic species, the largest female in a group usually changes sex following the disappearance of the dominant male (Warner 1984, 1988; Ross 1990; Kuwamura and Nakashima 1998), because she can increase her reproductive value by spawning with all the remaining females (Munday et al. 2006). Some females even change harems to advance their position in the size hierarchy, and in doing so increase their reproductive value because they end up monopolizing a harem sooner (Sakai et al. 200; Munday et al. 2006). Similarly, in species that exhibit resource defence polygyny, a large female will usually change sex following the disappearance of a territorial male (Warner and Swearer 1991 in Munday et al. 2006). Territorial males

can spawn with 50 females per day (Warner and Hoffman 1980), so the advantage to changing sex when a dominant position becomes available can be significant (Munday et al. 2006). Nevertheless, the largest female does not always change sex following the disappearance of a dominant male; this appears non-adaptive, because the largest female could spawn with all the remaining females if she did change sex. A modification of the size advantage model (Muñoz and Warner 2003) demonstrated that the largest female in a group would not increase her reproductive value by changing sex if the combined fecundity of the other females in the group is less than her current fecundity, and/or if sperm competition was intense (Munday et al. 2006). Under these circumstances, the larger individual would do better to remain female and one of the smaller females might gain the most by changing sex (Munday et al 2006). These predictions were revealed in a manipulative experiment with the bucktooth parrotfish *Sparisoma radians*, where the largest female usually declined to change sex following the removal of the dominant male, and it was one of the smaller females that became male (Muñoz and Warner 2003; Munday et al. 2006).

Some female fish sometimes change sex at a size where they appear to have little chance of breeding as a male (early sex change) and thus experience no immediate gain in reproductive success (Moyer and Zaiser 1984; Hoffman et al. 1985; Munday et al. 2006). Early sex change might be a viable alternative strategy if non-reproductive individuals have decreased mortality rates or increased growth rates (Iwasa 1991), so that a small decrease in current reproductive success results in a much larger gain in reproductive value (Munday et al. 2006). In fact, early sex change in the spotlight parrotfish *Sparisoma viride* appears to be favoured because non-reproductive males grow faster than do territorial males (Van Rooij et al. 1995) and do not appear to suffer markedly higher mortality rates (Van Rooij and Videler 1997).

There is an increasingly long list of fish species that are known to have multiple sex reversals (see Kuwamura and Nakashima 1998; Sakai et al. 2003; Liu and Sadovy 2004; Wittenrich and Munday 2005) and many of the ecological conditions that favour repetitive sex change are known (Nakashima et al. 1995; Munday 2002). The polychaete worm *Ophryotrocha puerilis* forms breeding pairs: in each pair, the largest individual functions as a female, enabling individuals to take advantage of the female size–fecundity relationship (Munday et al. 2006). Males grow quicker than females and reciprocal sex change occurs when the male becomes larger than the

female (Munday et al. 2006). In coral gobies, when one of the breeding pair dies (or the host coral dies) the survivor might move to find a new partner (Munday et al. 2006). Searching for a new mate is, however, risky and when new partnerships form, one individual will change sex if the new partners are the same sex (Nakashima et al. 1995; Munday 2002; Munday et al. 2006). Thus the advantage of bidirectional sex change is that an individual maximizes its reproductive value by searching as little as possible for a new mate (Nakashima et al. 1995; Munday 2002; Munday et al. 2006).

These different strategies can be unified by the principle that individuals change sex when it increases their reproductive value (Munday et al. 2006). The breeding tactics (male, female or non-breeder) adopted by individuals often appear to be adaptive responses to their own social-ecological context and variation in these conditions results in significant differences in the timing of sex change within and between species (Munday et al. 2006).

1.4.2. Sex change and determination in molluscs

The sex change phenomenon has received particular attention in prosobranch gastropods of the genus *Crepidula*, being explained by the size (age) advantage hypothesis (e.g. Ghiselin 1969; Charnov 1982; Warner 1988). An alternative hypothesis stated that sex change is independent of size and acts to correct locally skewed sex ratios (the sampling error hypothesis in Ghiselin 1969). The former is well accepted to explain protandry in limpets (Ghiselin 1969, 1987; Warner 1975, 1978, 1988; Hoagland 1978; Wright 1988; Collin 1995). Charnov's (1982) review of sex allocation theory presented quantitative models of the optimal size at sex change based on the size-advantage hypothesis. The size at sex change maximizing an individual's life time reproductive output is the size at which the number of offspring an individual could produce, by acting as the second sex, exceeds the one produced by acting as the first sex (Charnov 1982). This is true in many molluscs where female fecundity is related to large body size, but male fecundity is related to mobility and therefore often with small size (Hoagland 1978). Thus, to maximize its lifetime reproductive success, an individual should begin life as the sex with the least increase of reproductive output with size and subsequently change to the other sex. Genetic and environmental determination on the timing for this sex shift was

outlined by several authors (e.g. Orton 1927; Coe 1935, 1953; Bacci 1951, 1955; Ghiselin 1969; Hoagland 1978; Collin 1995; Warner et al. 1996; Hobday and Riser 1998).

Environmentally-mediated sex determination (or labile sex determination) in the Calyptraeidae was demonstrated early last century. Many authors (e.g. Gould 1917a,b; Ishiki 1936; Coe 1944) reported members of the genus *Crepidula* influenced physiologically and behaviourally by conspecifics. In *Crepidula fornicata* and *Crepidula plana*, the protandric males are not only attracted to females but attain the male phase earlier and remain as males longer when in contact with females (Coe 1953). Earlier studies correlated sex change via environmental determination with the mode of larval development and dispersal, and with substratum constraints (Hoagland 1978). Species with planktonic larval development are rarely substratum-limited, exhibit labile sex determination and have socially influenced sex ratios. Their gregarious behaviour and female-induced delay of sex change appear to be mediated by pheromones, while species lacking planktonic larvae also lack gregarious behaviour and sex change and sex ratio are not influenced by other members of the species (Hoagland 1978). Hoagland (1978) hypothesised that complementing the different reproductive and dispersal mechanisms, this genus might have evolved distinct mechanisms of sex change and sexual behaviour. Several experiments were set up in the laboratory and in the field to quantify species-level differences in sexual behaviour such as gregariousness, degree of mobility of young males and permanence of mating pairs. Size and timing of sex change were addressed by comparing isolated and crowded conditions responses. Hoagland (1978) classified *C. fornicata*, *Crepidula onyx* and *C. plana* as having environmentally determined sex change (which she correlated among other features with planktonic larval development) while *Crepidula convexa* was included in the non-environmental sex determination group.

Although protandry in slipper-limpets of the genus *Crepidula* is a common textbook example of sex change (Barnes et al. 1988; Kozloff 1990; Ruppert and Barnes 1994) it had not been studied within the framework of the sex allocation theory (Charnov 1982) until Collin's (1995) approach.

The work was performed in *C. fornicata* which is the ideal species to investigate sex change (Collin 1995). These sedentary animals form semi-permanent stacks in which smaller younger males attach to the shells of larger females. Only the bottom-

most male changes sex (Collin 1995), which results in a number of males on top of one to several females. As copulation occurs within a stack those can be viewed as independent mating groups among which the sex ratio, number and size of individuals vary (Collin 1995). Sex change is strongly influenced by an individual association with conspecifics (Hoagland 1978). Indeed, when females are removed from stacks, single males change sex immediately, while in stacks only the bottom-most male of a group changes (Coe 1938a). Females also affect sexual differentiation of juveniles: in the absence of females, newly settled juveniles sometimes differentiate directly into females (bypassing the male phase), while in the presence of females they always differentiate as males before becoming female (see Hoagland 1978). Individuals bypassing the functional male phase, had been explained by environmental influence (Hoagland 1978), rather than genetic determinism. Coe (1935; 1938 a,b) on the contrary claimed that some individuals of *Crepidula* species are "true males" incapable of change sex.

Based upon this knowledge Collin (1995) developed and tested three models of the optimal size at sex change for *C. fornicata* that combined among-stack variation and the relationship between size and reproductive output. The models differed in how male reproductive output was related to male size and number.

All three models predicted that sex change should occur at smaller sizes than observed in the field. Delaying sex change is expected if a loss of immediate reproductive output, replaced for greater reproductive output in life, increases lifetime fitness (Charnov 1982; Iwasa 1991). Such delays might increase the fitness of an individual animal because sacrificing immediate reproductive output might allow it to grow faster and reach a larger size before becoming female (Collin 1995). Those results suggested advantages of remaining male resulting from factors not considered in the models such as differences between male and female growth (Collin 1995). Differences in growth rates can result in selection for sex change even in the absence of sex-specifc relationships between size and reproductive output (Iwasa 1991). Quantitative models with simplistic assumptions about growth, mortality, and reproductive output may not adequately reflect the tradeoffs involved in sex change (Collin 1995). Hence, efforts must be made to include relevant data (as the accurate relationship between male size and reproductive output, potential costs of sex change and seasonality of reproduction) with as much precision as possible. In sedentary animals with environmentally mediated sex determination, the optimal size

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at sex change is probably determined by local conditions in patches. Modelling it should focus on stimuli from that immediate environment rather than from factors that reflect distant or averaged conditions (Collin 1995).

That the advantages of being a male or female should depend on the size of the individual relative to others in the local mating group is in line with the size-advantage model (Ghiselin 1969; Wright 1988). For example, it may profit even a small individual to function as a female if it is the largest in the group; conversely, a large individual could benefit by staying a male if there are yet larger females available to inseminate. This idea was investigated by Warner et al. (1996) in a combination of field collections and laboratory experiments which revealed that sex change in the non-stacking *Crepidula norrisiarum* is strongly affected by the size of the individual and the composition of the local group.

Since the largest solitary male found was about 16 mm in length they assumed that individuals not influenced by social conditions tended to change sex when that length was reached. Large males were used to determine the circumstances under which social conditions delayed sex change. Each individual of the both types (large-delay and small-not delay) was placed into the following treatments: 1-alone, 2-with relatively smaller males and no larger individuals, 3-with a larger female and no other individual, 4-with five smaller empty limpet shells glued to the host shell to test whether the presence of live individuals was necessary to induce sex change.

In accordance with the predictions, males changed sex when they became the largest member of a group regardless of their initial size. Individuals changed sex faster in the presence of smaller males than when alone, which was proposed as a response to the potential for competition for dominance status among the larger individuals in the local population. If being a large female conveys high fitness, and if females can suppress sex change of smaller individuals, then a rapid conversion to female may be favoured (Warner et al. 1996). Warner et al. (1996) also reported sex-change in individuals who had significantly higher growth rates (see Iwasa 1991). Comparisons among groups (non-sex-changing males in groups vs non-sex-changing solitary males and sex-changed solitary males vs sex-changed males in groups of smaller individuals) suggested that growth differences are conditional and not simply due to the presence of feeding competition from larger individuals. In *C. norrisiarum* rapid growth was almost invariably associated with sex change (Warner et al. 1996).

An alternative to increasing fitness via sex change in *C. norrisiarum* was explored by Hobday and Riser (1998). They proposed the increase in fitness due to individuals changing its social situation. Being aware that *C. norrisiarum* can change its social condition through movement (e.g. Warner et al. 1996), experiments were made to assess the ability of this species to move as a function of size and sex. *C. norrisiarum* of all sizes and both sexes were observed to have some ability to reattach to a host if separated, but only males move on and between hosts. Most of those movements resulted in an increase in reproductive potential (Hobday and Riser 1998). Immediate benefits from moving occur when males find an available female in the proximity. Indeed moving males were implicated in several successful fertilizations in *C. fornicata* (Gaffney and McGee 1992).

One potential problem is using the size and sex of solitary individuals to determine the size at sex change in the absence of social influences (see Warner et al. 1996 in Hobday and Riser 1998). As solitary males may have recently moved from conspecifics or had companion males leave, much of the variance in the size at sex change may be attributable to the influence of "ghost of *Crepidula* past" (Hobday and Riser 1998). This was pointed out as an outstanding feature to include in models of sex change which consider the size at sex change in varying social conditions, often assumed to be static (e.g. Collin 1995). The use of "social-free size at sex change" as obtained from field data (see Warner et al. 1996) should be avoided and the rearing of single individuals in guaranteed isolation a priority (Hobday and Riser 1998).

Collin (2006) used data from 27 populations of 19 species of calyptraeid gastropods to evaluate three predictions of sex change theory and compare the patterns observed in this monophyletic family to those of a compilation of animals from the literature (Allsop and West 2003a, 2004). The tested predictions were (Collin 2006):

- Sex ratios are biased toward the first sex;
- Size at sex change/maximum size is less variable than expected at random; and
- Intraspecific variation in size at sex change is associated with social environment, such that species with more variation in the social environments to which different individuals are exposed show more variation in size at sex

change than species in which all individuals are exposed to similar social environments.

The most common assumption in sex change studies made in the field is that the individuals sampled represent a single population and that there is a single optimal size at sex change across that population (Collin 2006). However, sex changing species often have populations with structured mating groups: labrid fishes often form harems, anemone fish live in breeding pairs, and calyptraeid gastropods often form clusters or stacks (Collin 2006). A large sample of individuals, as would be collected to assess population sex ratio, probably represents a pool of several groups. In species with labile sex change, where animals change sex in response to cues from conspecifics, a population sample that includes several groups would be expected to show considerable within-population variation in size at sex change which is common among calyptraeid gastropods (Collin 1995, 2000, 2006).

As in previous research (see Charnov 1993; Charnov and Skúladóttir 2000; Allsop and West 2003a,b) the size at which 50% of the animals were female was considered to be the size at sex change (L_{50}) and maximum size was the size of the largest individual in the samples collected (L_{max}). In Chapter 3 this methodology is applied to the investigation of the sex phenomenon in *P. vulgata* and to my knowledge, no previous study has used the L_{50} (Collin 2006) approach in other patellids.

In order to determine if intraspecific variation in size at sex change is associated with intraspecific variation in social environment, Collin (2006) used a correlative approach. The standardized size overlap between males and females was used as a proxy for variation in size at sex change and was calculated for each species as the difference in size between the largest male and smallest female divided by the average size. Variation in social environment was assessed by four different measures (stack size, average number of snails in a stack, the range in stack size, and the standard deviation in stack size) because it is unknown how calyptraeids assess their social situation (Collin 2006).

The prediction that sex ratio should be biased toward the first sex is based on minimal assumptions and is not altered by size-specific mortality or growth rates (Charnov and Bull 1989). The main underlying assumption is that both male and female fertility increases with size (Charnov and Bull 1989). Data on egg production for several species of calyptreaids make it clear that female fertility increases with size (e.g., Collin 1995, 2000; Chaparro et al. 1999; Chaparro and Flores 2002). A

male-biased sex ratio, consistent with the idea that both sexes have increasing fertility with size, was observed for several calyptraeids. Nevertheless, female-biased sex ratios observed for other species imply that male fertility at the size of sex change is less than average male fitness suggesting that male fertility decreases with size in these species (Collin 2006). Such a decrease could be explained by the difference in mobility between large and small males that has been suggested for some species (Warner et al. 1996). Whatever the mechanism, the shape of the relationship between male fertility and size probably differs between species, increasing in some and decreasing in others (Collin 2006). The predictions of first-sex-biased sex ratios could also be altered by high mortality during the transition phase. This is unlikely to explain the lack of fit for calyptraeids, as laboratory studies have not found different mortality rates in transitional animals (Collin et al. 2005).

The considerable, unpredicted variation in sex ratio and L_{50}/L_{max} in samples from a single taxon suggested that calyptraeids violate the basic assumptions of sex allocation theory (Collin 2006). The theoretical derivation of L_{50}/L_{max} as an invariant relies on the assumptions that k/M, $\eth$, and $\propto M$ (where k is the von Bertalanffy coefficient, M is adult mortality rate, $\propto$ is age at first breeding, and $\eth$ is a coefficient relating male fertility to size) are constant across the species examined. The large variation in L_{50}/L_{max} among calyptraeids suggested that at least one of these three values is not constant across species. The significant differences in L_{50}/L_{max} between different populations of the same species of calyptraeids suggested that these values may not even be constant across different populations of the same species (Collin 2006). These findings are in line with other research that questioned the use of regression slopes alone to test life-history invariants (Buston et al. 2004; Cipriani and Collin 2005; De Jong 2005; Nee et al. 2005; Linde and Palmer 2008; Molloy et al. 2010). Comparing log-log plots of L_{50} and L_{max} of harvested and protected populations is not sufficient to establish whether relative size at sex change can be invariant in response to harvesting pressure (Fenberg and Roy 2012). Due to the underlining controversy, Fenberg and Roy (2012) suggested that the sex change invariance hypothesis within a species should examine the co-variation between L_{50}/L_{max} and other biological traits such as mean body size and age across populations.

P. vulgata has been the protandric species most widely studied in Europe, but some data exist for sex change in *Patella caerulea* (Montalenti 1958; Bacci 1965)

Patella ferruginea (Frenkiel 1975; Espinosa et al. 2009a; Rivera-Ingraham et al. 2011) in the Mediterranean; *C. oculus* (previously *Patella oculus,* Branch 1974a) in South Africa; and *Patella rustica* (Bacci 1975) in Italy.

In most of the cases, knowledge of sex change patterns comes mainly from the interpretation of sex-age/size data from field collections. Indeed, the preponderance of males in smaller size classes and females in the larger ones suggests that other species may also be protandric hermaphrodites. However, it does not conclusively prove the existence of protandry. Change of sex ratio with age due to females having faster growth, and/or earlier maturation of males and/or higher mortality rate may produce then same effect (Thompson 1979; Branch 1981). In this sense *Patella ulyssiponensis* has the appearance of a protandric hermaphrodite but the conclusive proof of sex change requires oogenis to be observed in animals known to be or to have been male (Thompson 1979). Individuals that appeared to be functionally male and female simultaneously, have been recorded in a number of species including all three British *Patella* spp. (Dodd 1956), and in *Patella candei gomensii* (Cunha et al. 2007) but were considered to be unrelated to a change of sex (Branch 1981; Cunha et al. 2007). In *P. caerulea* the change from male to female gonad occurs during the resting period (Bacci 1947; Pellegrini 1948). In fact one major difference in the breeding cycle of *Patella depressa* compared with the protandric *P. vulgata* is the absence of a resting period (Orton and Southward 1961). Whether its absence obstructs *P. depressa* from being a sex changer is still unknown. Few reports are known in this genus of sex change phenomenon being addressed using the experimental approach of contemporary intertidal ecology. This might have to do with the difficulty of following sex change through an individual's life. Unlike in the genus *Crepidula* there are no visible external sexual characters and removing an individual from the substratum has often great influence on its survival. Determining the sex in these limpets usually implies killing the animals. To overcome this, Wright and Lindberg (1979) presented a non-fatal method of sex determination for patellacean gastropods that has been adopted by several researchers. It requires the extraction of a gonad sample by the use of a syringe with a hypodermic needle. The method was developed in *L. gigantea* and the critical aspects of the technique are the possible damages in other organs and the removal of limpets from the rock which would affect their survival. The application of this method in the *Patella* spp. would allow the design of experiments testing specific possible cues of sex change (Le

Quesne and Hawkins 2006). However, before its utilization, previous studies of its influence upon the biology of the species should be done in order to minimize possible impacts. Chapter 3 presents an innovative way of assessing protandry in *P. vulgata*. The sex change was investigated at the end of the experiment comparing several parameters of *P. vulgata* sexual biology.

Le Quesne and Hawkins (2006) reported direct observations of protandric sex change in *P. vulgata* following the method of Wright and Lindberg (1979). In October 2003, 200 limpets covering the size-range over which sex change was thought to occur (15-25mm) were measured to the nearest mm and marked with small plastic numbered discs attached with epoxy resin, and their sex determined by taking a biopsy of the gonad with a hypodermic needle inserted into the gonad through the posterior mantel (Le Quesne and Hawkins 2006). In the following year only 37 of the initially 200 marked limpets were found. The low number of collected limpets could be the result of increased mortality due to the biopsy procedure, tag loss and problems with limpets re-attaching, in addition to natural mortality (Le Quesne and Hawkins 2006). From the 37 limpets found, nine of the 31 limpets (29%) that had initially been male were female at the time of the second observation, and one of the eight limpets that had initially been female was male at the time of the second observation. The remaining 22 male and seven female limpets were the same sex at both observations. Le Quesne and Hawkins (2006) concluded that *P. vulgata* can undergo a male to female sex change and can be considered a protandric hermaphrodite and that the single observation of a female becoming male could be the first observation of two-way sex change in the superfamily Patellidae.

The occurrence of sex change in a giant endemic limpet, *Patella* (now *Scutellastra*) *kermadecensis*, from the Kermadec Islands in New Zealand was reported by Creese et al. (1990) who collected individuals three times each year during 1984 and 1985 at Raoul Island in the Kermadecs group. Small limpets were predominantly male, while amongst larger ones about half were female (Creese et al. 1990). The presence of hermaphroditism in *P. kermadecensis* was confirmed by microscopic examination of gonad material from over 50 limpets, which revealed a high percentage of individuals with both male and female gametes in their gonads (Creese et al. 1990). Branch (1981) acknowledged two forms of hermaphrodites in *C. oculus:* transitional and mosaic. The first type had morphologically uniform gonads which were predominantly male, but which had small oocytes scattered throughout

and arising from the germinal epithelium. Eventually, all sperm were either shed or reabsorbed, and the limpet became entirely female (Creese et al. 1990). This situation has been reported for *C. oculus* (Branch 1974a) and *P. caerulea* (Bacci 1947). The high incidence of this form of hermaphrodite gonad in *P. kermadecensis* was interpreted as an intermediate stage in the transformation of a male gonad into a female one (Creese et al. 1990). Mosaic hermaphrodites, however, contained some patches of gonad that were functional female and some that were functional male (i.e., simultaneous hermaphrodites). This form of hermaphroditism has a small, sporadic incidence in many patellid species from South Africa (Branch 1981), England (Dodd 1956) and elsewhere, but this is not necessarily related to sex change (Branch 1981). Creese et al. (1990) found only one mosaic hermaphrodite in the samples of *P. kermadecensis*, and argued that this was almost certainly associated with sex change: a transitional gonad in which reabsorption of sperm had not occurred for some reason and a hermaphroditic condition had been retained. Since the transitional hermaphrodites in *P. kermadecensis* were found at three different times of the year, Creese et al. (1990) reasoned that sex change was unlikely to be closely linked with spawning. Being that the case, it would represent a different pattern of sex change from three other well-documented patellid limpets (Branch 1981): in *P. vulgata* the transition takes place in the resting phase between spawning and transitional hermaphroditic gonads are never encountered, and in *C. oculus* and *P. caerulea* transitional gonads are only encountered after spawning (Creese et al. 1990).

In line with the size advantage model (Ghiselin 1969, 1974), Creese et al. (1990) postulated that protandric sex change in *P. kermadecensis* is a phenomenon related to a change in habitat from the backs of large shells (limpets less than approximately 50 mm) to bedrock (larger limpets). In fact, *P. kermadecensis* can attain sizes about 155 mm, hence the relative reproductive advantage for large females is likely to be particularly pronounced. On the other hand, it may be disadvantageous to be a small female because it is energetically more expensive to produce eggs than sperm (Branch 1981; Hughes 1986; in Cresse et al. 1990). In a situation where young *P. kermadecensis* at the time of sexual maturity (25 to 40 mm) may have limited supply of energy for reproduction due to the limited grazing surface available on adult shells, production of sperm would be favoured (Creese et al.1990). By rapidly and inexpensively developing a male gonad, young individual *P. kermadecensis* could

participate in a non-trivial way in at least some spawning activity before making the probably hazardous transition from adult shell to rock surface (Creese et al.1990).

When comparing the pattern of sex change in *P. kermadecensis* with that from other species of limpet one could argue that a reproductive system consisting of gonochoristic males and sequential hermaphrodites may be particularly advantageous for a large, slow-growing species that has a very restricted geographical range, (Creese et al. 1990). Although recognizing the need for more sampling, the authors indicated that like *L. gigantea* (Lindberg and Wright 1985; Wright 1989) protandric sex change in *P. kermadecensis* may be at least partly controlled by environmental factors, being unlikely to be just a function of growth, as the sizes of the hermaphroditic limpets varied considerably; between 31 and 143 mm (Creese et al. 1990).

Besides the genus *Patella,* sex change is also known to occur in the Lottidae. The effects of intraspecific density and agonistic interactions on the sex change in *L. gigantea* were investigated by Wright (1989) using the hypodermic needle methodology. This species is a large intertidal gastropod, with planktonic larval phase, whose adults graze microalgae from bare rock surfaces, and defend feeding territories from competing herbivores (Galbraith 1965; Stimson 1970). Territory acquisition is accompanied by a marked change in the individual behavioural strategy from an exploitative competitor that flees from conspecific contact (Wright 1989) to an interference competitor, which aggressively defends its territory against other limpets (Stimson 1970, 1973; Wright 1982). The sex change in *L. gigantea* appears to be facilitated by territory acquisition with up to two years delay (Lindberg and Wright 1985). Wright (1989) evaluated the existence of environmentally mediated sex change in *L. gigantea* and the relative importance of density versus agonistic interaction as ecological cues to sex change. The environment of individual males was experimentally manipulated and their sex observed after one year (Wright 1989). Initially the experiment was set to vary simultaneously dominance status and intraspecific density. Dominance status was controlled by containing each limpet in a ring with either a larger ringmate or a smaller one. Density was manipulated by containing the two limpets in rings of two different sizes that approximated the natural range of effective densities extremes. Nevertheless, after six months the experiment continued with only the ring size treatment: the author had realised that

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within three months the smaller limpet of many pairs was found outside the ring presumably due to the territorial effects of the large one.

The outcome of this one-year field experiment was that limpets in the larger rings (which mimicked low density), were more likely to change sex than those in the small rings (which mimicked high density) (Wright 1989). In addition, relatively large initial pre-experimental size appeared to enhance the probability of sex change. As an effort to discover density and initial size effects, sex-age distributions of populations covering a range of intraspecific densities were determined for small populations (Wright 1989). Females appeared at the youngest age in the lowest density, as predicted by the density effect discovered in the ring size experiment. However, female age distribution did not rise monotonically with increased intraspecific density (Wright 1989). Therefore, some other influence appeared to be acting in addition to intraspecific density. Wright (1989) suggested variation in the level of female aggression, and hence territorial status, as a possibility to be investigated. When estimating the possible additional influence on sex change of dominance status it was observed that more females performed territorial behaviour than males. In contrast evasive behaviour was shown almost exclusively by males. Wright (1989) concluded that dominant territorial status might be a good clue for the initiation of sex change since it correlates with the size and therefore egg-producing capacity of an individual relative to its neighbours. Pooling his results with those of two previous studies (Wright and Lindberg 1982; Lindberg and Wright 1985) confirmed that sex change in *L. gigantea* is enhanced by low density.

1.5. PROXIMAL QUESTIONS ON SEX CHANGE – GENETIC *VERSUS* ENVIRONMENTAL CONTROL

One of the most important questions in understanding the adaptive value of a given pattern of sex allocation is the elucidation of its ecological correlates (Wright 1989); for it is important to distinguish whether allocation is strictly genetically determined, or whether environmentally mediated phenotypic plasticity "smears" the genetic programming. Some individuals of every *Crepidula* species may possibly be true males incapable of sex change (Coe 1935; 1938a,b) and others observed in

natural populations and in laboratory experiments bypassed the male phase
(Hoagland 1978).

   Limpet populations probably therefore have individuals that never change sex and
remain all their adult lives as male or female. Others, after sexual maturation might
experience several sex-changing stages (Figure 1.1).
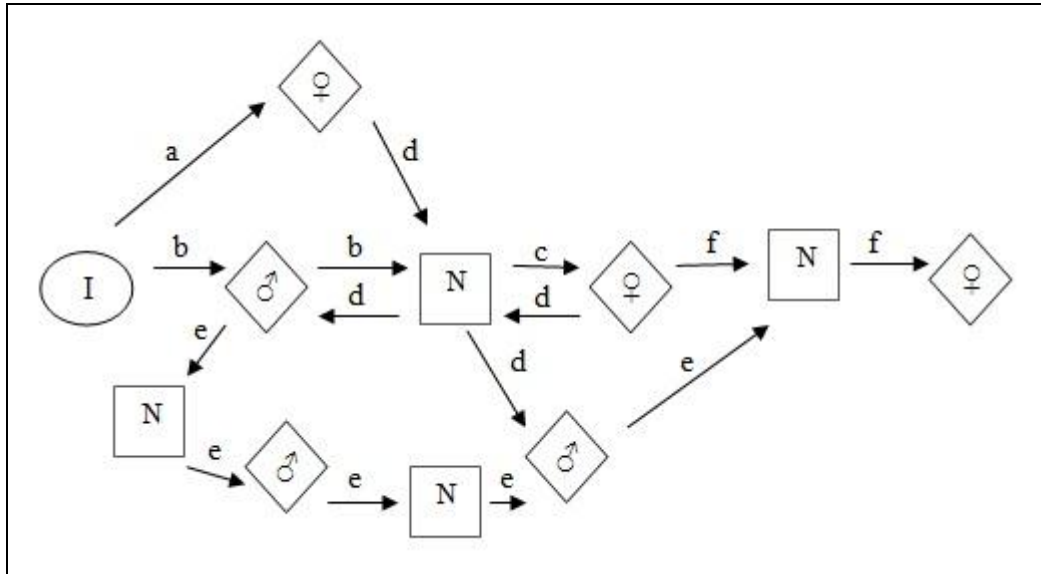
Figure 1.1. Possible sex change sequences in limpets with a resting phase, such as *P. vulgata* (I, immature; N, neuters; ♂, males; ♀, females): (a)- maturation from immature to female; (b)- immature individuals change to male and pass by a neuter (resting) phase; (c)- male individuals, change sex to female after a neuter (resting) phase; (d)- sex reversal back to male after a neuter (resting) phase; (e)-possibility of multiple years as male, with neuter (resting) phases before becoming female;(f)- individuals are female with neuter (resting) phases.

   Phenotypically plastic environmentally mediated sex change has been known in
gastropod molluscs since the beginning of last century (e.g. Gould 1919; Hoagland
1978; Breyer 1980). General interest in the phenomenon has arisen as a result of
work on sex allocation theory (e.g. Warner et al. 1975; Leigh et al. 1976; Charnov
and Bull 1977; Charnov 1982; Policansky 1982; Collin 1995; Warner et al. 1996;
Hobday and Riser 1998). It allows an individual of a sex-changing species to
enhance the adaptive value of its sex change by responding to local changes on its
environment even if the genetic constitution of local individuals reflects a much

broader gene pool from a larger range of environments. This is particular helpful for planktonically dispersed species whose adults are constrained, either by dispersion (i.e., very patchy distributions) or a lack of mobility, to live in small persistent groups as in many species of molluscs (Ghiselin 1969; Charnov and Bull 1977; Hoagland 1978; Charnov 1982; Wright 1989).

The experiments reported by Wright (1989) confirmed the hypothesis (Lindberg and Wright 1985) that sex change in *L. gigantea* can be environmentally mediated. Most accounts of sex change in other patellacean species (e.g. Branch 1974a,b; Webber 1977; Russell-Hunter 1979; Fretter 1984) emphasized the role of polymorphic genetic control of sex change to explain the large overlap in sizes between male and female limpets (Orton 1927; Bacci 1947, 1965; Montalenti and Bacci 1951). In this sense, there might be several genetically determined ages of sex change in a population, including a non-changing pure-male/female genotype. The pure-male genotype hypothesis predicts that relatively older males would consist to a large degree of the pure-male genotype, since most individuals with the sex change genotype would have previously left the male ranks by changing to female (Wright 1989). Thus it would predict the rates of sex change among old males to be relatively lower than that of the intermediate-aged. This was opposite to the pattern found by Wright (1989) where the initially larger males had the highest probability of changing sex. Wright (1989) proposed a less extreme genetic polymorphism in the age of sex change in *L. gigantea* than had been suggested for other species (e.g. initial work by Orton 1927; Bacci 1947, 1965; Montalenti and Bacci 1951). Environmental variability was suggested as a more parsimonious explanation of the overlap in size between males and females rather than genetic determination (Wright 1989). However, the latter might support the variation in age of sex change within relatively homogeneous isolated habitats (Wright 1989). Indeed the existence of such a genetically based variation in age of sex change has been proved conclusively for the polychaete *O. puerilis* (Bacci 1978; Wright 1989). Collin (1995) suggested three possible reasons for the variation in size at sex change commonly seen as the overlap of male and female sizes in field studies of several species (e.g. Wright 1989; Soong and Chen 1991; Sewell 1994), which are:

1. Animals may change sex based on cues like age, which may not correlate exactly with size.

2. Individual genetic variation could cause scatter around the optimal size at sex change. However she stressed that the large degree of variation found in size at sex change when compared to variation in other major life histories traits weaken this hypothesis.

3. Size at sex change may be environmentally determined by factors that vary spatially within a patchy environment. This was also pointed out by Collin (1995) as the most important explanation for the co-occurrence of males and females over a wide size range in sedentary animals living in a heterogeneous environment such as benthic molluscs. Differences among patches in size distributions, growth rates, mortality rates, or mating opportunities may lead to among patch variation in size at sex change. Combining data from many patches would then result in the apparent overlap of male and female size (Collin 1995).

Further studies, complementing experimental work in the environmental determination of sex, still need to be made investigating the genetic basis for age ranges of sex change and to look for possible pure-male/female genotypes.

Wright (1989) demonstrated that reduced density in *L. gigantea* can accelerate sex change. Intraspecific density was proposed as used by individuals to assess local mortality rate, been a highly valuable cue for the proper timing of sex change. As low density implies high mortality and a reduced number of individuals of the second sex (females), anticipating sex change would grant reproductive advantages to those able of that performance. The author suggested as possible proximal signals of density "sounded" by limpets, the frequency of contact, movement area availability, food intake, gut distension, growth rate and pheromonal information. The latter would involve water-borne chemicals or substances laid on the substratum as limpets move. Some mesogastropod limpets of the genus *Crepidula* appear to assess local sex ratio via water-borne pheromones and gauge the proper age of sex change accordingly (Gould 1919, 1952; Coe 1953; Hoagland 1978). Nonetheless, a pheromone hypothesis is weakened by the results of Wright's (1989) ring-size experiment. The sex change frequencies in the two ring sizes were enormously different despite sex-ratio equivalence: only males within the rings and equivalent sex ratio outside the rings. This led him to conclude that the density effect could only be complemented by a sex-ratio pheromone hypothesis. Nevertheless the high-energy intertidal area in which *L. gigantea* lives would probably dilute and smear

pheromonal signals (Wright 1989). Hence, substances imbedded in the mucus of limpets as they move over the substratum might provide another kind of chemical cue for local sex ratios (Wright 1989). Coe (1938a, 1948a) reported starved animals that tended to remain male and Hoagland (1978) referred that the cause could be the insufficient stored energy to accomplish the remodelling required to change from male to female. If higher densities prevent food intake this might be one reason for sex change to be restrained.

1.6.   NOMENCLATURE USED

The family Patellidae and the former genus *Patella* have been subject to much taxonomic, phylogenetic and phylogeographic investigation in recent years (see Ridgway et al. 1998; Koufopanou et al. 1999; Weber and Hawkins 2002, 2005; Sá-Pinto et al. 2005, 2007, 2008). I have used the original name used by the author when reviewing the literature, but where possible indicated new names after (e.g. *Patella* (now *Cymbula*) *oculus*). In Macaronesia I have followed Weber and Hawkins (2005) and called the Macaronesian (Azores, Canaries, Madeira) species *Patella aspera*, retaining *Patella ulyssiponensis* for continental populations. Current names follow Ridgway et al. (1998) especially for South African species. The genus *Patella* should be strictly reserved for North Atlantic species. Both protandric and protandrous are used as adjectives in the literature. I have followed Orton (1928), and used protandric throughout this thesis.

1.7.   AIMS AND OBJECTIVES

The understanding of species biology is essential for its conservation. In limpet species that change sex, there is a major gap due to the poor knowledge of proximal constraints. To my knowledge, no previous study has investigated simultaneously the implications of harvesting pressure in *P. vulgata* biology (and sex change) and upon the shore macro-community structure. The aim of this thesis is to investigate the influence of environment and harvesting upon the biology of target species (*Patella* spp.) and consequences for macro-community structure (non-target species).

Specifically, the objectives of this thesis are:

1. To investigate variation in sex with size as indicator of protandry in two species of *Patella* (*P. vulgata* and *P. depressa*) with some incidental data on *P. ulyssiponensis*. Comparisons were made between the centre range edges of distribuition (for *P. vulgata* southern limit in Portugal, range centre southern England; for *P. depressa* range edge in southern England, centre in Portugal). A broad-scale field survey was undertaken in 2012 to gather information on size frequencies, densities, biomass, mean and smallest sizes, proportion of females and gonad development. (Chapter 2).

2. To investigate the effects of simulated human exploitation of limpets (*Patella* spp.) on the ecology of *P. vulgata* (the target species). Two levels of exploitation of the bigger limpets were simulated in two shores in the southwest of England and different parameters were analysed through and at the end of the experiment. The effects of harvesting pressure on *P. vulgata* biology were assessed by comparing data on limpet sex change rate; limpet growth through increments in shell major length; food availability through the assessment of microalgal film abundance; recruitment and limpet migrations into and out of the experimental plots; limpet density and size frequency (Chapter 3).

3. To investigate the effects of simulated human exploitation of limpets (*Patella* spp.) presented on Chapter 3, upon the composition of the rocky shore macro-community structure (non-target species). During the experiment the abundance, spatial dispersion of key species (*Fucus* spp., *Mytilus* spp., barnacles and limpets), species richness and shore macrobiotic structure were recorded. Changes over the macro-community were followed in time and treatment plots compared at the end of the experiment (Chapter 4).

4. To investigate the status of the limpet stocks (*P. aspera* and *Patella candei crenata*) in the Canary Islands. A field survey was conducted in 1999 and its data compared a previous unpublished survey from 1992. I quantified spatial variability in limpet catches abundances in weight between and within islands related to human population density and aspect of shores respectively (Chapter 5).

The results are integrated in Chapter 6 in the context of the management and conservation of limpet species – some of which have probably gone extinct (*Scutellastra mexicana*) and are endangered (e.g. Macaronesian endemic species such as *P. aspera*).

CHAPTER 2

BROAD-SCALE PATTERNS OF SEX RATIOS OF *PATELLA* SPP.: A COMPARISON OF THE BRITISH ISLES AND PORTUGAL

2.1.   ABSTRACT

The occurrence of protandry in *P. vulgata* was first reported in the British Isles by Orton and co-workers. Most other studies to date have been made on single populations with protandry being inferred from a switch in sex ratios from males in smaller size classes to females in larger size classes. A broad-scale survey of limpets by sex and size classes covering a range of latitudes from Zambujeira do Mar (southern Portugal) to Jennycliff (Devon, southern England) is presented here. The primary aim of the survey was to put the populations experimented upon in Chapter 3 into context and to explore changes in sex ratio within the range of the species studied: *P. vulgata* (southern limit in Portugal, range centre in the British Isles) and *P. depressa* (poleward limit in the British Isles, range centre in Portugal). As a protandric population approaches its range boundary, the alternative hypotheses to test are: (i) sub-optimal conditions (due to the environment or increased interspecific competition) delay promotion from male to female resulting in fewer females; (ii) relaxation of resource limitation resulting from reduced intraspecific competition will enhance promotion from males to females resulting in higher number of females; (iii) at range edges recruitment is intermittent leading to more older larger animals which are female.

Evidence was found for the occurrence of protandry in non-harvested *P. vulgata* populations from the south of England, with females predominating in larger size classes; cumulative frequency distributions of males and females were different; sex ratios were biased towards the first sex and smallest sizes of males were smaller than the smallest sizes of females. In *P. depressa* populations from England and Portugal: females were interspersed across most size classes; cumulative frequency distributions of males and females and smallest sizes of males and females did not

differ. This was also displayed in *P. vulgata* populations from Portugal. *P. depressa*, however, also showed some patterns indicating the possibility of slight protandry occurring in Portugal. The test of sex ratio variation with latitude revealed that *P. vulgata* sex ratios could be involved in determining the species range limit, particularly at the equatorward limit since the likelihood of being male rises with latitude from Alentejo (Portugal) through to Plymouth (England). Thus at the southern range limit sperm could be in short supply.

2.2.   INTRODUCTION

The abundance and distribution of a species throughout its geographic range are influenced by several ecological processes, including rates of reproductive output and recruitment success affecting demography, habitat availability and quality, dispersal, competition with other species, predation and human impacts (Brown 1995; Hyder et al. 2001; Gaston 2003, 2009; Sagarin et al. 2006; Gaines et al. 2009; Fenberg and Rivadeneira 2011). These differences in ecological processes will have evolutionary consequences through selection and drift, especially in range edge populations (Sexton et al. 2009; Phillips et al. 2010; Phillips 2012). Differences in climate and hence environmental regime experienced will also have an influence on survival, growth and reproduction of individuals and hence population processes. At geographic range limits, viability can be determined by physical barriers to dispersal (Gaines and Gaylord 2007), habitat quality (Herbert et al. 2003, Herbert and Hawkins 2006), biological interactions such as competition (Poloczanska et al. 2008) and predation (Sanford 2002). Dispersal variability will also influence the genetic potential for local adaptation of peripheral populations (Holt et al. 2005; Moore et al. 2007; Gaston 2009; Sexton et al. 2009; Dawson et al. 2010; Fenberg and Rivadeneira 2011; Herbert et al. 2009).

Little is known about how the above mechanisms differentially affect species in different parts of their range (e.g. southern and northern limits versus the centre) and how they relate to abundance and population structure across the range (Fenberg and Rivadeneira 2011). The abundance and range limits of species may be reflected in population structure, life history, and genetic traits (see Gilman 2005, 2006; Gaston 2009; Dawson et al. 2010; Rivadeneira et al. 2010; Herbert et al. 2009). For example,

low abundances near range limits may be a result of recruitment limitation, potentially caused by distances between individuals causing Allee effects or between populations due to habitat variability (Zacherl et al. 2003; Gilman 2006; Sexton et al. 2009) or poor larval supply due to hydrographic factors (Gaines and Gaylord 2007). Low survival of larvae or juveniles because of other physical factors such as temperature stress are also likely to be important (Zacherl et al. 2003; Gilman 2005, 2006; Byers and Pringle 2006; Sanford et al. 2006; Fenberg and Rivadeneira 2011; Herbert et al. 2009). Temperature stresses will be influenced by oceanographic regime (i.e. upwelling) and timing of tides (e.g. Helmuth et al. 2002; Seabra et al. 2011). In exploited species such as limpets, these patterns might be masked by the impact of size-selective human predation (Fenberg and Roy 2012).

Protandry is widespread in patellid limpets of the genera *Patella, Cymbula* (Orton 1920, 1928, 1946; Bacci 1952, 1965, 1975; Dodd 1956; Orton et al. 1956; Montalenti 1958; Orton and Southward 1961; Branch 1974a; Frenkiel 1975; Espinosa et al. 2009a; Rivera-Ingraham et al. 2011) and *Lottia (*Lindberg and Wright 1985; Wright 1989; Fenberg and Roy 2012). Protandry was first investigated in the Bristish Isles by Orton and co-workers (Orton 1920, 1928, 1946; Dodd 1956; Orton et al. 1956; Orton and Southward 1961) but some early work was also done in Italy (Bacci 1965). In most studies protandry was inferred from analysis of size-frequency data where females predominate in larger size classes and used single populations. Little work has been done on variation between populations (but see work on calyptraeids by Collin (2006) showing considerable ranges in sex ratio).

I aimed to investigate sex ratios across multiple populations in the British Isles and Portugal. The overall aim of the survey was to compare *P. vulgata* at its southern range limit with populations at the centre of their distribution in the British Isles. Conversely, *P. depressa* was compared at its northern limit in the British Isles with populations at the centre of the range in Portugal. The Portuguese and English coasts respectively provide a sharp gradient of abundance of P. *vulgata* approaching its equatorward limits and *P. depressa* approaching one of its poleward range edges (Crisp and Southward 1958; Southward et al. 1995; Boaventura et al. 2002c). The survey was intended to explore the following hypotheses:

(i) The increasingly sub-optimal environment at range edges leads to slower growth and hence delayed promotion from male to female. Sub-optimal conditions could be due to the environment or increased competition from congeneric species

(see Boaventura et al. 2002b) at centre of their range (i.e. *P. depressa* in Portugal, *P. vulgata* in the British Isles). The consequences of this hypothesis being correct would be that Allee effects due to the absence of females could be important in setting range limits. This assumes that interspecific competition is stronger than intraspecific competition.

Alternatively to (i), (ii) Relaxation of resource limitation resulting from reduced intraspecific competition at lower density (see Boaventura et al. 2003) will allow faster promotion from males to females (i.e. sex change is density dependent).

(iii) An alternative explanation to both (i) and (ii) is that at range edges recruitment is intermittent and in protandric species this leads to more older, large animals which are female. This could lead to Allee effects due to sperm limitation.

Without manipulative experiments it is impossible to unambiguously test these alternative hypotheses. However, the survey provided a background to the rest of the thesis including experimental approaches (Chapter 3). The patterns observed should provide further inference enabling hypotheses (i) to (iii) to be ruled likely or unlikely. Care must be taken in interpreting these data as little exploitation of *Patella* spp. occurs in the British Isles, but this is common in Portugal and could bias sex ratios.

The design of the survey included both density (no.m^{-2}) and biomass (g. m^{-2}) as metrics of abundance. Gonad staging along the Channel coast also allowed testing of the hypothesis that gonad development would be delayed at range edges in *P. depressa*, and more advanced in *P. vulgata* due to colder conditions. Incidental data for *P. ulyssiponensis* are included from exposed sites, particularly Highcliff where the small tidal range leads to much overlap in distribution of the three species.

2.3. MATERIAL AND METHODS

2.3.1. Study sites

The study was made on the south of England and in Portugal (Figure 2.1) from September to November 2012.
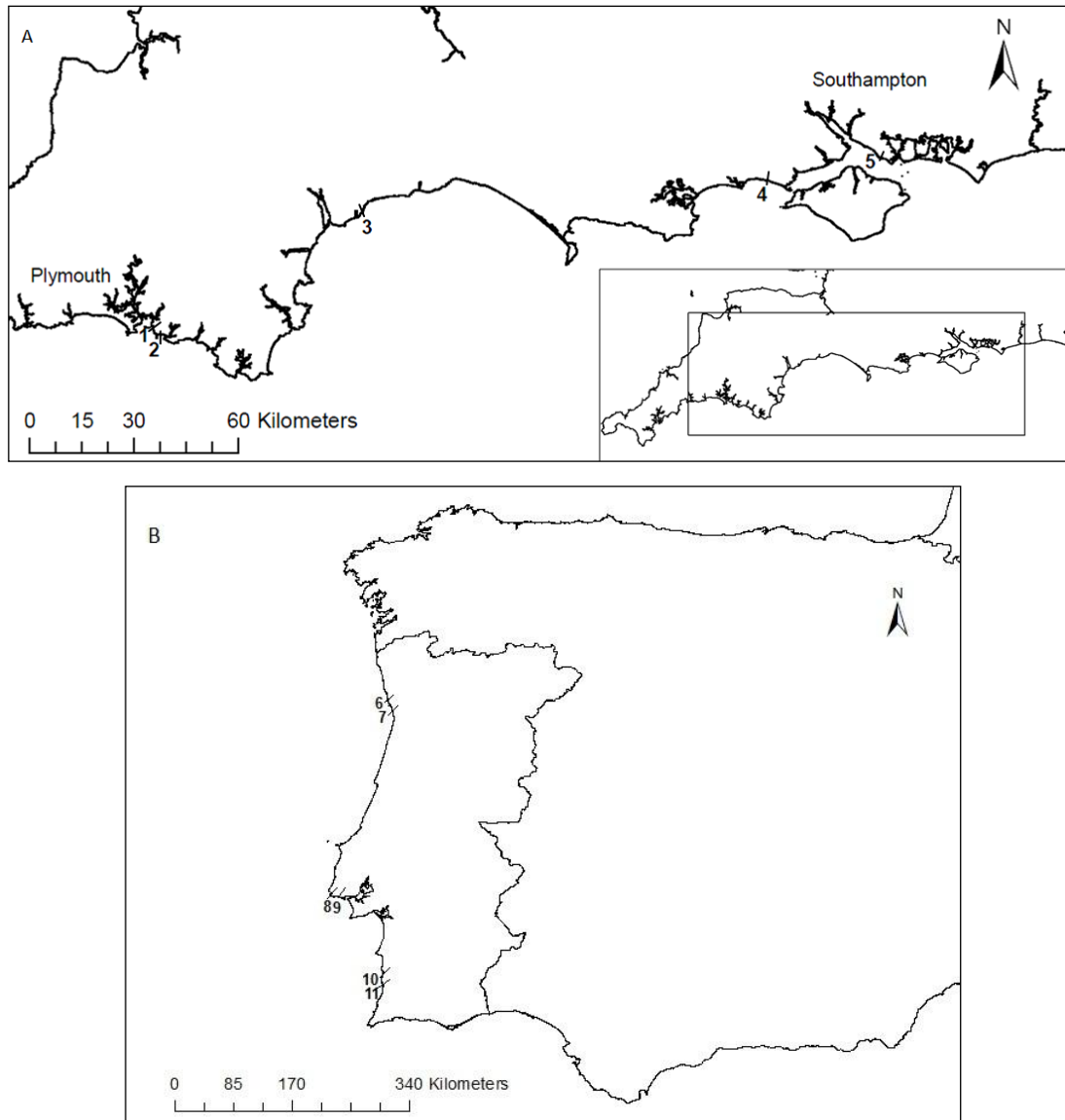
Figure 2.1. A. Map showing the south coast line of England and relative position of the five study site locations. 1, Jennycliff Bay; 2, Heybrook Bay; 3, Sidmouth; 4, Highcliff; 5, Lee-on-the-Solent. Inset map shows the position of sites in the south of England. B. Map showing the coast line of Portugal and relative position of the six study site locations. Porto: 6, Cabo do Mundo; 7, Foz; Cascais: 8, Cabo Raso; 9, Avencas; Alentejo: 10, Almograve; 11, Zambujeira do Mar.

2.3.2. Geographic distributions and basic biology of study species

Three limpet species, *P. vulgata, P. depressa* and *P. ulyssiponensis* are found in both the British Isles and Portugal.

 Excluding the Swedish coast, *P. vulgata* has a continuous European distribution from the Artic Circle near Tromso in Norway to its southern biogeographic limit in Southern Portugal (Bowman and Lewis 1986; Guerra and Gaudencio 1986; Southward et al. 1995; Jenkins et al. 2001; Marclim 2002). *P. vulgata* is also present throughout Britain and Ireland (Southward and Crisp 1954; Bowman and Lewis 1977; Baxter 1982, 1983; Bowman and Lewis 1986; Delany 1998; Jenkins et al. 2000, Marclim 2002). *P. vulgata* is considered a protandric species (Orton et al. 1956, Orton and Southward 1961; Le Quesne and Hawkins 2006).

The eastern limits of *P. depressa* and *P. ulyssiponensis* in Britain occur close to the Isle of Wight and are thought to be set by soft substratum, exposure and hydrography influencing larval supply (Crisp and Southward 1958; Lewis 1964, 1986; Marclim 2002).

P. depressa is restricted to the south and west coasts of Britain, with its northern biogeographic limit reached in northern Wales, on Anglesey (Bowman and Lewis 1986; Southward et al. 1995). Eastern limits in the English Channel were the Isle of Wight (Crisp and Southward 1958) but recently a small range extension has occurred on the sea defences to Hayling Island and Elmer near Bognor Regis (Hawkins, *pers. comm.*; Marclim 2002; Keith et al. 2011). *P. depressa* is absent from Ireland (Marclim 2002). In continental Europe, populations do not penetrate north of Cap de la Hague in France but extend south to Senegal, West Africa (Southward et al. 1995; Marclim 2002). *P. depressa* is considered a gonochoristic species (Orton and Southward 1961).

P. ulyssiponensis has a northern boundary at Bohuslan, Norway (Lewis et al. 1982). Populations occur around the British coast excluding the south east region from east Yorkshire to the Isle of Wight (Crisp and Southward 1958; Bowman and Lewis 1986; Southward et al. 1995; Marclim 2002). Recent range extensions have been found as far end as Beachy Beach (Hawkins unpublished; Mieszkowska et al. 2006; Keith et al. 2011). In mainland Europe, *P. ulyssiponensis* is recorded from Cap de la Hague in France to Portugal, throughout the Eastern Atlantic Islands (Marclim 2002). Its southern range boundary is found in West Africa Mediterranean (Fischer-

Piette 1955; Guerra and Gaudencio 1986; Lewis 1986; Southward et al.1995; Bulleri et al. 2000; Marclim 2002; Weber and Hawkins 2005). *P. ulyssiponensis* is considered a possible protandric species (Thompson 1979).

2.3.3. Data collection

On each shore, 250 limpets were collected using a 0.5×0.5 m sampling quadrat from the mean tide level (the spatial coordinates for each shore are presented in Table A1.1 located in appendix 1). In England, Jennycliff and Heybroock Bay, being respectively a moderately sheltered and a moderately exposed shore, were compared to assess local differences. Data for Highcliff were duplicated on different groynes (Highcliff 1 and Highcliff 2) in order to test for spatial variability in the collected data.

2.3.3.1. Size-frequency distributions and sex ratios

In the laboratory the collected limpets were separated by species, weighed, their maximum length measured and dissected to identify sex and stage of reproductive activity. Size-frequency data were plotted by species and visually compared. Kolmogorov-Smirnov tests (Sokal and Rohlf 1998) were performed on size distributions of males and females for each species from the shores in England and Portugal.

The overall proportion of females at each sampled shore was calculated as the (number of females) / (number of males + number of females). It was expected that protandric *Patella* species should show a male biased sex ratio in contrast to gonochoristic species that should have equal proportions of males and females.

2.3.3.2.   Statistical analysis: Male/female sizes

A two-factor split-plot design (equivalent to model 5.6 in Doncaster and Davey, 2007) was used to test the hypothesis that in potentially protandric species males were smaller than females on average in body size, measured as length. The model was applied to species from England and Portugal separately.

The design had two levels of the fixed sex factor (Sx), at each level of a random site factor (St, with six levels for England and Portugal for *P. vulgata*; with five levels for England and six for Portugal for *P. depressa*). Replicate random quadrats (Qt, 50 for England and Portugal for *P. vulgata*; 26 for England and 58 for Portugal for *P. depressa*) were nested in each level of St. This design was analysed with the General Linear Model (GLM) model:

Y= Sx|Qt´(St′)   model 1

where a prime represents a random factor, vertical line means 'crossed with' and parenthesis means 'nested in'. In the Minitab statistics package the analysis of terms = Sx|St + Sx|Qt(St) − Sx*Qt(St) was requested. It produced an output with the structure described by Table 2.1. During all analyses a visual inspection of the residuals was performed to check for the underlying assumptions of normality and homoscedasticity.

If a species is protandric males are expected to be smaller than females. Additionally the smallest male and female individuals for each sampled population were calculated as the lowest 2.5% quantile for each size-frequency data and compared with a two-sample permutation test.

Table 2.1 . GLM model 1, degrees of freedom (d.f.), components of variation and MS for *F* ratios. P´ denotes replicate observations.

Mean square	d.f.	Components of variation estimated in population	*F*- ratio
Between quadrats			
St´	a-1	P´(Sx*Qt´(St´)) + Qt´(St´) + St´	St´/ Qt´(St´)
Qt´(St´)	(n-1)a	P´(Sx*Qt´(St´)) + Qt´(St´)	No test
Within quadrats			
Sx	b-1	P´(Sx*Qt´(St´)) + Sx*Qt´(St´) + Sx*St´+ Sx	Sx /Sx*St´
Sx*St´	(b-1)(a-1)	P´(Sx*Qt´(St´)) + Sx*Qt´(St´) + Sx*St´	Sx*St´/ Sx*Qt´(St´)
Sx*Qt´(St´)	(b-1)(n-1)a	P´(Sx*Qt´(St´)) + Sx*Qt´(St´)	No test
P´ (Sx*Qt´(St´))	0	P´(Sx*Qt´(St´))	-

2.3.3.3. Sex ratios and density of single species and all *Patella* spp. combined

The density of limpets by shore was calculated as the number of individuals per sampling quadrat and data were presented for each species (to understand possible intraspecific competition) and total species (to understand possible interspecific competition). Sex change was expected to be enhanced by low densities therefore the proportion of females was plotted against species density (intraspecific) and total species density (interspecific).

2.3.3.4. Species biomass

Species biomass (measured as full body wet weight) per sampling quadrat was assessed to compare the relative weight of species at each location and between locations as another measure of density.

2.3.3.5.   Sex ratio variation with latitude

For each species a global binary logistic regression of sex versus shore (two factor level) and location (four level factor) was run to investigate the influence of latitude on the sex ratio. Shores ranged northwards from Zambujeira do Mar and Almograve in Alentejo, Portugal to Jennycliff and Heybrook Bay in Plymouth, England and along the south coast of England.

2.3.3.6.   Gonad stage

Gonad ripeness was inspected for the shores in the south coast of England by dissecting away the limpet foot to reveal the gonad, and assessed according to the semi-quantitative scale of Orton et al. (1956). Their scheme scored gonad development, with states ranging from neuter, where limpet sex and gonad are indistinguishable, through filling stages I–V and emptying stages V–I (Moore et al. 2011). Each location along the Channel was evaluated for the proportion of limpets in advanced stages of gonad development (stages IV: filling and emptying stages (respectively IV+ and IV-); and fully ripe V).

2.4. RESULTS

2.4.1. Size-frequency distributions and sex ratios

Size-frequency distributions for *P. vulgata* along the English Channel and for *P. ulyssiponensis* from Highcliff (Figure 2.6), accorded with the predictions of protandry, since bigger individuals were predominantly female (Figure 2.2). *P. vulgata* size-frequency distributions from Portugal did not show this pattern (Figure 2.3). Recruitment was also more pronounced in England than in Portugal, except for the artificial stone groyne at Lee-on-the-Solent (Figures 2.2 and 2.3).

P. depressa both in England and Portugal showed sexes interspersed in most of the size classes suggesting a gonochorist life-history (Figures 2.4 and 2.5).

The Kolmogorov-Smirnov tests indicated that for *P. ulyssiponensis* and *P. vulgata* from England male and female distributions differed from each other ($\alpha < 0.05$) (Figures 2.7 and 2.8). For these species the cumulative frequency graphs also showed that the median size of neuters was smaller than median size of males and these median sizes were smaller than the median sizes of females. For *P. vulgata* from Portugal and *P. depressa* from both England and Portugal male and female distributions did not differed from each other ($\alpha > 0.05$) (Figures 2.9, 2.10 and 2.11). The cumulative frequency graphs also showed the median sizes of males was equal or very similar to the median sizes of females.

The proportion of females varied considerably between sites and between species (Figures 2.12 and 2.13). However, *P. vulgata* from England showed lower proportions of females than in Portugal which was confirmed by a two-sample permutation test ($p = 0.009$, $n = 6$ shores per sample). For *P. depressa* from England and Portugal no differences were detected on a two-sample permutation test for the proportion of females ($p = 0.238$, $n = 5$ shores per sample).

At Sidmouth in England both species showed a higher proportion of neuters in larger size classes than expected, probably due to parasite infestation (see Crewe 1951; Thomas 1965; Copeland et al. 1987; Kollien 1996; Prinz et al. 2010).
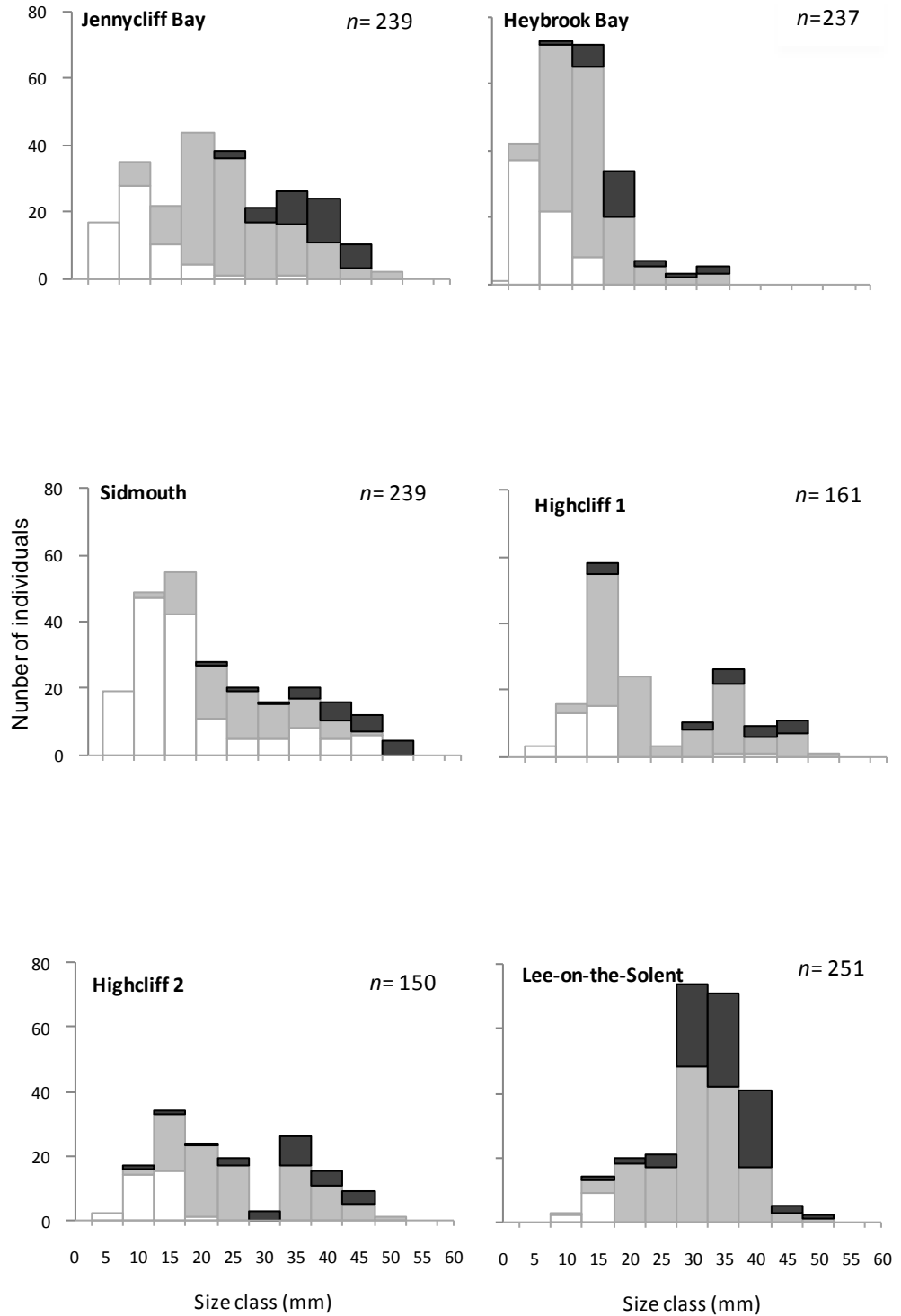
Figure 2.2. Size-frequencies of *P. vulgata* neuters (clear bars), males (grey bars) and females (black bars), from sample sites in the south of England. *n* refers to number of individuals.
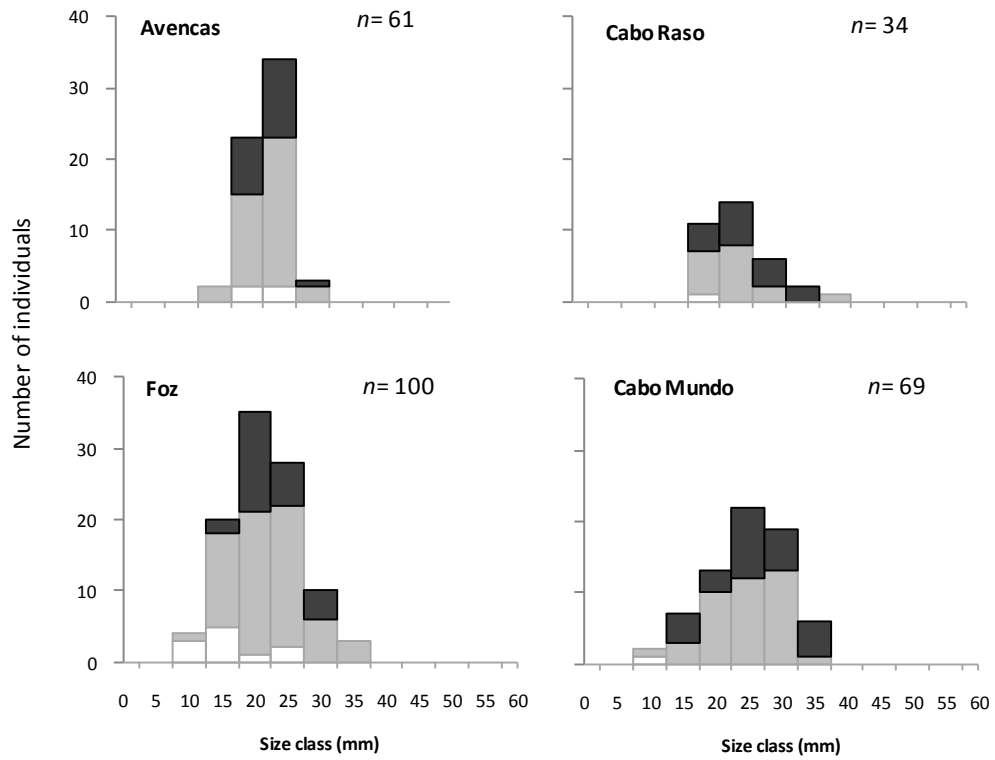
Figure 2.3. Size-frequencies of *P. vulgata* neuters (clear bars), males (grey bars) and females (black bars), from sample sites in Portugal. Notice that no data are available for Almograve and Zambujeira do Mar since only a few individuals were found. *n*, refers to number of individuals.
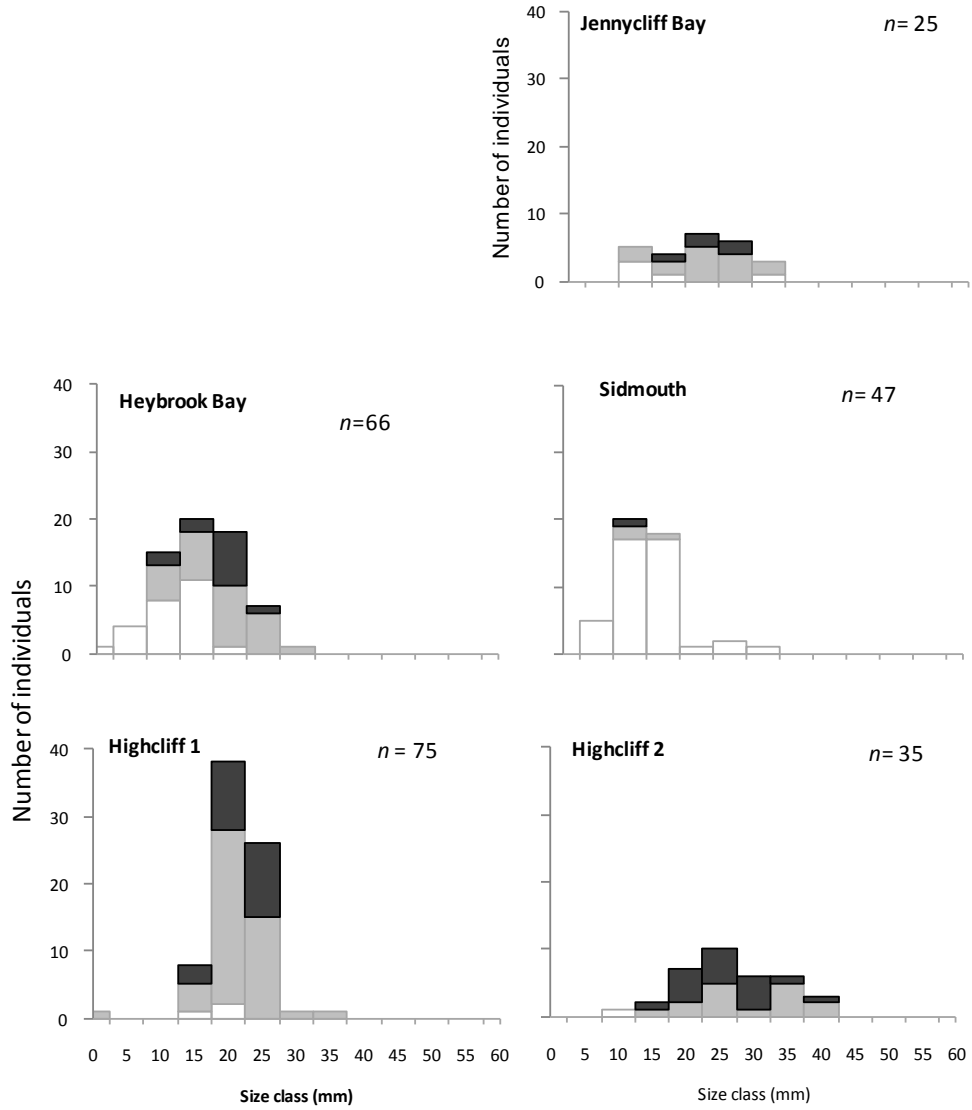
Figure 2.4. Size-frequencies of *P. depressa* neuters (clear bars), males (grey bars) and females (black bars), from sample sites in the south of England. *n* refers to number of individuals.
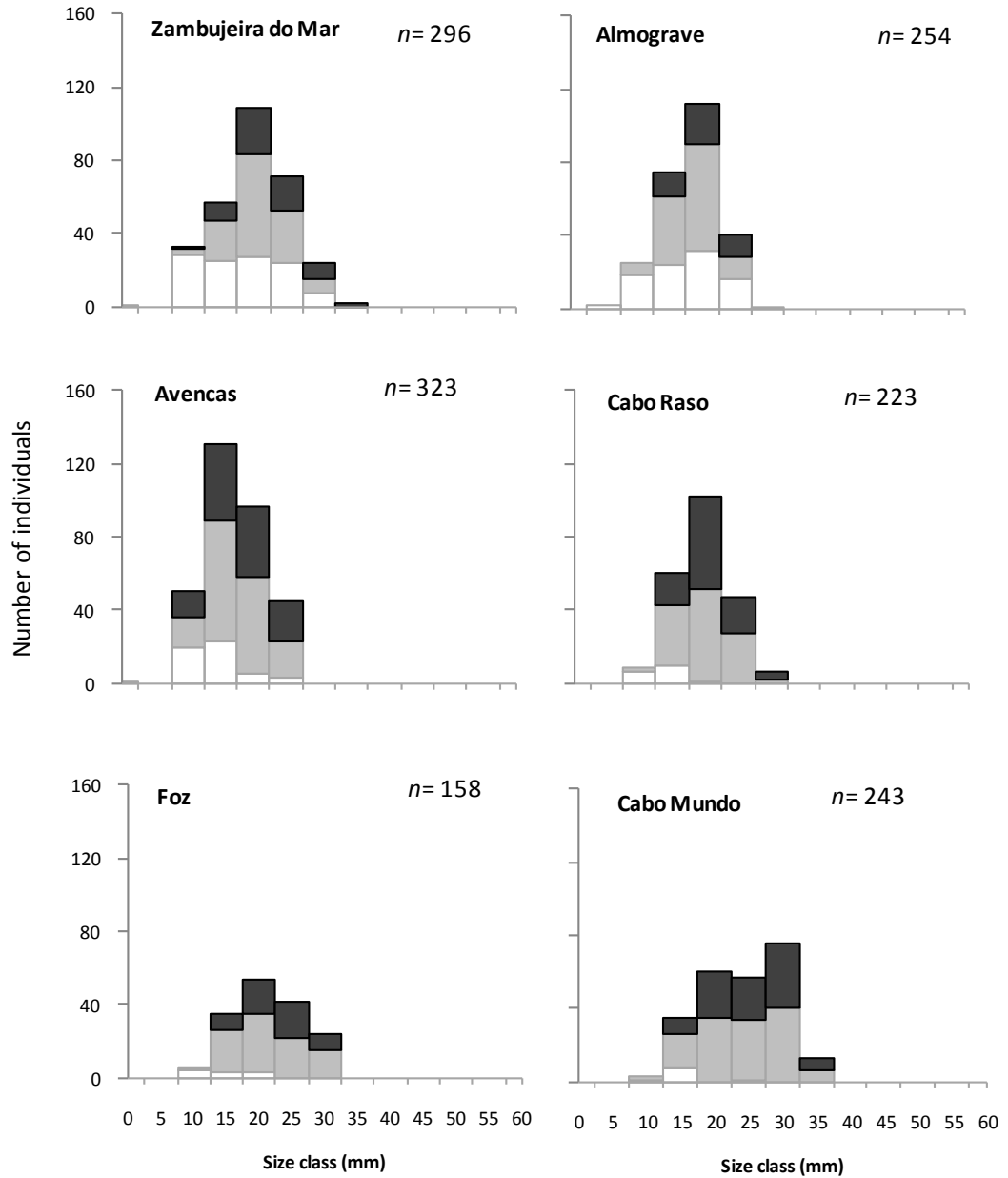
Figure 2.5. Size-frequencies of *P. depressa* neuters (clear bars), males (grey bars) and females (black bars), from sample sites in Portugal. *n,* refers to number of individuals.
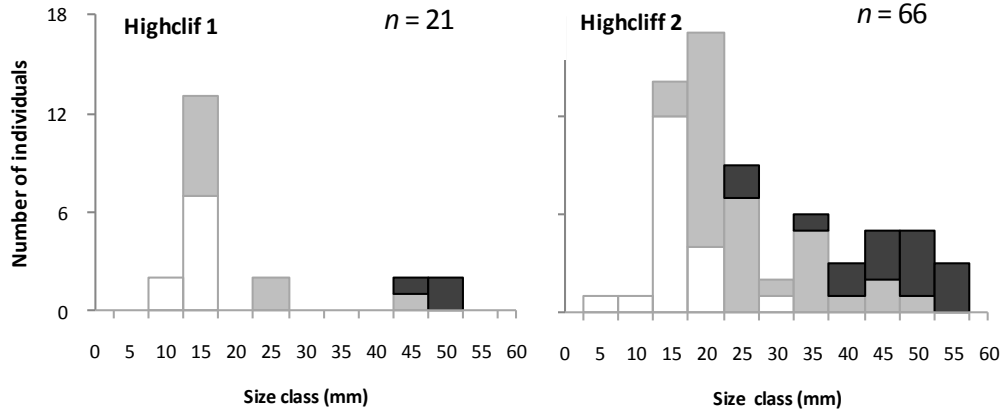
Figure 2.6. Size-frequencies of *P. ulyssiponensis* neuters (clear bars), males (grey bars) and females (black bars), from Highcliff in the south of England. *n,* refers to number of individuals.
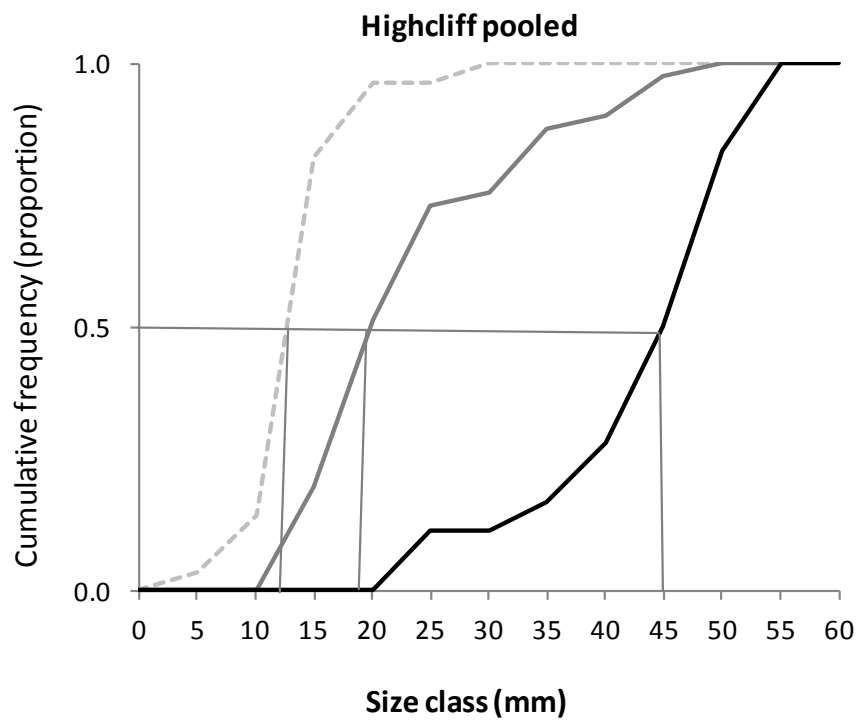
Figure 2.7. Cumulative frequencies for *P. ulyssiponensis* neuters (grey broken line), males (grey line) and females (black line) from Highcliff pooled data in the south of England.
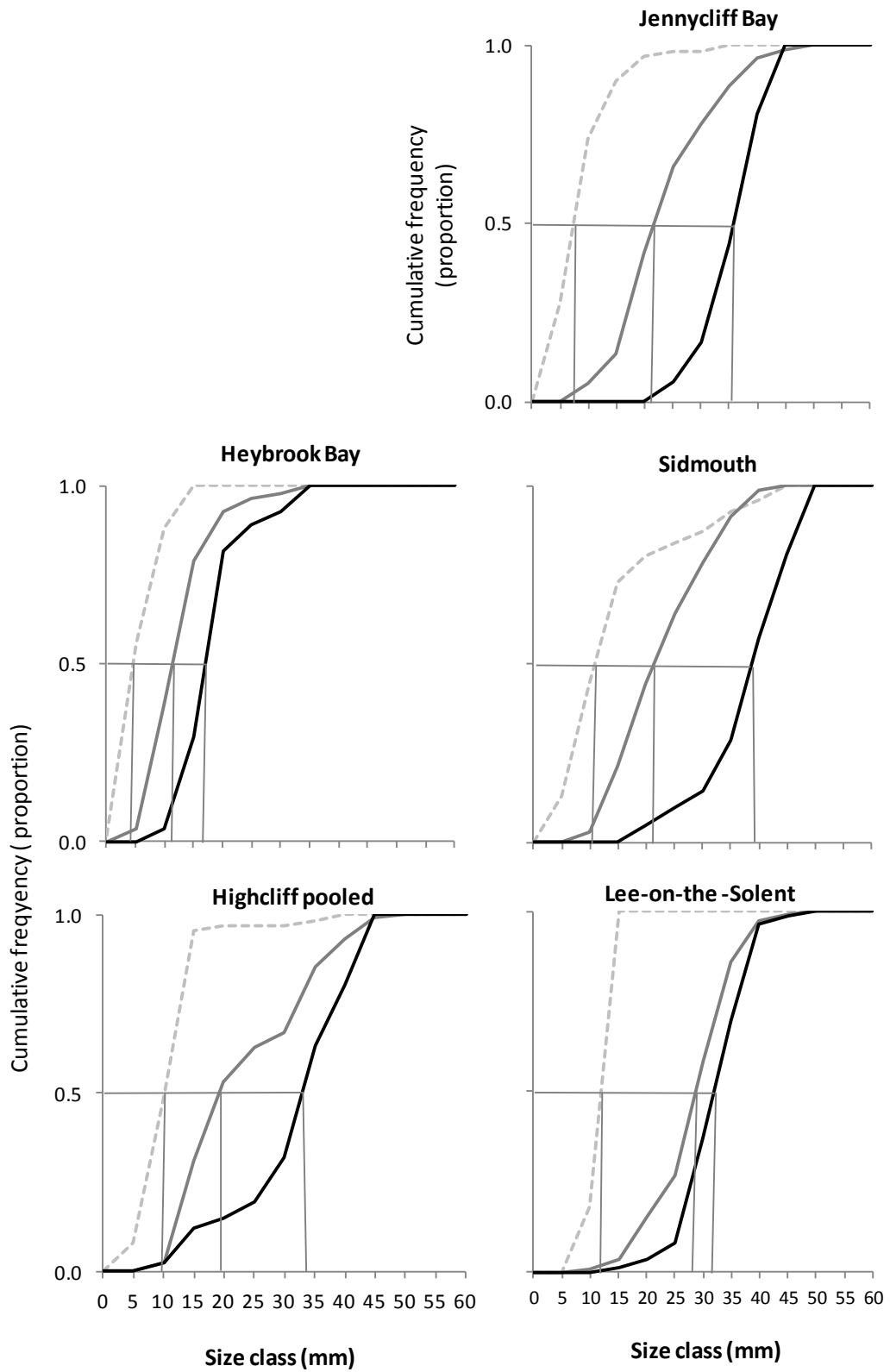
Figure 2.8. Cumulative frequencies for *P. vulgata* neuters (grey broken line), males (grey line) and females (black line) from sample sites in the south of England. N.B: Data from Highcliff were pooled.
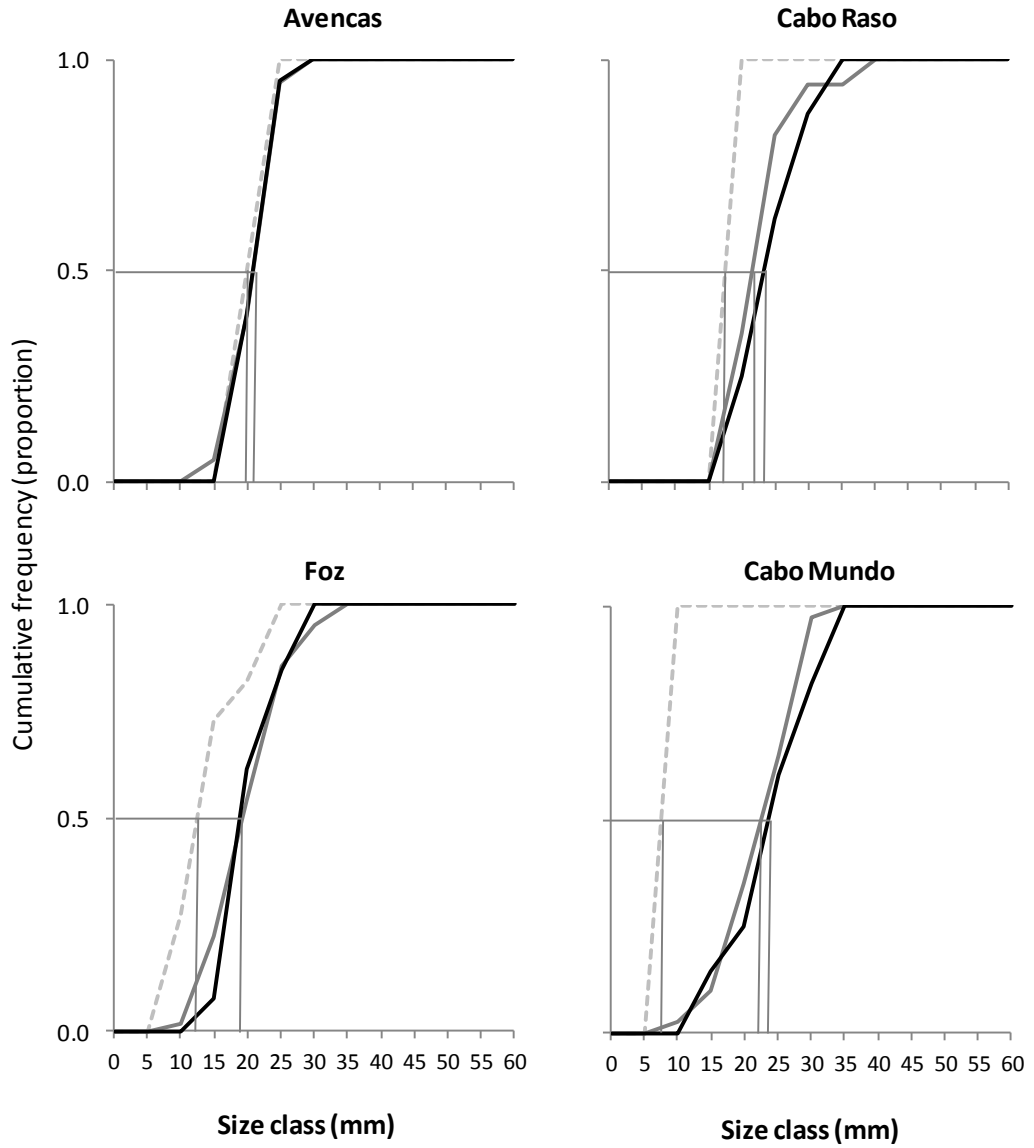
Figure 2.9. Cumulative frequencies for *P. vulgata* neuters (grey broken line), males (grey line) and females (black line) from sample sites in Portugal.
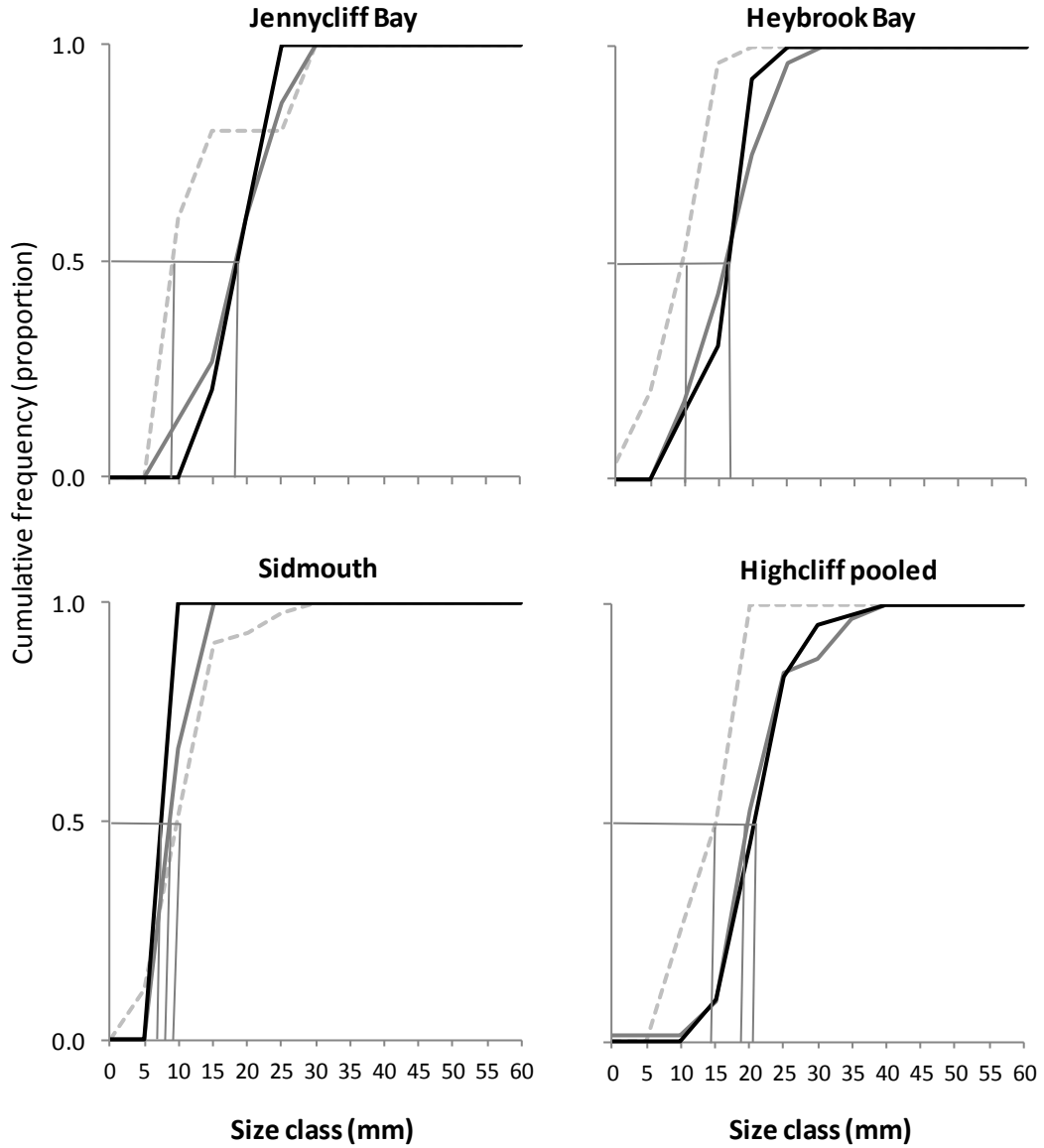
Figure 2.10. Cumulative frequencies for *P. depressa* neuters (grey broken line), males (grey line) and females (black line) from sample sites in the south of England. N.B: Data from Highcliff were pooled.
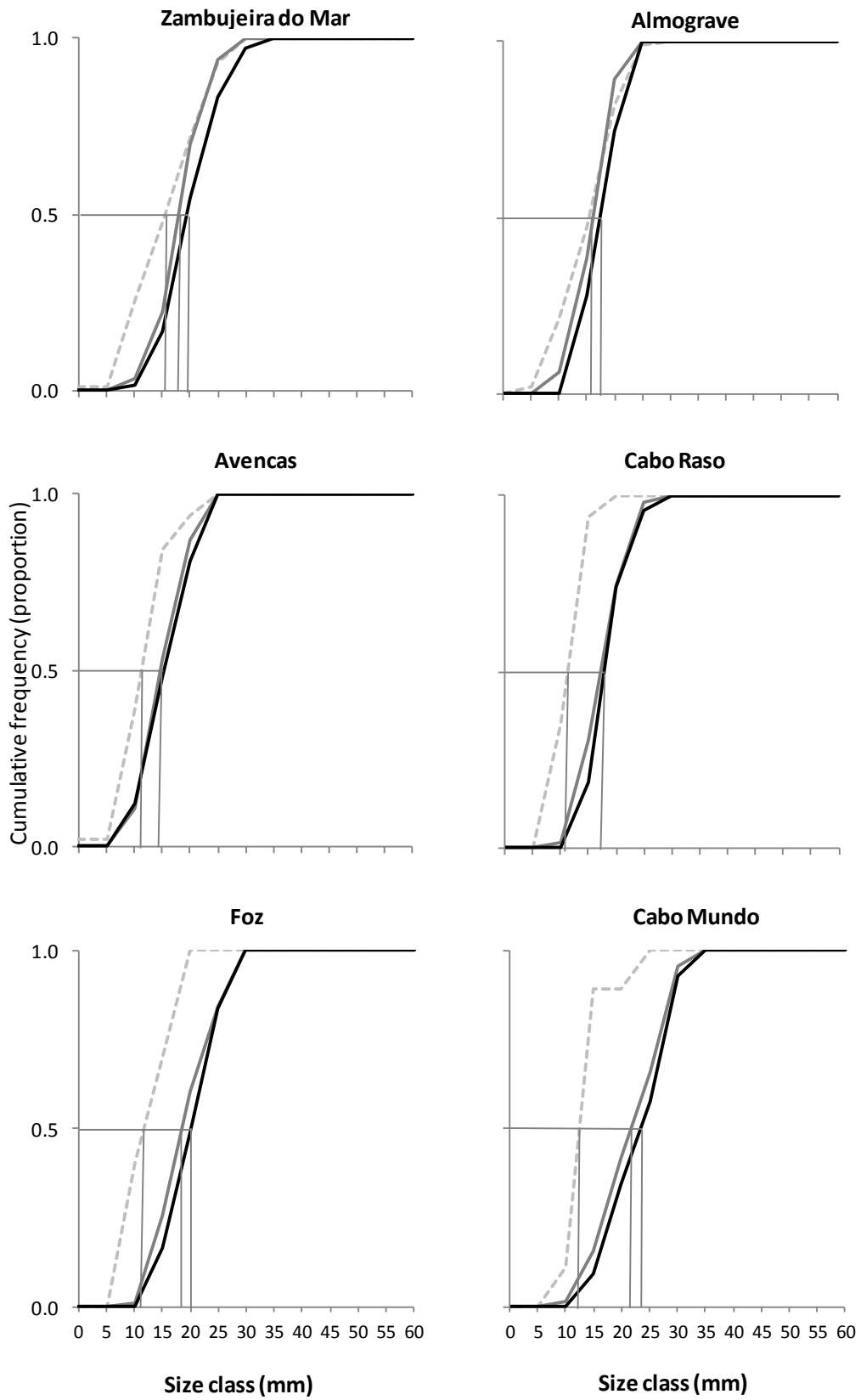
Figure 2.11. Cumulative frequencies for *P. depressa* neuters (grey broken line), males (grey line) and females (black line) from sample sites in Portugal.
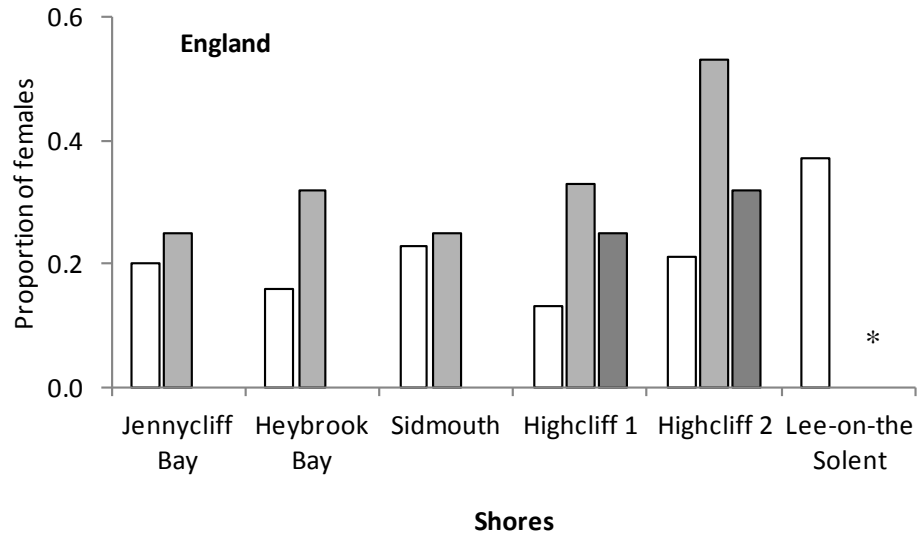
Figure 2.12. Proportion of females of *P. vulgata* (clear bars), *P. depressa* (grey bars) and *P. ulyssiponensis* (dark grey bars), from the sample sites in the south of England (* *P. depressa* absent).
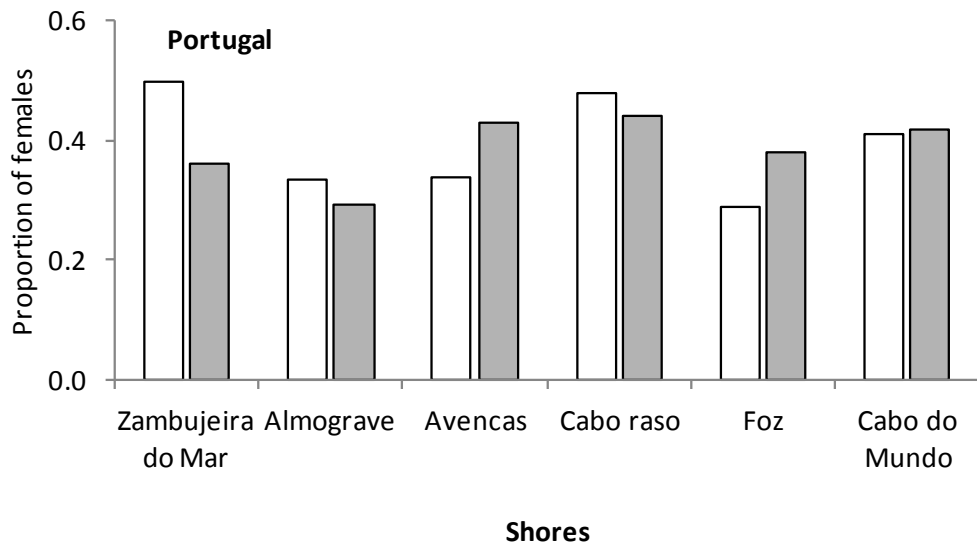
Figure 2.13. Proportion of females of *P. vulgata* (clear bars) and *P. depressa* (grey bars), from the sample sites in Portugal.

2.4.2. Statistical analysis: Male/female sizes

Figure 2.14 suggests that in England *P. vulgata* females were larger than males as also shown by the cumulative frequency graphs and Kolmogorov-Smirnov tests. The GLM analysis of *P. vulgata* mean body size in Table 2.2 revealed a significant sex*site interaction ($F_{5, 38}$ = 4.60, p = 0.002) and main effects of sex and site ($F_{1, 5}$ = 27.32, p = 0.003, and $F_{5, 44}$ = 12.60, $p < 0.001$ respectively). The overall smaller size of males than females therefore had a site-dependent magnitude of difference. For *P. vulgata* from Portugal (Figure 2.15, Table 2.3) the GLM detected no differences by sex or by site.

For *P. depressa* from England (Figure 2.16) differences were only detected by site ($F_{1, 5}$ = 27.32, p = 0.003; Table 2.4) while for *P. depressa* from Portugal (Figure 2.17) differences were detected by sex ($F_{1, 5}$ = 49.84, p = 0.001; Table 2.5) and site ($F_{5, 52}$ = 13.58, $p < 0.001$; Table 2.5).

P. ulyssiponensis data were insufficient for statistical GLM testing but Figure 2.18 suggests that females had bigger mean sizes than males, as shown by the cumulative frequency graphs and Kolmogorov-Smirnov tests, and consistent with protandry inferred in other populations (Thompson 1979).
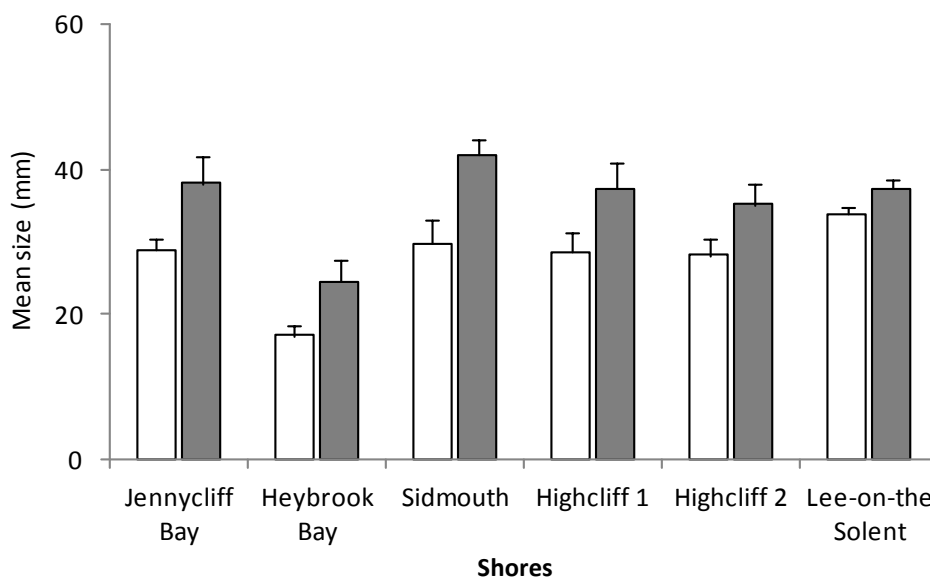
Figure 2.14. Mean sizes (mm) (+ SE, *n*= 5 to 12 quadrats per site) of *P. vulgata* males (clear bars) and females (grey bars) from the sample sites in the south of England.

Table 2.2. *P. vulgata* GLM on mean size (mm) by sex (Sx) and site (St´) in sample quadrats (Qt´) in the south of England.**Terms with $p < 0.05$ are in bold.**

Source	d.f.	Seq SS	Seq MS	F	p
Between quadrats					
St´	5	2175.19	435.04	12.60	**<0.001**
Qt´(St´)	44	1519.78	34.54	-	
Within quadrats					
Sx	1	1635.14	1635.14	27.32	**0.003**
Sx*St´	5	299.24	59.85	4.60	**0.002**
Error	38	494.70	13.02		
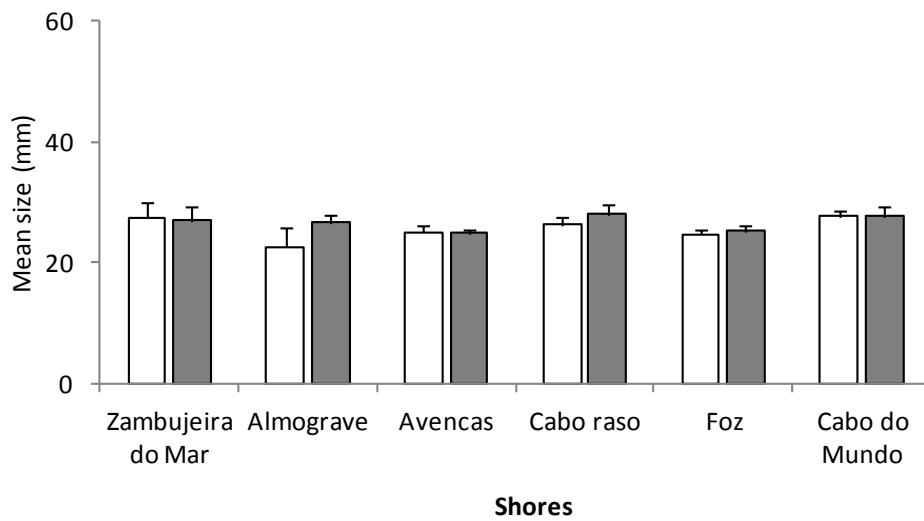
Figure 2.15. Mean sizes (mm) (+ SE, *n*= 4 to17 quadrats per site) of *P. vulgata* males (clear bars) and females (grey bars) from the sample sites in Portugal.

Table 2.3. *P. vulgata* GLM on mean size (mm) by sex (Sx) and site (St´) in sample
quadrats (Qt´) in Portugal.

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between quadrats					
St´	5	147.03	29.41	2.16	0.076
Qt´(St´)	44	599.77	13.63	-	
Within quadrats					
Sx	1	10.40	10.40	1.93	0.223
Sx*St´	5	26.93	5.39	0.33	0.892
Error	37	599.19	16.19		
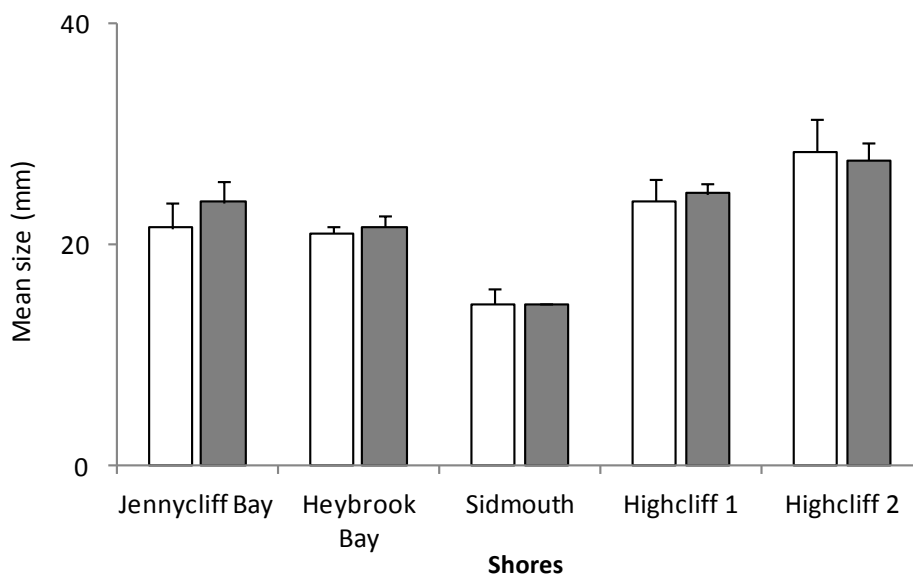
Figure 2.16. Mean sizes (mm) (+ SE, *n*= 5 to 12 quadrats per site) of *P.depressa* males
(clear bars) and females (grey bars) from the sample sites in the south of England.

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Table 2.4. *P. depressa* GLM on mean size (mm) by sex (Sx) and site (St´) in sample quadrats (Qt´) in the south of England. **Terms with *p* < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between quadrats					
St´	4	583.39	145.85	6.69	**0.001**
Qt´(St´)	21	457.14	21.77		
Within quadrats					
Sx	1	14.74	14.74	4.27	0.108
Sx*St´	4	13.80	3.45	0.22	0.926
Error	16	255.74	15.98		
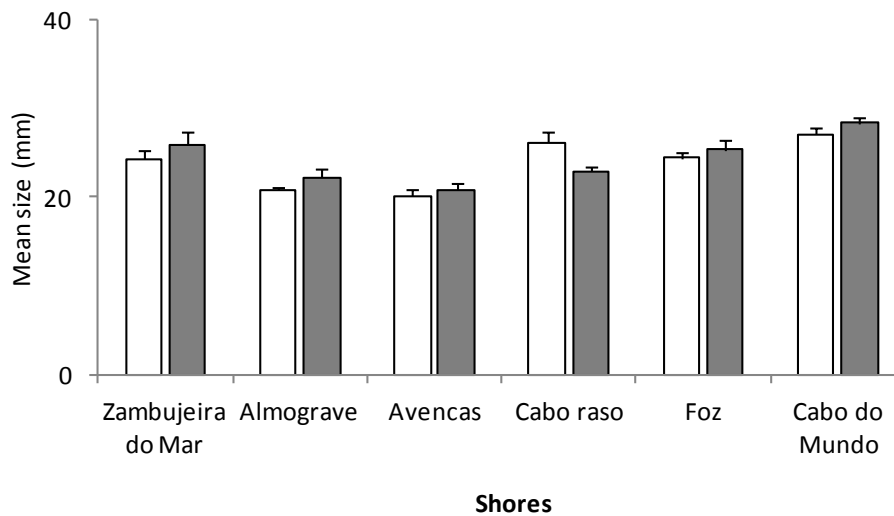
Figure 2.17. Mean sizes (mm) (+ SE, *n*= 4 to17 quadrats per site) of *P. depressa* males (clear bars) and females (grey bars) from the sample sites in Portugal.

Table 2.5. *P. depressa* GLM on mean size (mm) by sex (Sx) and site (St´) in sample quadrats (Qt´) in Portugal. **Terms with *p* < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between quadrats					
St´	5	748.83	149.77	13.58	**< 0.001**
Qt´(St´)	52	573.73	11.03	-	
Within quadrats					
Sx	1	35.89	35.89	49.84	**0.001**
Sx*St´	5	3.61	0.72	0.27	0.930
Error	51	137.96	2.71		
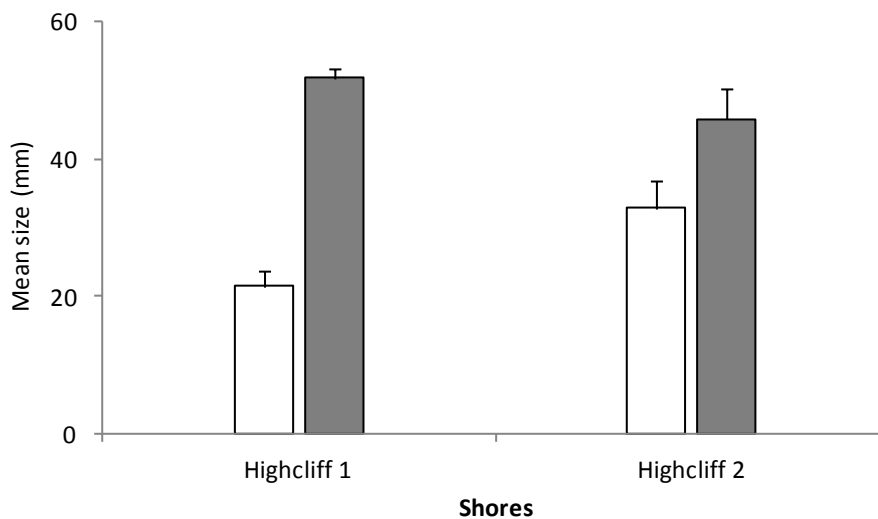
Figure 2.18. Mean sizes (mm) (+ SE, *n*= 5 and 9 quadrats per site) of *P. ulyssiponensis* males (clear bars) and females (grey bars) from Highcliff in the south of England.

Figure 2.19 suggests that in England, *P. vulgata* males were smaller than the females. This was confirmed by a two-sample permutation test for the smallest sizes of males and females ($p = 0.042$, $n = 6$ individuals per sample). For *P. vulgata* from Portugal (Figure 2.20) no differences were detected on a two-sample permutation test for the smallest sizes of males and females ($p = 0.091$, $n = 6$ individuals per sample).

For *P. depressa* from England and Portugal (Figure 2.21 and 2.22) no differences were detected on a two-sample permutation test for the smallest sizes of males and females ($p = 0.778$ and $p = 0.082$, $n = 5$ and $n = 6$ individuals per sample respectively).

P. ulyssiponensis data were insufficient for statistical testing but Figure 2.23 suggests that females had larger smallest sizes than males.
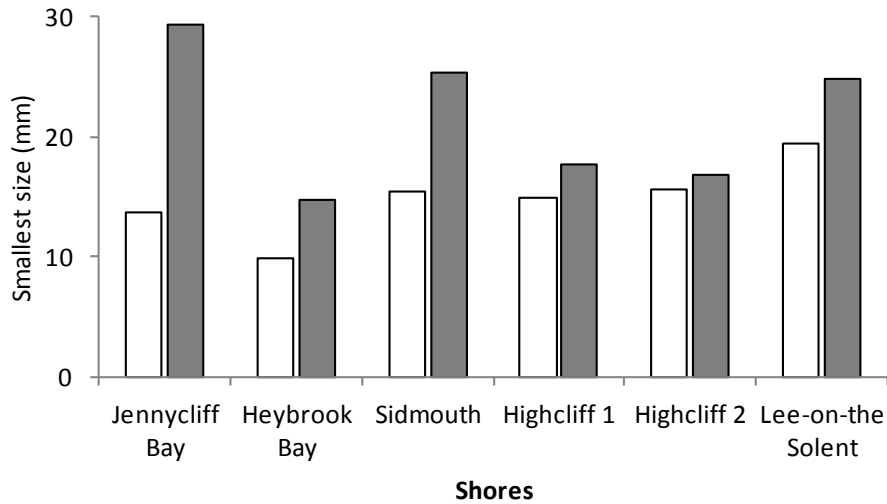
Figure 2.19. Smallest sizes (mm) of *P. vulgata* males (clear bars) and females (grey bars) from the sample sites in the south of England.
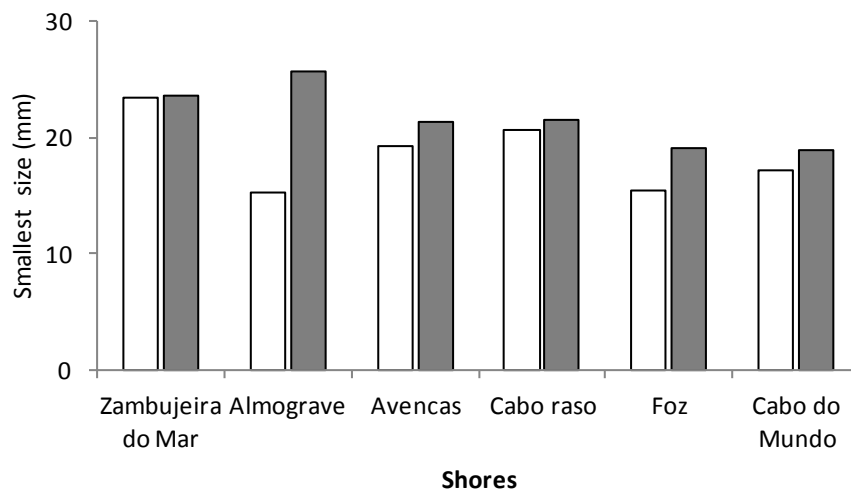
Figure 2.20. Smallest sizes (mm) of *P. vulgata* males (clear bars) and females (grey bars) from the sample sites in Portugal.
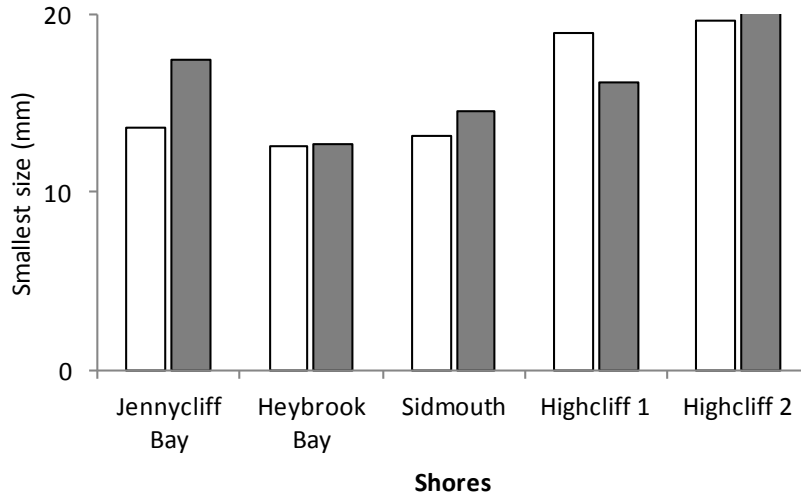
Figure 2.21. Smallest sizes (mm) of *P. depressa* males (clear bars) and females (grey bars) from the sample sites in the south of England.
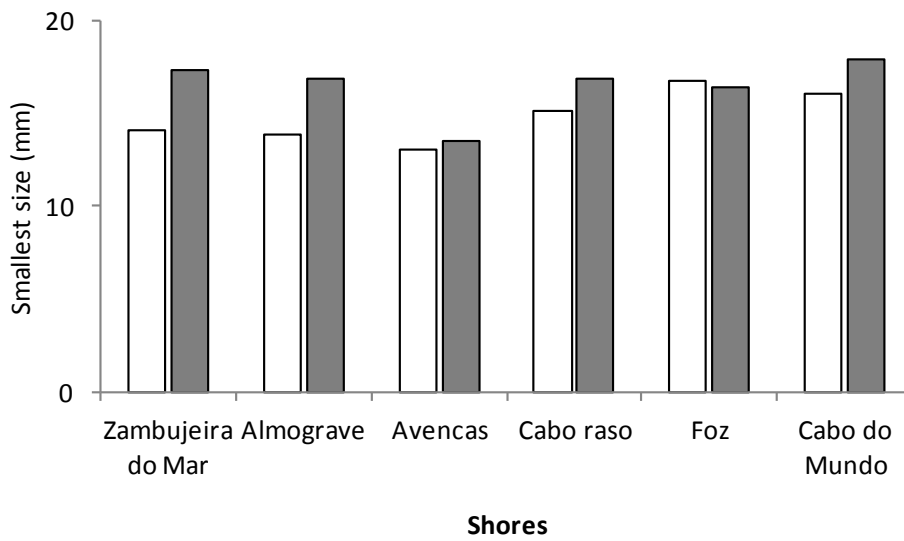
Figure 2.22. Smallest sizes (mm) of *P. depressa* males (clear bars) and females (grey bars) from the sample sites in Portugal.

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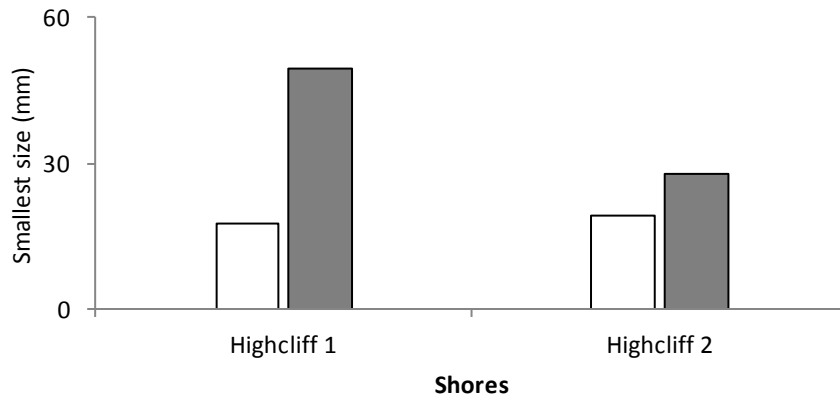
Figure 2.23. Smallest sizes (mm) of *P. ulyssiponensis* males (clear bars) and females (grey bars) from Highcliff in the south of England.

2.4.3. Sex ratios and densities of single species and all *Patella* spp. combined

Densities of *P. vulgata* were higher in England than in Portugal while *P. depressa* had higher densities in Portugal than in England (Figures 2.24 and 2.25). In England, *P. vulgata* showed greater variability in densities. The moderately exposed shore at Heybrook Bay had higher numbers of individuals of both *P. vulgata* and *P. depressa* than the moderately sheltered shore at Jennycliff Bay. In Portugal, *P. vulgata* densities increased from south to north while *P. depressa* numbers decreased. This accorded with the known distribution of both species where *P. vulgata* is a boreal cold temperature species more abundant in northern water and *P. depressa* is a southern warm water species.

In England, the total density of *Patella* spp. was higher on the more exposed shores. The low density at Lee-on-the-Solent probably reflected the lack of nursery grounds such as pools and crevices (Bowman and Lewis 1977) on an artificial structure (Moschella et al. 2005) (Figure 2.26). In Portugal, the density of *Patella* spp. was higher on southern than northern shores (Figure 2.27).

The pooled data from England and Portugal indicated that the proportion of females decreased with increasing density of *P. vulgata*, but not with increasing density of all *Patella* spp. (Figure 2.28: $r^2 = 0.60$, $F_{1,10} = 15.14$, $p = 0.003$ and Figure

2.29: $r^2 = 0.04$, $F_{1,10} = 0.45$, $p = 0.519$ respectively). The proportion of females of *P. depressa*, in contrast, showed no relationship with density of *P. depressa* alone, nor all *Patella* spp. combined (Figure 2.30: $r^2 = 0.001$, $F_{1,9} = 0.01$, $p = 0.913$ and Figure 2.31: $r^2 = 0.15$, $F_{1,9} = 1.64$, $p = 0.233$ respectively).
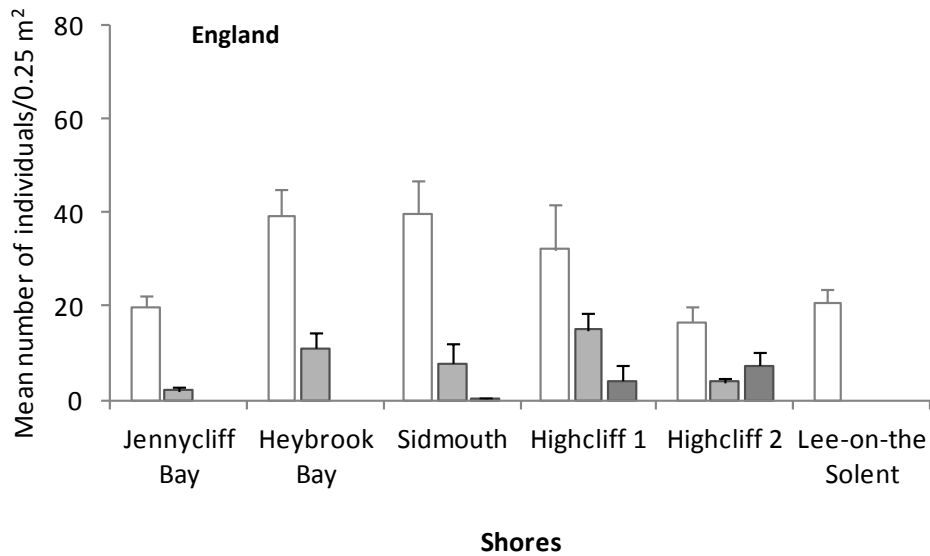
Figure 2.24. Density (mean number of individuals/0.25 m2) (+ SE, *n*= 5 to 12 quadrats per site) of *P. vulgata* (clear bars), *P. depressa* (grey bars) and *P. ulyssiponensis* (dark grey bars) from the sample sites in the south of England.
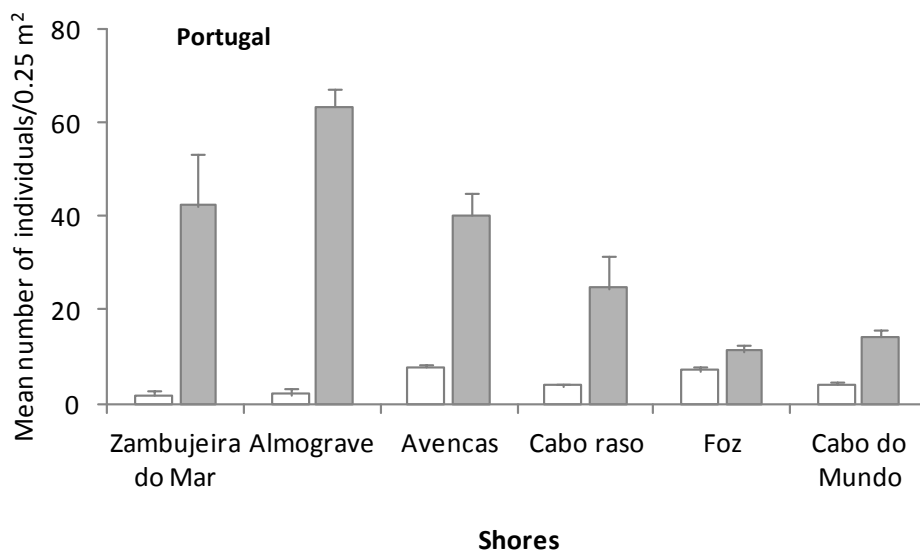
Figure 2.25. Density (mean number of individuals/0.25 m^2) (+ SE, *n*= 4 to17 quadrats per site) of *P. vulgata* (clear bars) and *P. depressa* (grey bars) from the sample sites in Portugal.

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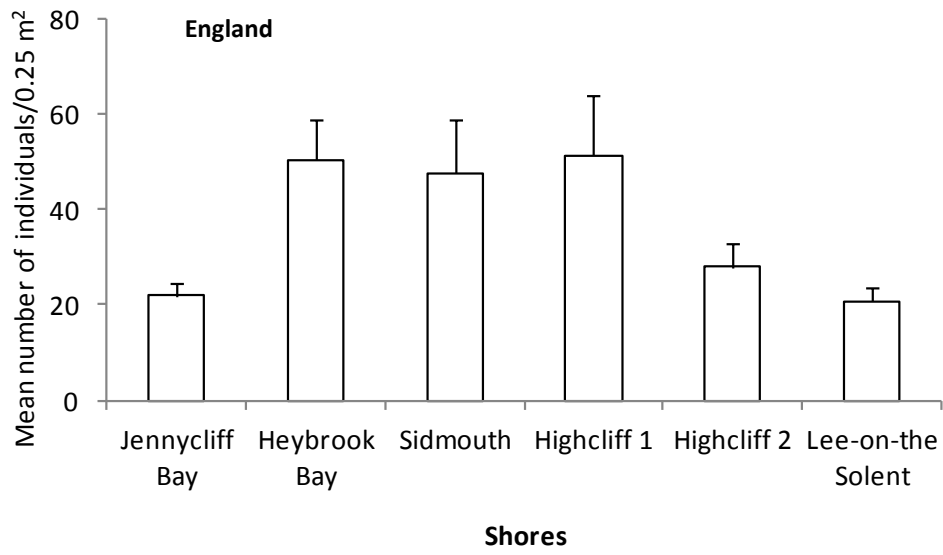
Figure 2.26. Density (mean number of individuals/0.25 m^2) (+ SE, *n*= 5 to 12 quadrats per site) of *Patella* spp. from the sample sites in the south of England.
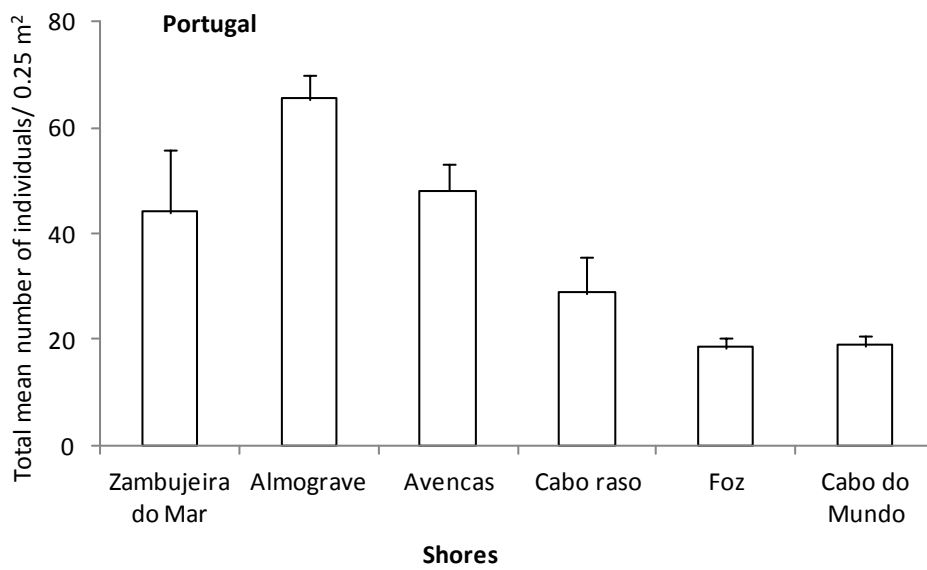
Figure 2.27. Density (mean number of individuals/0.25 m^2) (+ SE, *n* = 4 to17 quadrats per site) of *Patella* spp. from the sample sites in Portugal.
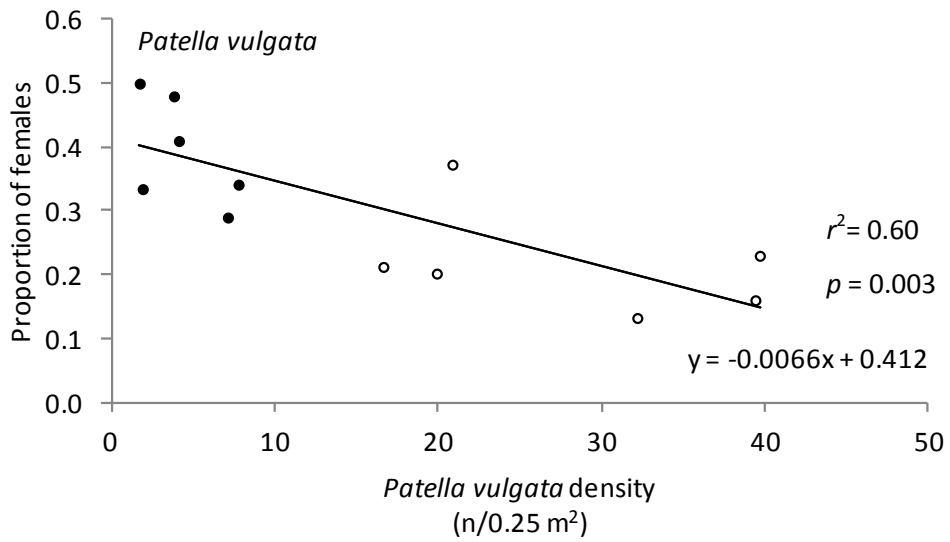
Figure 2.28. *P. vulgata* proportion of females by *P. vulgata* density (number of individuals/0.25 m^2) for the sample sites in the south of England (○) and Portugal (●).
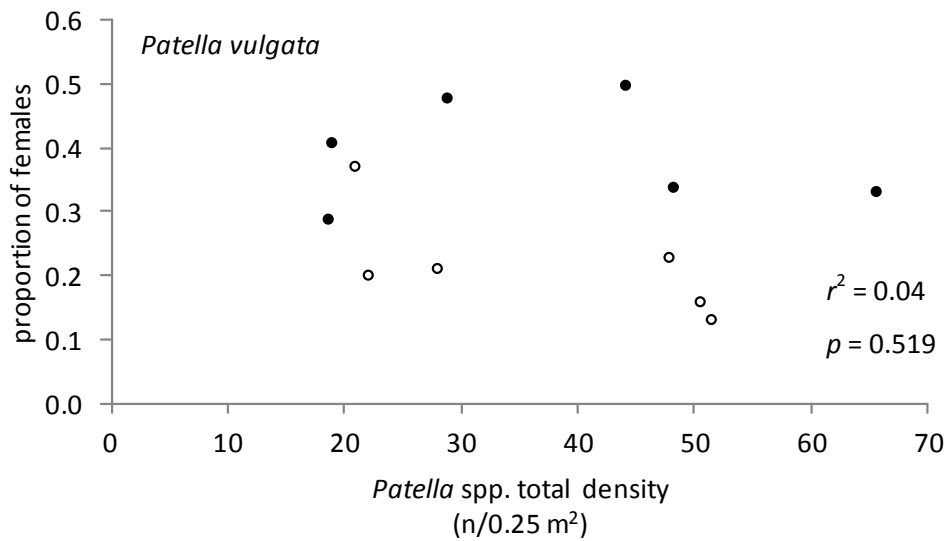
Figure 2.29. *P. vulgata* proportion of females by *Patella* spp. density (number of individuals/0.25 m^2) for the sample sites in the south of England (○) and Portugal (●).
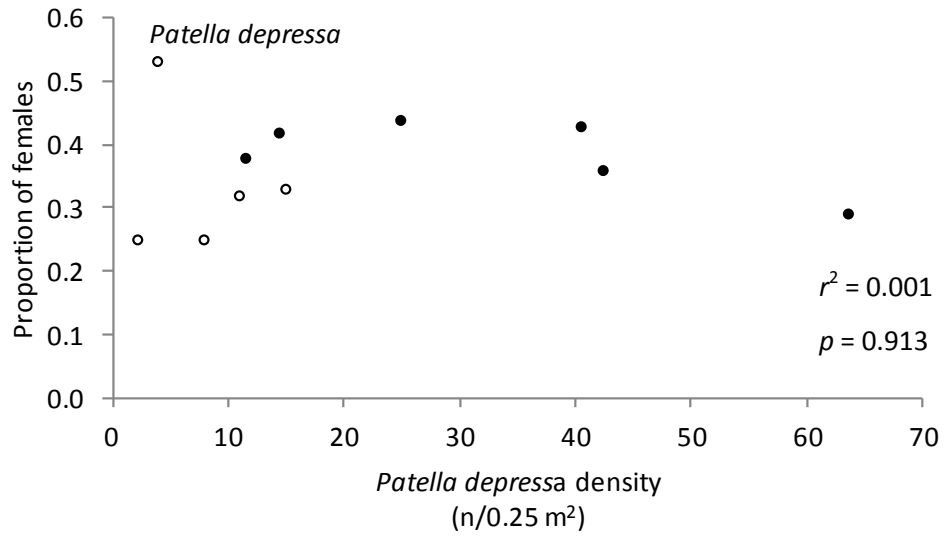
Figure 2.30. *P. depressa* proportion of females by *P. depressa* density (number of individuals/0.25 m^2) for the sample sites in the south of England (○) and Portugal (●).
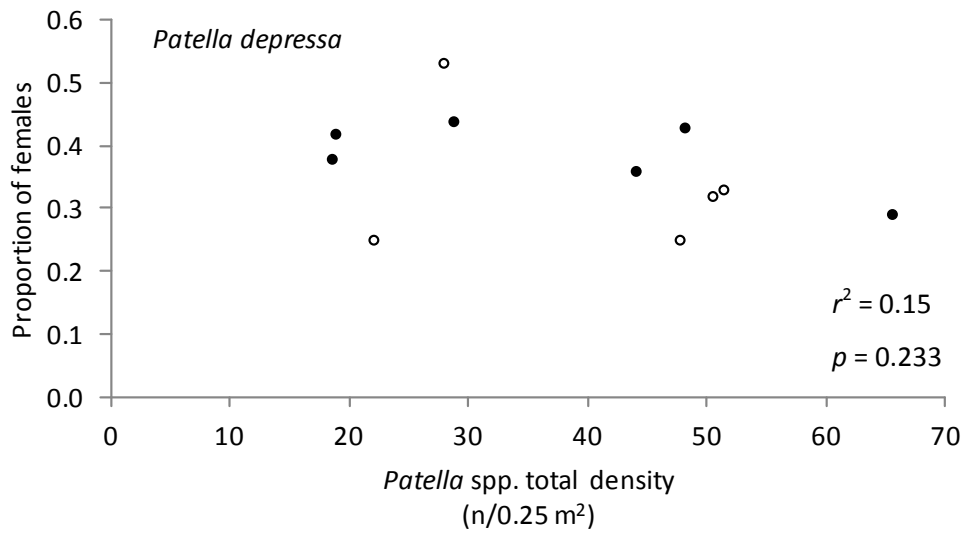
Figure 2.31. *P. depressa* proportion of females by *Patella* spp. density (number of individuals/0.25 m^2) for the sample sites in the south of England (○) and Portugal (●).

2.4.4. Species biomass

In England the highest biomass was attained by *P. vulgata*, followed by *P.ulyssiponensis* (Figure 2.32). At Jennycliff Bay *P. vulgata* biomass was higher than at Heybrook Bay despite the smaller densities (see Figure 2.24). In Portugal *P. depressa* had the highest biomass and the differences between species were more noticeable towards the south (Figure 2.33). The pooled data both for *P. vulgata* and *P. depressa* from England and Portugal of proportion of females plotted against biomass per 0.25m^2 of the species alone and *Patella* spp. combined showed no relationship and is not shown.
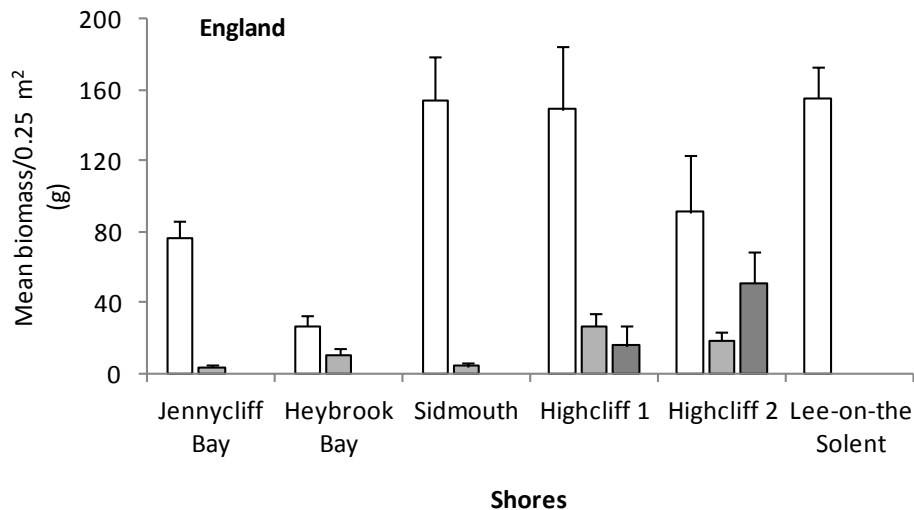
Figure 2.32. Mean biomass (g/0.25 m^2) (+ SE, *n*= 5 to 12 quadrats per site) of *P. vulgata* (clear bars), *P. depressa* (grey bars) and *P. ulyssiponensis* (dark grey bars) from the sample sites in the south of England.
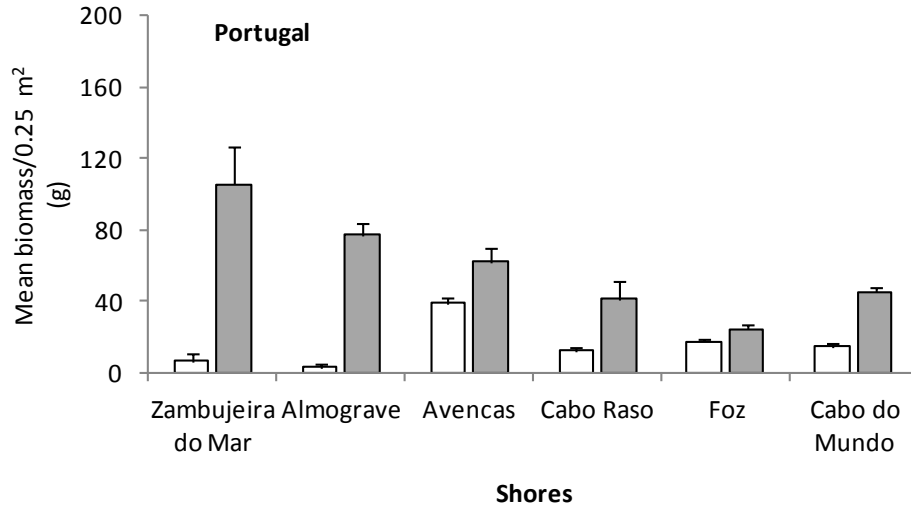
Figure 2.33. Mean biomass (g/0.25 m^2) (+ SE, *n*= 4 to17 quadrats per site) of *P. vulgata* (clear bars) and *P. depressa* (grey bars) from the sample sites in Portugal.

2.4.5. Sex ratio variation with latitude

For *P. vulgata*, the odds of being male rise with latitude to 3.94 times higher at Plymouth than at Alentejo (*Z* = 2.08, *p* = 0.037; Table 2.6). For *P. depressa* in contrast, the odds ratios show no trend with latitude (|*Z*| < 1.62, *p* > 0.1 for all comparisons; Table 2.7), with only the suggestion of a switch from male to female bias from Alentejo to Cascais (odds ratio = 0.76, *Z* = -1.40, *p* = 0.160; Table 2.7), and back to male bias at Plymouth (odds ratio with respect to Alentejo = 1.69, *Z* = 0.98, *p* = 0.329; Table 2.7).

Table 2.6. *P. vulgata* global binary logistic regression of sex versus shore (two factor level with Alentejo as reference) and location (four level factor). **Terms with p < 0.05 are in bold.**

	Z	*p*	Odds ratio
Location= L			
Cascais	0.93	0.352	1.90
Porto	1.31	0.189	2.42
Plymouth	2.08	**0.037**	3.94
Shore= Sh			
2	0.65	0.518	2.00
L*Sh			
Cascais*2	-1.10	0.272	0.28
Porto*2	-1.09	0.277	0.29
Plymouth*2	-0.37	0.715	0.67

Table 2.7. *P. depressa* global binary logistic regression of sex versus shore (two factor level with Alentejo as reference) and location (four level factor).

	Z	*p*	Odds ratio
Location = L			
Cascais	-1.40	0.160	0.76
Porto	-0.29	0.775	0.94
Plymouth	0.98	0.329	1.69
Shore = Sh			
2	1.35	0.176	1.37
L*Sh			
Cascais*2	-1.33	0.184	0.67
Porto*2	-1.62	0.106	0.60
Plymouth*2	-0.98	0.327	0.52

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2.4.6. Gonad stage

Figures 2.34 and 2.35 indicate that on the sampled shores along the English Channel both *P. vulgata* and *P. depressa* had reproductively active animals. The proportion of ripe *P. vulgata* limpets increased from west to east, indicating more advanced reproductive development in the colder eastern channel (Figure 2.34). The proportion of *P. depressa* populations in advanced states of gonad development varied along the English Channel possibly reflecting the species multiple periods of spawning (Moore et al. 2011).
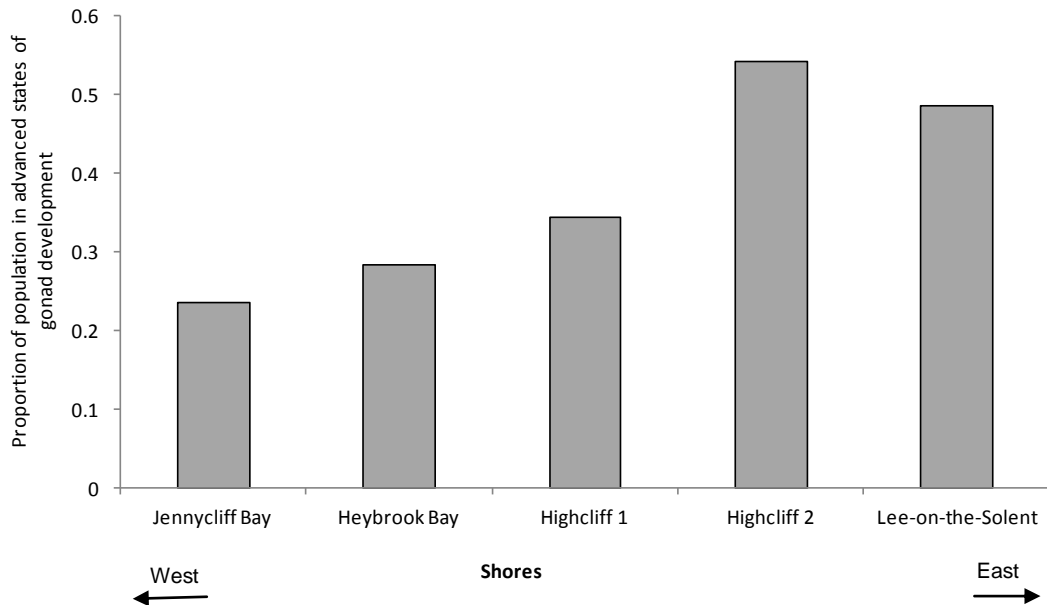
Figure 2.34. Gonad stages (IV+, V+. IV-, following Orton et al. (1956) scale) proportion of *P. vulgata* from the sample sites in the south of England.
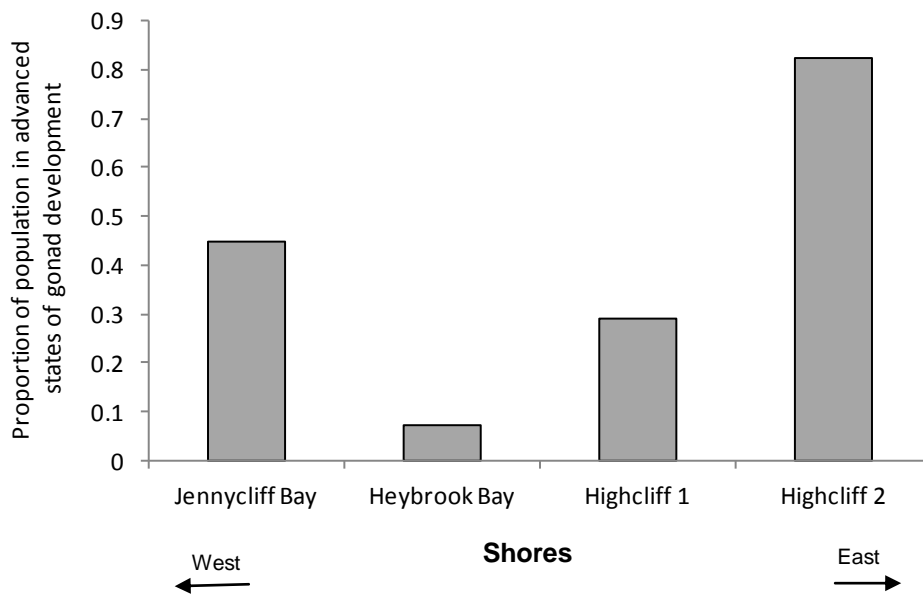
Figure 2.35. Gonad stages (IV+, V+. IV-, following Orton et al. (1956) scale) proportion of *P. depressa* from the sample sites in the south of England.

2.5. DISCUSSION

2.5.1. Protandry

My survey confirmed that protandry appears to be happening strongly in *P. vulgata* especially from England and indicated a possibility of slight protandry also for *P. depressa* from Portugal.

There is strong indirect evidence of the occurrence of protandry in non-harvested *P. vulgata* populations from the south of England: females predominated in larger size classes; cumulative frequency distributions of males and females were different; sex ratios were biased towards the first sex and smallest sizes of males were smaller than the smallest sizes of females. For *P. vulgata* populations from Portugal no such patterns were found: females were interspersed across most size classes; cumulative frequency distributions of males and females did not differ; the proportion of females was higher than in England and smallest sizes of males and females did not differ.

The above observations raise the possibility that *P. vulgata* from Portugal were not protandric. These populations had low densities, and an alternative explanation

would be that due to selective predation by humans on bigger females, males are changing sex at smaller sizes at a high rate to compensate for the female losses. This could result in higher numbers of females, hence the higher proportion of females at low limpet densities (Figures 2.28). Alternatively the same patterns could result from low recruitment occurring in Portugal, generating populations with larger older females with few smaller males. Previous studies on *P. vulgata* from England and Scotland indicated that sex ratios varied between populations (Das and Seshappa 1948; Ballantine 1961; Blackmore 1969; Lewis and Bowman 1975; Baxter 1983) with the majority of small, mature individuals being male and the number of females increasing with size. The sex ratios found ranged from male biased (Ballantine 1961; Baxter 1983) to numbers of males and females approximately equal (Das and Seshappa 1948; Lewis and Bowman 1975). In Portugal, a predominance of females was found in *P. vulgata* samples from the northern and central coast (Ribeiro et al. 2009) while previous studies had mentioned male biased sex ratios (Guerra and Gaudêncio 1986; Castro 2004). In any case, the density especially of new recruits of *P. vulgata* seems to have more influence in determining female numbers suggesting that sex change could be density dependent (Figure 2.28). In other studies, intraspecific competition has appeared to have more influence on *P. vulgata* performance than interspecific competition with *P. depressa* (Boaventura et al. 2002b). The GLM on body size partially corroborated these results: no differences by sex were detected for *P. vulgata* from Portugal but in England there was a sex*site significant interaction. Apart from the GLM of *P. vulgata* size from Portugal, all the other GLMs detected differences by site, emphasizing the likelihood that other variables at the scale of site were determining the sex differences in body size. At Jennycliff Bay *P. vulgata* biomass was higher than at Heybrook Bay but limpet density was smaller. The former shore is moderately sheltered with abundant macro-algae allowing larger sizes (Jenkins and Hartnoll 2001; Davies et al. 2007).

Low densities have promoted sex change in *L. gigantea* with individuals being more likely to change sex in artificially lowered densities (Wright 1989). Wright's (1989) field survey found that females in low densities occurred at younger ages. In a recent study, *L. gigantea* individuals from harvested populations changed sex at smaller sizes and grew at slower rates compared to individuals from protected populations (Fenberg and Roy 2012). A similar mechanism was described for *P. ferruginea*, which seems to be able to adjust sex change to local density of larger

individuals (Rivera-Ingraham et al. 2011). *P. ferruginea* individuals in populations with low density of larger individuals switched to female at smaller sizes (Rivera-Ingraham et al. 2011). Such sex-change plasticity seems also to be occurring in *P. vulgata* when subjected to exploitation (see Chapter 3).

P. depressa both from England and Portugal exhibited patterns expected of a gonochoristic species: females were interspersed across most size classes; cumulative frequency distributions of males and females and smallest sizes of males and females were similar. These results were partially corroborated by the GLM on body size since for *P. depressa* from England where no influence of sex on mean size was detected while in Portugal size differed slightly by sex. Mean size and smallest size graphs (Figures 2.17 and 2.22) also suggest that males were generally slightly smaller than females for *P. depressa* from Portugal. However, these tendencies could be due to differential growth or mortality between males and females rather than protandry or low recruitment. A predominance of males in *P. depressa* from Portugal was mentioned in previous studies (Guerra and Gaudêncio 1986; Castro 2004; Ribeiro et al. 2009) although some data exist on equal numbers of males and females (Brazão et al. 2003). In fact, Boaventura et al. (2003) have suggested that the higher percentages of males found in increased *P. depressa* density treatments could possibly be explained by a suppression of protandry due to competition. Thus the possibility of some protandry occurring in this species remains.

The variability encountered in sex ratios on the present and other studies emphasises that protandry should not be inferred solely from those data. Studies should combine different analyses such as male/female distributions comparisons, microscopy and manipulative experiments.

2.5.2. Geographic range edges

The distributions and relative abundances of *P. vulgata* and *P. depressa* were in accordance with the known range limits for these species. *P. vulgata* was more abundant in the south of England and *P. depressa* was more abundant in Portugal. The test of sex ratio variation with latitude revealed that distorted sex ratios of *P. vulgata* might be involved in setting the species southern range limit, since the likelihood of being male rise with latitude from Alentejo (Portugal) through

Plymouth (England) (Table 2.6). Hypothesis ii and iii could explain the higher proportions of females in populations from Portugal since resource relaxation due to lower intraspecific competition would allow higher rates of promotion of males to females; alternatively intermittent and low recruitment leave populations with few males and older larger females. In England *P. vulgata* is at the centre of its range and sex change is probably occurring due to the male-biased sex ratio found across those populations. A male-biased sex ratio was observed for several protandric limpets (Collin 2006). If skewness in sex at lower latitudes compromises the species reproductive success then the species occurrence would be limited by Allee effects due to the reduction of the numbers of females (e.g., Boukal and Berec 2002; Kent et al. 2003; Walker et al. 2001; Lee et al. 2010). Towards the south the excess of females could also be a compensatory mechanism to human selective predation. *P. depressa* showed no tendency of change in sex ratio with latitude, therefore sex ratios possibly do not influence the species northern limit as it is not protandric.

Climate change is widely recognized as presenting a major threat to global biodiversity (Helmuth et al. 2006; Rosenzweig et al. 2007; Keith et al. 2011). Several studies indicate that species are responding to this threat through changes in behaviour (Post et al. 1999), phenology (Sims et al. 2001) and abundance (Barbraud and Weimerskirch 2001; Moore et al. 2011; Jenouvrier et al. 2012), with consequences for community composition (Holbrook et al. 1997; Walther et al. 2002; Hiddink and ter Hofstede 2008; Keith et al. 2011). The distributional ranges of many species also appear to be shifting to track suitable climatic conditions through space and time (Walther et al. 2005; Mieszkowska et al. 2006; Hawkins et al. 2008, 2009; Keith et al. 2011). Resilience by limpet species to such events could be facilitated by sex change if recruitment is not compromised. Rising temperatures may cause differential mortality rates among individuals but sex-changing populations could then modulate sex change to compensate the losses and balance sex ratios given that survival of recruits is assured. These possibilities suggest that sex changing limpets under these circumstances might be better prepared to face climate change than gonochoristic species, and that this phenomenon should be incorporated in future predictions.

CHAPTER 3

INFLUENCE OF SIMULATED EXPLOITATION ON THE SEX CHANGE AND POPULATION DYNAMICS IN *PATELLA VULGATA*

3.1.   ABSTRACT

Today shellfish are heavily exploited in many parts of both the developed and developing world where some species are now gourmet items on the menu. Mobile grazing herbivores are important target species for human exploitation in many systems and limpets have been harvested commercially for both local consumption and export in several countries. This chapter describes a field experiment designed to simulate human exploitation of limpets in order to evaluate the effects of human exploitation on the ecology of *P. vulgata* (the target species). *P. vulgata* is not a major target species for human consumption today, but due to the occurrence of sex change was considered an appropriate model for investigating the consequences of predation by humans upon the sex change phenomenon. Limpet populations were exploited by systematic removal for 18 months, during which records were kept of limpet density, size frequency and sex ratios, and shore macro-community structure. The hypothesis tested was that selective removal through time of largest limpets would influence the timing and magnitude of several biological processes, including sex change, growth, migration and density regulation. There was strong evidence that the size at sex change (L_{50}) decreased in response to the exploitation treatment, given by analysis of the averaged L_{50} extracted from the logistic regression on each treatment plot. The observed L_{50} at controls (50.5 ± 6.4 mm) suggests that sex change was occurring at a bigger size than expected from the overlap in male and female size classes. The relative size at sex change was invariant across the studied populations, which changed sex when they reached 90% of their maximum size. The study sites located in the south-west of England had considerable differences in community

structure: Trevone was a moderately exposed shore with barnacles and *Fucus* spp. dominated community while Constantine can be considered an exposed shore with a *Mytilus* spp. dominated shore community. Thus the sex change response could be context-dependent and influenced by the community on the rest of the shore. The apparent overlap of male and female size results from the combination of data from many patches. To further understand the sex change in *P. vulgata* it is essential to design experiments that consider differences among patches in size distributions, growth rates, density, mortality rates, sex ratio and mating group opportunities. The knowledge of sex change dynamics in exploited sex changing limpet populations would allow management procedures to counter population decline (being essential to rocky shore conservation and resource management).

3.2.   INTRODUCTION

Shorelines have been exploited for food since prehistoric times as evidenced by shellfish middens at many archaeological sites (McKusic and Warren 1959; Meighan 1959; Waechter 1964; Roubet 1969; Voight 1973; Roche and Texier 1976; Buchanan et al. 1978, 1984; Volman 1978; Schweitzer 1979; Yesner 1984; Buchanan 1988; Cunliffe and Hawkins 1988; Stiner 1994; Yates 1998; Henshilwood et al. 2001; Halketta et al. 2003). Shellfish continue to be heavily exploited around the world where some species are now gourmet items on restaurant menus (Raffaelli and Hawkins 1996). In many developing countries, subsistence collecting of shellfish has been a principal source of protein (see Branch 1975; Siegfried et al. 1985; Duran and Oliva 1987; Hockey et al. 1988; Lasiak 1992; Kyle et al. 1997). Pressure on resources has increased with the rising global human population, particularly in coastal areas, putting many stocks at risk, and changing shellfish collection in many countries from a subsistence activity to a highly profitable commercial operation (Raffaelli and Hawkins 1996).

Many of the harvested species are mobile grazing herbivores (e.g. Kingsford et al. 1991). Limpets have been harvested commercially for both local consumption and export in several countries including Hawaii, South Africa, the Azores and Madeira in Portugal, Chile, Mexico and California in the U.S.A. (Kay and Magruder 1977; Oliva and Castilla 1986; Hockey and Bosman 1986; Martins et al. 1987a,b; Hockey

et al. 1988; Duran and Castilla 1989; Santos et al. 1989; Eekhout et al. 1992; Santos et al. 1995; Pombo and Escofet 1996; Kyle et al. 1997; Hawkins et al. 2000).

The effects of humans as top predators on rocky intertidal communities has been the focus of many studies, particularly in South Africa (Branch 1975; Siegfried et al. 1985; Hockey and Bosman 1986; Hockey and Bosman 1988; Lasiak and Dye 1989; Lasiak 1991,1992), Chile (Moreno et al. 1984, 1986; Castilla and Duran 1985; Castilla 1986; Moreno 1986; Oliva and Castilla 1986; Castilla and Bustamante 1989; Bustamante and Castilla 1990), Costa Rica (Ortega 1987) and Australia (Catteral and Poiner 1987). Although some studies have analysed the implications of harvesting in terms of biomass and gonadal outputs (Branch 1975; Blake 1979; McLachlan and Lombard 1981; Eekhout et al. 1992) most effects of exploitation on populations of intertidal invertebrates have been addressed by comparing sites with and without exploitation, before and after human exclusion. In most cases collectors preferred bigger specimens presenting an extra constraint for protandric species conservation. As the bigger individuals, mainly females, were removed; the reproductive output of the population was being compromised.

This chapter describes a field experiment designed to simulate human exploitation of limpets in order to evaluate the effects of human exploitation on the ecology of *P. vulgata* (the target species). The experiment was carried out in the south-west of England on two replicate shores (Constantine and Trevone) identified as little disturbed by people, having similar tidal range and shore extension and where limpets were not human target species.

The focal species for the experiment, *P. vulgata,* is the species most widely studied in Europe. Although it changes sex from male to female as it grows in size, in common with many exploited species (e.g. some *Patella* spp., *C. oculus* and *L. gigantea*), few studies have addressed experimentally the potential implications of human exploitation on demographic sex ratios (e.g. see Rivera-Ingraham et al. 2011; Fenberg and Roy 2012). This might have to do with the difficulty of following sex change in an individual through its life. Unlike in the genus *Crepidula* spp. there are no visible external sexual characters and removing an individual from the substratum often greatly impacts on its probability of survival. Determining sex in these limpets therefore usually involves killing the animals.

P. vulgata is not a major target species for human consumption today, but due to the occurrence of sex change was considered an appropriate model for investigating

the consequences of predation by humans upon the sex change phenomenon. Since other protandric limpets (*P. ulyssiponesis*, *P. caerulea*, *P. ferruginea*) are at risk from overexploitation and habitat degradation, understanding sex change in the Patellidae can inform future conservation strategies for European rocky shores. Limpet populations were exploited by systematic removal over an 18 month period, during which records were kept of limpet density, size frequency and sex ratios, microalgal film abundance and shore macro-community. The hypothesis tested was that selective removal through time of largest limpets would influence the timing and magnitude of several biological processes, including sex change, growth, migration and density regulation. Since limpets are keystone grazers (Jones 1948; Southward 1964; Hawkins 1981a; Hawkins and Hartnoll 1983; Jenkins et al. 2005, 2008; Coleman et al. 2006), removals would be likely to affect the whole rocky shore community, and this was assessed by recording the abundances of macro-algae and macro-benthic invertebrates (see Chapter 4).

3.3. MATERIAL AND METHODS

3.3.1. Pilot study

An initial pilot study was performed along the south-west coast of England to determine the two rocky shores to use for the experiment. Representativeness of the sampling methods was determined by recording densities, size/sex frequencies and mortality of tagged *Patella* spp. The pilot study led to implementation of the following:

- Plot extension was defined as 3×3 m, on the basis of the available surface found.
- Paint tagging sufficed to record individual histories, on the basis of no mortality being recorded due to paint application, from 20 tagged limpets.
- 10 quadrats per plot, each 0.5×0.5 m, were sufficient to assess limpet density, on the basis of the plotted density against number of quadrats.
- Five quadrats per plot, each 0.5×0.5 m, were sufficient to assess macro-algae and macro-benthic invertebrates abundances, on the basis of time available and recommendations of colleagues.

3.3.2. Study organisms and sites

The limpet *P. vulgata* is the dominant grazer in the mid-intertidal zone of the south-west of England and is distributed across the wave exposure gradient. *P. depressa* also occurs on open rock and *P. ulyssiponensis* can be encountered in mid-shore depressions and rock pools but is far more abundante lower on the shore (Raffaelli and Hawkins 1996; Little and Kitching 1998). These species feed primarily on the microalgal film that coats the rocky intertidal shore, although recent work has shown that they can also consume adult macro-algae (Davies et al. 2007). Food availability is indirectly linked to the rock surface available, season of the year and the shore hydrodynamics. Individual *P. vulgata* pass through a neuter immature stage which can be followed by a male and subsequently a female stage. Some individuals may remain the same sex throughout their live, as both small females and

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large males are occasionally found. Indeed this phenomenon of protandry (sequential sex change from male to female) in the Patellidae has been known since the early work of Orton (1920, 1928, 1946) and colleagues (Dodd 1956; Orton et al. 1956; Orton and Southward 1961) and cytological work by Bacci (1952, 1965). *P. vulgata* is the sex changing species that has been most widely studied in Europe, although data exist also for sex change in the edible *P. ulyssiponesis* (Thompson 1979), *P. caerulea* (Montalenti 1958; Bacci 1965), *P. ferruginea* (Frenkiel 1975; Espinosa et al. 2009a) and *P. rustica* (Bacci 1975). In most of the cases knowledge of sex change patterns comes mainly from the interpretation of sex-age/size data from field collections. Indeed, the preponderance of males in smaller size classes, and females in the larger ones, suggests that other species may also be protandric hermaphrodites. That being the case, removal of bigger limpets (predominantly females) may possibly induce the sex change of the resident males.
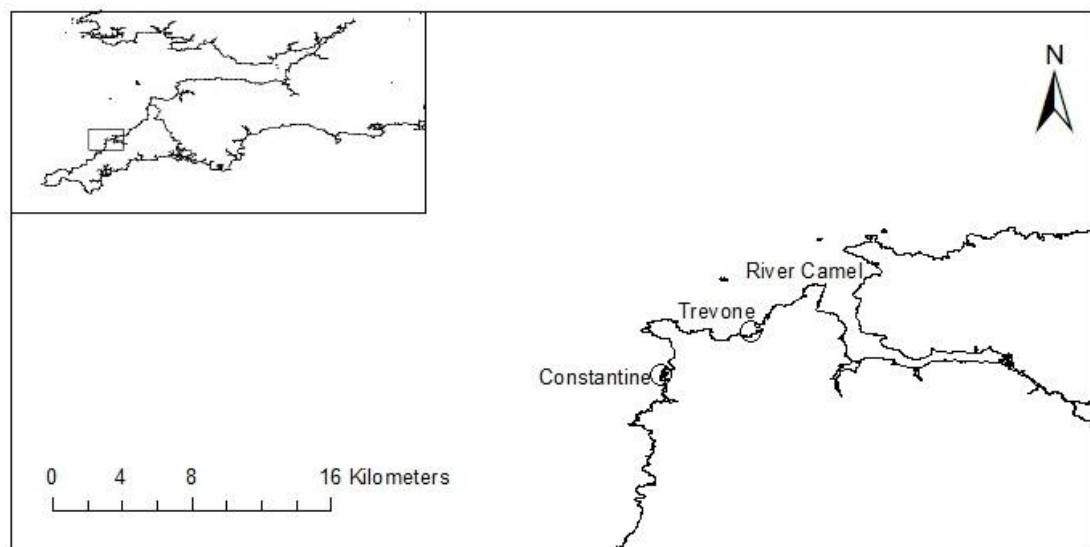
Figure 3.1. Map showing coast line of Cornwall and the locations of the two study sites: Constantine and Trevone (circle symbols). Inset map shows the position of the sites in the south-west of England.

The study was carried out at Constantine (50º31'52.02" N, 5º01'33.32" W) and Trevone (50º32'40.48" N, 4º58'50.08" W) on the west coast of Cornwall, UK (Figure 3.1). The simulated exploitation treatment involved removing the biggest limpets at two different rates in accordance with what happens in most exploited limpet populations, where bigger sizes are preferentially removed by collectors (e.g.

Branch 1975; Duran and Oliva 1987; Hockey et al. 1988; Espinosa et al. 2009b). Thus on each shore, the three limpet species were targeted as a single resource. However, due to extremely low abundances during the experiment of *P. depressa* and *P. ulyssiponensis*, here I only present responses of *P. vulgata* populations.

Before running the experiment, measures were taken from one 0.5×0.5 m sampling quadrat in each 3×3 m plot of the un-manipulated density, size and sex structure of limpet populations and also of macro-benthic invertebrates, and macro-algal abundances.

The simulated exploitation was performed every three months from March 1999 to October 2000. Data were collected on:

- Limpet sex change rate.
- Limpet growth through increments in shell major length.
- Food availability through the assessment of microalgal film abundance.
- Recruitment and limpet migrations into and out of the experimental plots.
- Limpet density and size frequency.
- Macro-algae and macro-benthic invertebrate abundances.

All results are presented at the scale of the sampling quadrat.

3.3.3. Limpet removal treatments

At the high exploitation treatments (henceforth referred to as 'H') all limpets with a base length shell ≥ 25 mm were removed at each visit. At the low exploitation treatments (L), all limpets with a base length of shell ≥ 25 mm were removed from half of the available area, by sampling from alternate 18 squares in a grid of 0.5×0.5 m quadrats covering the plot, starting from one randomly attributed quadrat. No limpets were removed from the control treatments (C). The 18-month experiment included six limpet removal events, one every three months.

3.3.4. Experimental design

At each of the two shores, three adjacent blocks of shore, about 20 m wide were defined at the mean tide level (MTL). In each block, three unfenced treatment plots

of 3×3 m were randomly placed on the available rock surface (Figure 3.2). Plots were sited away from large rock pools where possible. Since limpets can reach up to 1.21 m from their home scar (Hartnoll and Wright 1977), to ensure independence of plots, they were all separated by at least 5 m and no limpet was observed to move from one plot to another. Below I describe the statistical analysis, and the five different measures of response to the treatments.
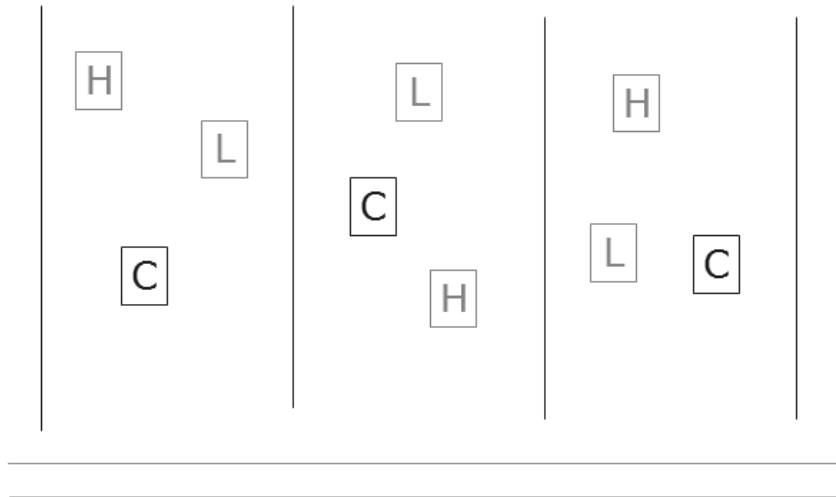
Figure 3.2. Diagrammatic representation of the experimental design. At each shore, three adjacent blocks of shore (~ 20 m wide) were defined at the mean tide level (MTL) facing the sea (unbroken horizontal lines), and included three unfenced treatment plots of 3x3 m, randomly allocated to one of two rates of exploitation: high (H), low (L), or an unexploited control (C).

3.3.4.1.   Statistical analysis

A two-factor split-plot design (equivalent to model 5.6 and 6.3 iii in Doncaster and Davey, 2007) was used to test the hypothesis for the end of the experiment, that selective removal through time of largest limpets would influence timing and magnitude of several biological processes (sex change, growth, migration and density responses: model 1, Table 3.1).

This design had three levels of the fixed treatment factor (T_3), at each of two random levels of a location factor (L'_2). Three replicate random blocks (B'_3) were

nested in each level of L and responses of observations over time were taken on each block at each level of T. This design was analysed with the ANOVA model:

$$Y = T|B'(L') \qquad\qquad\qquad\qquad \text{model 1}$$

where henceforth a prime represents a random factor, vertical line means 'crossed with' and parenthesis means 'nested in'. Using the Minitab statistics package, the model was tested by requesting analysis of terms: $= T|L' + T|B'(L) - T*B'(L)$, producing an output with the structure described by Table 3.1.

The use of Location as a random factor was dictated by the aim to extrapolate the final results to the south-west coast of England from which the two locations were selected. The residual variation ($P'(T*B'(L'))$) could not be estimated due to the absence of treatment replicates in each block. Therefore the interaction of treatment with block [$T*B'(L')$] was not testable, but its mean square (MS) provided the error term for testing the treatment-by-location interaction ($T*L'$).

Although the treatment effect was tested with only 2 error degrees of freedom, if the interaction between Location and Treatment was not detectable at $\alpha = 0.25$, it was pooled with the error term to provide a more powerful test for the treatment effect with 10 error degrees of freedom (*post hoc* pooling, following Underwood 1981, 1997).

Table 3.1. ANOVA model 1 terms, degrees of freedom (d.f.), components of variation and MS for *F* ratios.

Mean square	d.f.	Components of variation estimated in population	F-ratio
Between subjects			
L´	1	P´(T*B´(L´))+B´(L´)+L´	L´/B´(L´)
B´(L´)	4	P´(T*B´(L´))+B´(L´)	No test
Within subjects			
T	2	P´(T*B´(L´))+T*B´(L´)+T*L´+T	T/ T*L´
T*L´	2	P´(T*B´(L´))+T*B´(L´)+T*L´	T*L´/T*B´(L´)
T*B´(L´)	8	P´(T*B´(L´))+T*B´(L´)	No test
P´(T*B´(L´))	0	P´(T*B´(L´))	-

The hypothesised sex change response was that manipulating the rate of exploitation would alter the pattern of sex change. If this did not cause heavily exploited plots (bigger limpets being selectively removed at higher rates) to have the most truncated frequency distributions of *P. vulgata* body size and sex, it would suggest that limpets had changed sex during the experiment to compensate for the loss of females (Hypothesis A). In this case, mean size and size at sex change (L_{50}) would be smaller in exploitation treatments and size frequency less skewed than expected if no sex change was occurring.

Alternatively, more heavily truncated size and sex distributions under harvesting would suggest that limpets had not changed sex quickly enough during the experiment to compensate for the loss of females (Hypothesis B). In this case, mean size of females and L_{50} could be explained by alternative processes:

− Some sex change having occurred: some (or all) the bigger males switching to females (but at too slow a rate to compensate the female loss), and/or

− No sex change having occurred: the observed females are the ones that escaped predation.

In order to test these predictions, model 1 was expanded to include a fixed cross factor of sexual stage (S_3) with levels of neuter, male, female. Stage-specific differences in body size and abundance in response to selective predation were investigated with the three-factor split-plot ANOVA model:

$$Y = S|T|B'(L') \qquad\qquad \text{model 2}$$

In Minitab the following analysis of terms was requested: $= S|T|L' + S|T|B'(L) − S*T*B'(L)$, producing an output with the structure described by Table 3.2.

Two sets of planned *a priori* contrasts were used to detect differences between Treatment and Stage effects. Treatment contrasts partitioned the sums of squares of the exploitation treatment, with a first contrast of no exploitation (NE: control) and exploitation (EX: low and high pooled), and a second contrast of exploitation levels (low vs. high). Stage contrasts partitioned the sums of squares of sexual stages, with a first contrast of no sex (NS: neuters) to sex (SE: males and females pooled) and a second contrast between the sexes (males vs. females).

Because the removal of limpets increased the available space, model 1 was applicable to the investigation of variation in the microalgal film and consequently its impact on limpet growth. The model was augmented with a fixed cross factor of

Table 3.2. ANOVA model 2 terms, degrees of freedom (d.f.), components of variation and MS for *F* ratios.

Mean square	d.f.	Components of variation estimated in population	F-ratio
Between subjects			
L´	1	P´(S*T*B´(L´))+B´(L´)+L´	L´/B´(L´)
B´(L´)	4	P´(S*T*B´(L´))+B´(L´)	No test
Within subjects			
T	2	P´(S*T*B´(L´))+T*B´(L´)+T*L´+T	T/T*L´
T*L´	2	P´(S*T*B´(L´))+T*B(L´)+T*L´	T*L´/T*B´(L´)
T*B´(L´)	8	P´(S*T*B´(L´))+T*B´(L´)	No test
S	2	P´(S*T*B´(L´))+S*B´(L´)+S*L´+S	S/S*L´
S*L´	2	P´(S*T*B´(L´))+S*B´(L´)+S*L´	S*L´/S*B´(L´)
S*B´(L´)	8	P´(S*T*B´(L´))+S*B´(L´)	No test
S*T	4	P´(S*T*B´(L´))+S*T*B´(L´)+S*T*L´+S*T	S*T/S*T* L´
S*T*L´	4	P´(S*T*B´(L´))+S*T*B´(L´)+S*T*L´	S*T* L´/S*T*B´(L´)
S*T*B´(L´)	16	P´(S*T*B´(L´))+S*T*B´(L´)	No test
P´(S*T*B´(L´))	0	P´(S*T*B´(L´))	-

distance (D_2). This factor tested for differences in microalgal film abundance from the edge to the centre of the plot, which could be due to outsiders limpets foraging on the edge of the plots. The design was a three-factor split-plot ANOVA model:

$Y = D|T|B'(L')$ model 3

In Minitab the following analysis of terms was requested: $= D|T|L' + D|T|B'(L) - D*T*B'(L)$, producing an output with the structure described by Table 3.3.

Limpet density responses were investigated with a three-factor model with repeated measures on two factors: season (Se) and treatment (T):

$Y = Se|T|B'(L')$ model 4

In Minitab the analysis of terms: $= Se|T|L' + Se|T|B'(L) - Se*T*B'(L)$ was requested producing an output with the structure described by Table 3.4.

For all ANOVAs, visual inspection of the residuals following transformations indicated normal distributions and homogeneous variances.

All three-factor ANOVA analyses (models 2, 3 and 4) used a restricted 'model 2' analysis of random factors, and assumed that all mean squares for block-by-treatment interactions measured the same quantity, being pooled into a single variance component for the error term (Doncaster and Davey 2007: http://www.soton.ac.uk/~cpd/anovas/datasets/Model%202.htm, 2011).

Table 3.3. ANOVA model 3 terms, degrees of freedom (d.f.), components of variation and MS for F ratios.

Mean square	d.f.	Components of variation estimated in population	F-ratio
Between subjects			
L´	1	P´(D*T*B´(L´))+B´(L´)+L´	L´/B´(L´)
B´(L´)	4	P´(D*T*B´(L´))+B´(L´)	No test
Within subjects			
T	2	P´(D*T*B´(L´))+T*B´(L´)+T*L´+T	T/T*L´
T*L´	2	P´(D*T*B´(L´))+T*B(L´)+T*L´	T*L´/T*B´(L´)
T*B´(L´)	8	P´(D*T*B´(L´))+T*B´(L´)	No test
D	1	P´(D*T*B´(L´))+D*B´(L´)+D*L´+D	D/D*L´
D*L´	1	P´(D*T*B´(L´))+D*B´(L´)+D*L´	D*L´/D*B´(L´)
D*B´(L´)	4	P´(D*T*B´(L´))+D*B´(L´)	No test
D*T	2	P´(D*T*B´(L´))+D*T*B´(L´)+D*T*L´+D*T	D*T/D*T*L´
D*T*L´	2	P´(D*T*B´(L´))+D*T*B´(L´)+D*T*L´	D*T*L´/D*T*B´(L´)
D*T*B´(L´)	8	P´(D*T*B´(L´))+D*T*B´(L´)	No test
P´(D*T*B´(L´))	0	P´(D*T*B´(L´))	-

Table 3.4. ANOVA model 4 terms, degrees of freedom (d.f.), components of variation and MS for *F* ratios.

Mean square	d.f.	Components of variation estimated in population	F-ratio
Between subjects			
L´	1	P´(Se*T*B´(L´))+B´(L´)+L´	L´/B´(L´)
B´(L´)	4	P´(Se*T*B´(L´))+B´(L´)	No test
Within subjects			
T	2	P´(Se*T*B´(L´))+T*B´(L´)+T*L´+T	T/T*L´
T*L´	2	P´(Se*T*B´(L´))+T*B(L´)+T*L´	T*L´/T*B´(L´)
T*B´(L´)	8	P´(Se*T*B´(L´))+T*B´(L´)	No test
Se	5	P´(Se*T*B´(L´))+Se*B´(L´)+Se*L´+Se	Se/Se*L´
Se*L´	5	P´(Se*T*B´(L´))+Se*B´(L´)+Se*L´	Se*L´/Se*B´(L´)
Se*B´(L´)	20	P´(Se*T*B´(L´))+Se*B´(L´)	No test
Se*T	10	P´(Se*T*B´(L´))+Se*T*B´(L´)+Se*T*L´+Se*T	Se*T/Se*T*L´
Se*T*L´	10	P´(*T*B´(L´))+Se*T*B´(L´)+Se*T*L´	Se*T*L´/Se*T*B´(L´)
Se*T*B´(L´)	40	P´(Se*T*B´(L´))+Se*T*B´(L´)	No test
P´(Se*T*B´(L´))	0	P´(Se*T*B´(L´))	-

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3.3.5. Limpet sex change response

At the end of the experiment all limpets regardless of their size were removed from the plots under the different exploitation pressures. The limpets were frozen and their sex determined in the laboratory by direct observation of the gonad aided with a microscope when visual inspection was inconclusive.

The influence of the exploitation treatment on sex change was tested for limpet mean sizes and size at sex change following Collin (2006) using relative abundances of sexual stages and sex-size frequencies.

With the removal of the bigger individuals mainly depleting the female population it was hypothesised that the mean size of remaining males would be smaller at the end of the experiment due the transition of the bigger males to females. Consequently, the mean sizes of neuters and females would also be smaller than in the controls. Probably sex change would occur at a smaller size resulting in smaller mean sizes of the different sexual stages relatively to control plots. It was further hypothesised that the sex change response would vary under different levels of exploitation, occurring at smaller body sizes where the simulated exploitation was higher (and hence fewer female limpets were left). The sex change response was analysed with a three-factor split-plot ANOVA model comparing of the mean sizes of the different sexual stages at the end of the experiment, detailed in section 3.4.1.1 below.

Collin (2006) developed the use of L_{50} as the size at sex change representing the size at which 50% of animals were female as a response variable. As males and females size classes overlap, the size at sex change (L_{50}) is the size at which 50% of animals are males and 50% females. I estimated this for *P. vulgata* using the logistic regression approach adopted by Allsop and West (2003a, b).

For each treatment plot a logistic regression of sexual stage (sex: males and females) versus (size) was performed to extract the L_{50} which was then averaged across plots. Neuters were excluded because the method of Collin (2006) only considers the mature sexual stages. The value of L_{50} is extrapolated from the following logistic regression equation on body size:

$$\text{Log }[P(\text{male})/P(\text{female})] = A - B \times \text{Size}$$

The L_{50} is the body size A/B at which an individual is as likely to be male as female, and therefore the log-ratio equals zero.

A Global binary logistic regression of log [P(male)/P(female)] versus size (covariate), treatment (three level factor) and site (two level factor) was run to investigate the influence of rate of exploitation on *P. vulgata* L_{50}, detailed in section 3.4.1.2 below. This analysis ignored blocks which would otherwise be treated in a logistic regression as a fixed rather than random factor. In effect, the logistic regression is consistent with an ANOVA restricted 'model 2' analyses, where negligible variation is assumed between blocks in the response to each lower-order treatment term (Doncaster and Davey 2007).

Any response in body size at sex change would also alter the relative abundances of the sexual stages. The loss of females under high and low exploitation would be compensated by a reduction of numbers in neuters and males. These would be predicted to switch to the next sexual stage faster (and hence at smaller sizes) relative to the control plots. Reduction in numbers of males could in turn be compensated by neuters changing prematurely to males and the depletion of neuters being offset by recruitment.

Size frequencies for the different sexes in the different treatments were plotted to illustrate the shifts in size and numbers of the sexual stages.

I tested whether the relative size at sex change is invariant across these two limpet populations by plotting log (L_{50}) against log (L_{max}) for each treatment plot from both shores on the null prediction of a slope of 1.0. L_{max} was calculated as the top 2.5% quantile for each size-frequency data. The averaged value across treatments of L_{50}/L_{max} will be the relative averaged size of sex change.

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3.3.6. Responses of limpet growth and micro-algae film abundance

Between 30 and 40 *P. vulgata* individuals with shell lengths of 17.5 ± 2.50 mm (between 15 and 20 mm) were double tagged at each treatment plot at the beginning of the experiment at Constantine and Trevone. Growth in body size was measured from 1999 to 2000 once in each of spring, summer and autumn. Two numbers were stuck to each individual with cyanocrylate glue to reduce the probability of losing tags. Due to losses of individuals and tags, the number of tagged individuals used to calculate the growth rate of each plot was reduced to the minimum number of six individuals found in all three sampling seasons (spring, summer, autumn) in 1999 (considered a representation of the whole experiment). For each individual, its major-axis shell length was recorded on a sheet of paper with a pointed compass which was then measured in the laboratory with an electronic calliper to 0.1 mm precision, since each measurement could have some degree of error due to the elliptic limpet shell shape. Growth rates were calculated for each individual from its total change in length between spring and autumn 1999, expressed in mm per month. The growth-rate response was compared across treatments with a two-factor split-plot ANOVA model (model 1, Table 3.1, as detailed in section 3.4.2 below).

The abundance of microalgal film was compared between different rates of exploitation in June 2000, using chlorophyll *a* as an index of standing crop for the plots from the two shores. The level of chlorophyll *a* in each sample was estimated using the method for rock substrata described by Thompson et al. (1999). Samples were initially washed and hydrated in filtered sea water. Extraction was made using cold methanol over a period of five to 24 hours and absorbance of the resulting solution determined at 665 and 750 nm. The surface area of each rock sample was determined by image analysis and the level of chlorophyll *a* per unit area of rock surface calculated using the following equation:

$$\text{Chlorophyll } a \text{ concentration } \mu g.mm^{-2} = (13.0 \times Å_{net} \times v) / d \times a$$

where $Å_{net}$ = net absorbance of chlorophyll solution ($Å665 – Å750$), v = volume of solution (ml), d = path length of cell (mm), a = surface area of sample (mm^2).

Initially a random sample from each plot of 18 rock chips free of barnacles and algal encrusted rock with a minimum surface area of 200 mm^2 was taken using a

hammer and a fine chisel (blade size, 10-20 mm). As no differences in standing crop were detected following the ANOVA model 1 (Table 3.1), another sample of nine rock chips was randomly taken from within the edge (500 mm) of each plot and other nine from the centre of each plot to investigate differences following a three-factor split-plot ANOVA model (model 3, Table 3.3).

3.3.7. Recruitment and limpet migratory response

The three sampling seasons of spring, summer, autumn in 1999 were considered representative of the whole experiment for testing movement patterns. Before each removal procedure, all incoming limpets were painted with a single different colour to established limpets (spring: white, summer: yellow, autumn: green) in order to look for patterns of movements amongst treatments. Ten random quadrats, each of 0.5×0.5 m and subdivided by fishing line into 49 intersection points, were used in each treatment plot to assess shell colour, size frequency data, and limpet species. Recruits into treatments plots were registered for summer and autumn 1999 and no limpet was observed to have moved out of its original plot.

3.3.8. Limpet density responses

Density and size frequencies for each treatment plot were assessed with 10 random quadrats of 0.5×0.5 m, using a pointed compass to measures size, during three main seasons (spring, summer and autumn) over the 18 months experiment. The density response through time to the treatments was investigated with repeated measures ANOVA (model 4, Table 3.4, as detailed in section 3.4.4 below).

3.3.9. Responses of macro-algae and macro-benthic invertebrates abundance

Macro-algal percentage cover and macro-benthic invertebrates abundance (mussels *Mytilus galloprovincialis, Mytilus edulis*, hybrids and barnacles*: Chthamalus stellatus, Chthamalus montagui*, *Semibalanus balanoides*) were

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recorded using five random quadrats, each of 0.5×0.5 m and subdivided by fishing line into 49 intersection points. Estimation of the percentage cover was done using the point intersect method and presences counted at the intersections. Records were additionally taken of any species present inside the quadrat that did not extend to an intersection point. Since shores are three-dimensional structures, whenever large algae (e.g. *Fucus* spp.) were present, sampling was stratified in different layers and canopy cover was distinguished from substrate cover. These percentage covers were used to assess the wave exposure of the sites, which might condition the responses investigated (see Chapter 4 for details).

3.4.   RESULTS

3.4.1. Limpet sex change

3.4.1.1   Mean size comparisons at the end of the experiment

Mean body size of *P. vulgata* neuters changed little under different levels of exploitation, while males and females both decreased markedly in size with exploitation (Figure 3.3).

Table 3.5 shows that the mean size in the unexploited control differed from the two exploitation treatments, which themselves differ between each other, both as main effects and in the interaction with stage (Figure 3.3). There were no detectable differences by location. In effect, *P. vulgata* was larger in the absence of exploitation (control: 31.5 ± 5.0 mm) than with exploitation (low and high exploitation levels pooled: 25.8 ± 2.2 mm, $F_{1, 2} = 40.46$, $p = 0.024$; Table 3.5).

On the other hand, the limpets were significantly bigger under low exploitation (27. 7± 3.8 mm) compared to high exploitation (23.9 ± 2.3 mm, $F_{1, 2} = 19.82$, $p = 0.047$; Table 3.5). Nevertheless the mean sizes of different sexual stages depended on an interaction with the exploitation treatment ($F_{4, 4} = 123.81$, $p < 0.001$).

Table 3.5 reveals that the S*T interaction included contrasts between neuter and sexual stages by treatment ({NS vs. SE}*T, $F_{2, 4} = 223.41$, $p < 0.001$), and between the sexes by treatment {males vs. females}*T, $F_{2, 4} = 23.45$, $p = 0.006$).

The stage main effect ($F_{2, 2} = 286.63$, $p = 0.003$; Table 3.5) had contrasts revealing that neuters (16.8 ± 0.5 mm) were smaller than mature individuals (male and females pooled: 33.1 ± 1.8 mm, $F_{1, 2} = 508.07$, $p = 0.002$). On the other hand males (30.1 ± 2.3 mm) were significantly smaller than females (36.2 ± 2.4 mm) ($F_{1, 2} = 64.74$, $p = 0.015$; Table 3.5).
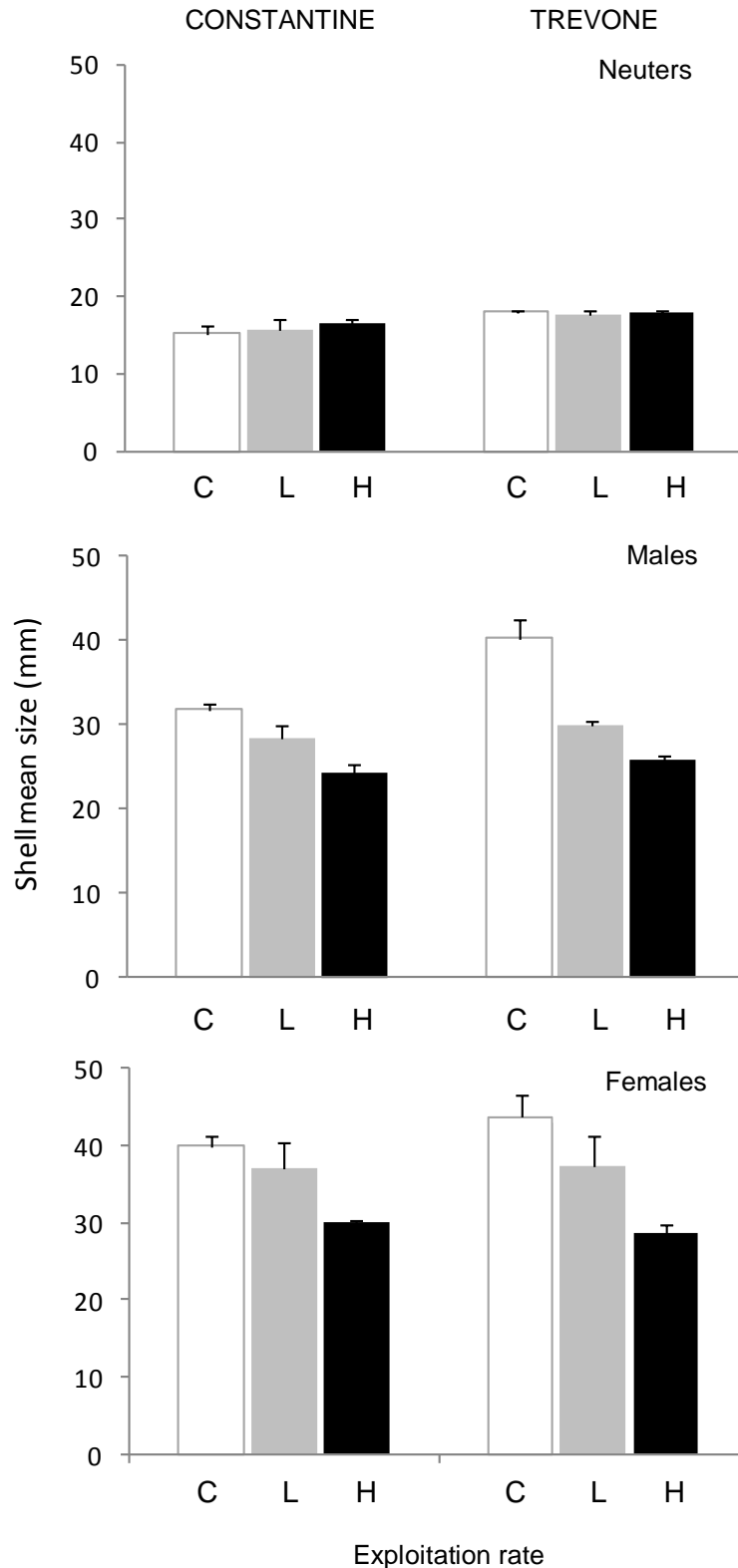
Figure 3.3. Mean body sizes (+ SE, n = 3 plots per sample) of *P. vulgata* neuters (top row), males (middle row) and females (bottom row) at the end of the experiment, by treatment (control (C): clear bars; low (L): grey bars; high (H): black bars) and location (left: Constantine, right: Trevone).

Table 3.5. Response of *P. vulgata* mean size at the end of the experiment (ANOVA model 2) at different sexual stages (neuters; males; females) by exploitation treatment (control; low; high) and location. Exploitation orthogonal contrasts are shown partitioning the treatment SS between no exploitation (NE: control) and exploitation (EX: pooled low + high) and between exploitation levels (low vs. high). Stage orthogonal contrasts are shown partitioning the stage SS between not sexed (NS: neuters) and sexed (SE: males and females pooled) and between the sexes (males vs. females).**Terms with p < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Location = L´	1	32.42	32.42	1.45	0.295
Block = B´(L´)	4	89.39	22.35	-	
Within subjects					
Treatment = T	2	388.78	194.39	30.16	**0.032**
NE vs. EX	1	260.94	260.94	40.46	**0.024**
low vs. high	1	127.84	127.84	19.82	**0.047**
L´*T	2	12.89	6.45	1.08	0.353
L´*{NE vs. EX}	1	11.80	11.80	1.97	0.170
L´*{low vs. high}	1	1.09	1.09	0.18	0.674
Stage = S	2	3379.62	1689.81	286.63	**0.003**
NS vs. SE	1	2997.63	2997.63	508.07	**0.002**
males vs. females	1	381.99	381.99	64.74	**0.015**
S*L´	2	11.79	5.90	0.98	0.385
{NS vs. SE}*L´	1	1.78	1.78	0.30	0.588
{males vs. females}*L´	1	10.01	10.01	1.67	0.206
S*T	4	251.79	62.95	123.43	**< 0.001**
S*{NE vs. EX}	2	146.71	73.36	143.84	**< 0.001**
S*{low vs. high}	2	105.08	52.54	103.02	**< 0.001**

...continued

{NS vs. SE}*T	2	227.87	113.94	223.41	**< 0.001**
{males vs. females}*T	2	23.92	11.96	23.45	**0.006**
S*L´*T	4	2.03	0.51	0.08	0.986
S*L´*{NE vs. EX}	2	1.29	0.65	0.11	0.896
S*L´*{low vs. high}	2	0.74	0.37	0.06	0.942
{NS vs. SE}*L´*T	2	1.28	0.64	0.11	0.899
{males vs. females}*L´*T	2	0.75	0.37	0.06	0.940
Error	32	191.58	5.99		

3.4.1.2   Size at sex change (L_{50})

The average L_{50} extracted from the logistic regression on each treatment plot shows that the size at sex change decreased with increased exploitation (control: 50.5 mm ± 6.4, low: 42.6 mm ± 1.5, high: 32.2 mm ± 0.2, Figure 3.4). This result was corroborated by the global multiple logistic regression showing a treatment*size interaction ($p < 0.01$, Table 3.6), given by the log [P(male)/P(female)] decreasing with increasing size in the high exploitation treatment relative to its change in size in the control (pooled across locations). The main effect of size ($p < 0.01$) shows an overall decrease in log [P(male)/P(female)] with each unit of increase in size, when pooling across treatments and locations.
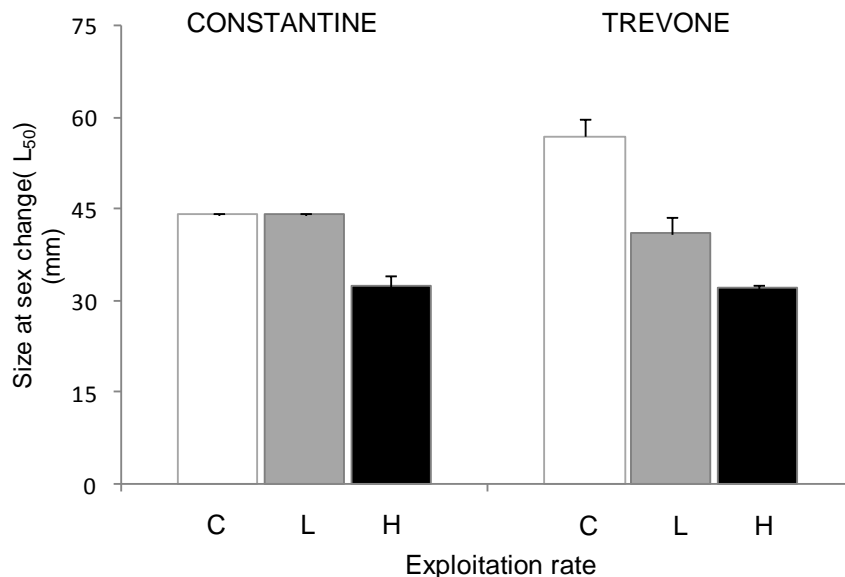
Figure 3.4. *P. vulgata* size at sex change (L_{50}) (+ SE, *n*= 3 plots per sample) at the end of the experiment by treatment plot (control (C): clear bars; low (L): grey bars; high (H): black bars) at Constantine (left) and Trevone (right). The L_{50} was obtained for each plot from a logistic regression of sexual stage (sex: males and females) versus (size).

Table 3.6. Global binary logistic regression of log [P(male)/P(female)] versus size (covariate), treatment (three level factor) and location (two level factor). Total number of observations = 2016.**Terms with p < 0.05 are in bold.**

	Coefficient	**SE Coefficient**	***p***
Constant	3.88	0.52	**< 0.01**
Size = Si	-0.09	0.01	**< 0.01**
Treatment = T			
low	-0.25	0.65	0.70
high	1.39	0.82	0.09
Location = L			
Trevone	-0.85	0.87	0.33
T*Si			
low	0.00	0.02	0.99
high	-0.08	0.03	**< 0.01**
L* Si			
Trevone	0.04	0.02	0.09
T*L			
low* Trevone	0.06	1.08	0.96
high*Trevone	-1.28	1.29	0.32
T*L*Si			
low* Trevone	-0.02	0.03	0.50
high*Trevone	0.03	0.04	0.42

3.4.1.3   Density comparisons at the end of the experiment

Table 3.7 shows a stage*location interaction ($F_{2, 32} = 21.92$, $p < 0.001$) and within this, an interaction of the NS vs. SE contrast with location ($F_{1, 32} = 42.56$, $p < 0.001$). Figure 3.5 correspondingly suggests a higher neuter abundance at Trevone which increases with increased exploitation. This was corroborated by Table 3.7 that shows an interaction of the NS vs. SE contrast with location and treatment ($F_{2, 32} = 4.40$, $p = 0.021$) despite the non-significant stage*location*treatment interaction. However, no differences were observed for the *P. vulgata* density under different rates of exploitation and at different sexual stages.
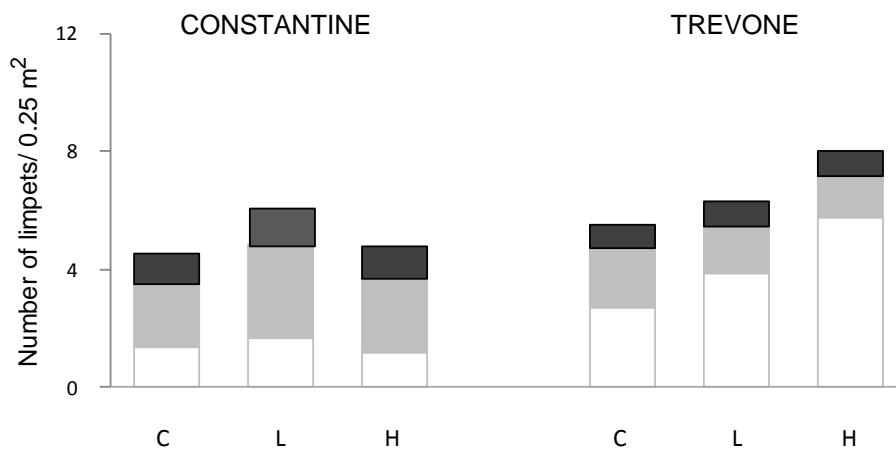
Figure 3.5. Total number (scaled to sampled quadrat) of *P. vulgata* neuters (clear bars), males (grey bars) and females (black bars) removed at the end of the experiment, by treatment of control (C) low exploitation (L), high exploitation (H), and by location at Constantine (left) and Trevone (right).

Table 3.7. Response of *P. vulgata* numbers per quadrat at the end of the experiment (ANOVA model 2) at different sexual stages (neuters; males; females) by exploitation treatment (control; low; high) and location. Orthogonal contrasts are shown partitioning the stage SS between not sexed (NS: neuters) and sexed (SE: males and females pooled) and between the sexes (males vs. females).**Terms with p < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Location = L´	1	3.28	3.28	0.87	0.403
Block = B´(L´)	4	15.02	3.75	-	
Within subjects					
Treatment = T	2	2.27	1.13	0.94	0.515
L´*T	2	2.41	1.20	1.54	0.230
Stage = S	2	28.77	14.39	0.84	0.544
NS vs. SE	1	17.16	17.16	1.00	0.423
males vs. females	1	11.61	11.61	0.68	0.496
S*L´	2	34.27	17.14	21.97	**< 0.001**
{NS vs. SE}*L´	1	33.27	33.27	42.65	**< 0.001**
{males vs. females}*L´	1	1.00	1.00	1.28	0.266
S*T	4	4.69	1.17	0.63	0.668
{NS vs. SE}*T	2	4.54	2.27	1.22	0.386
{males vs. females}*T	2	0.15	0.75	0.40	0.695
S*L´*T	4	7.45	1.86	2.38	0.072
{NS vs. SE}*L´*T	2	6.86	3.43	4.40	**0.021**
{males vs. females}*L´*T	2	0.59	0.29	0.37	0.693
Error	32	25.01	0.78		

3.4.1.4   Size frequencies at the end of the experiment

The sex/size-frequency structure at the end of the experiment (Figure 3.6) shows higher number of immature neuters at Trevone. Both shores showed evidence of the reduction in number of bigger individuals from low exploitation treatments to high exploitation treatments, and relative to the control (Figure 3.6 and 3.7).
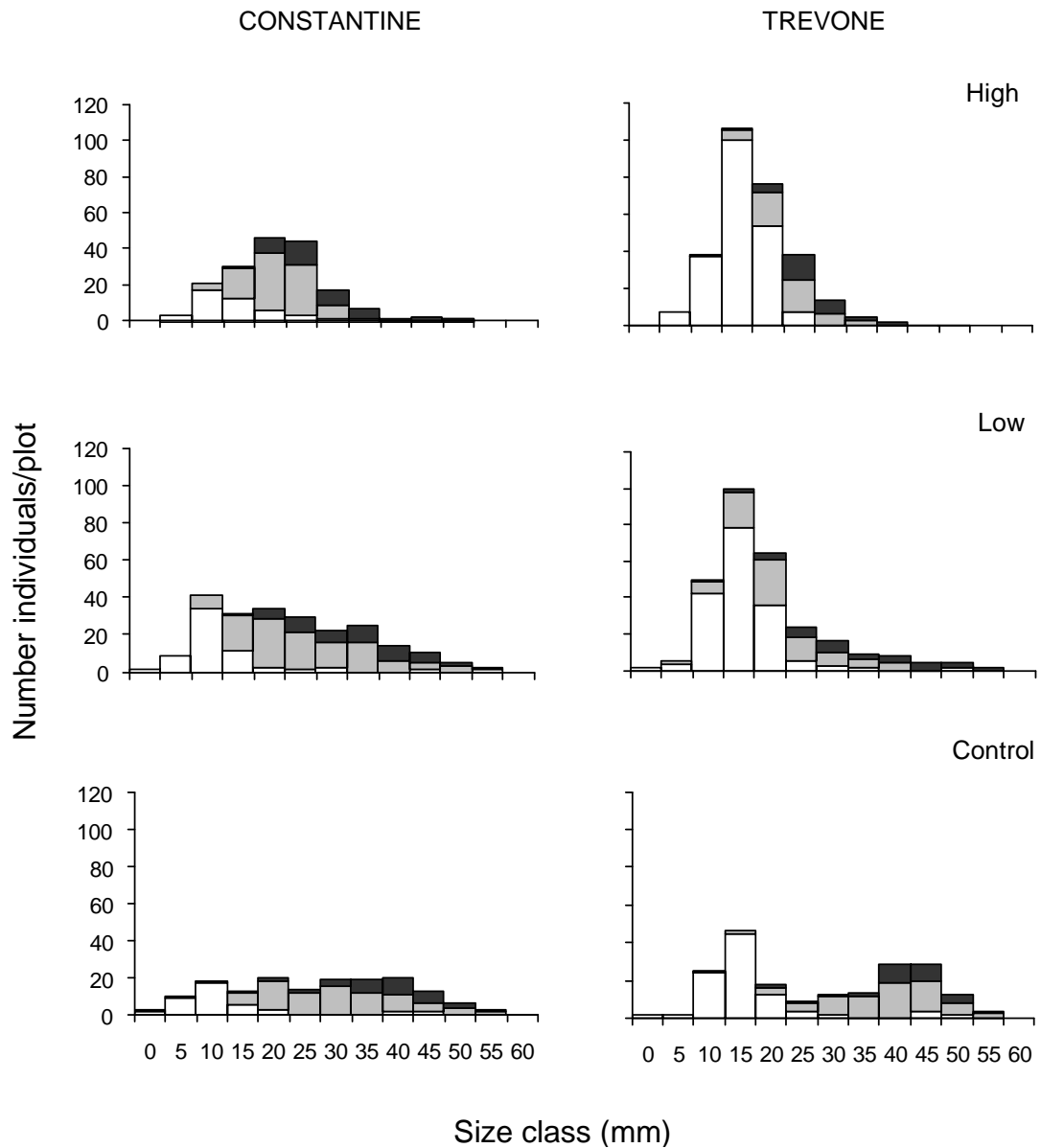
Figure 3.6. Size-specific abundances at the end of the experiment scaled to 3 x 3m treatment plot for *P. vulgata* neuters (clear bars), males (grey bars) and females (black bars), by no-exploitation control (bottom row), low exploitation (middle row) and high exploitation (top row), at Constantine (left) and Trevone (right).
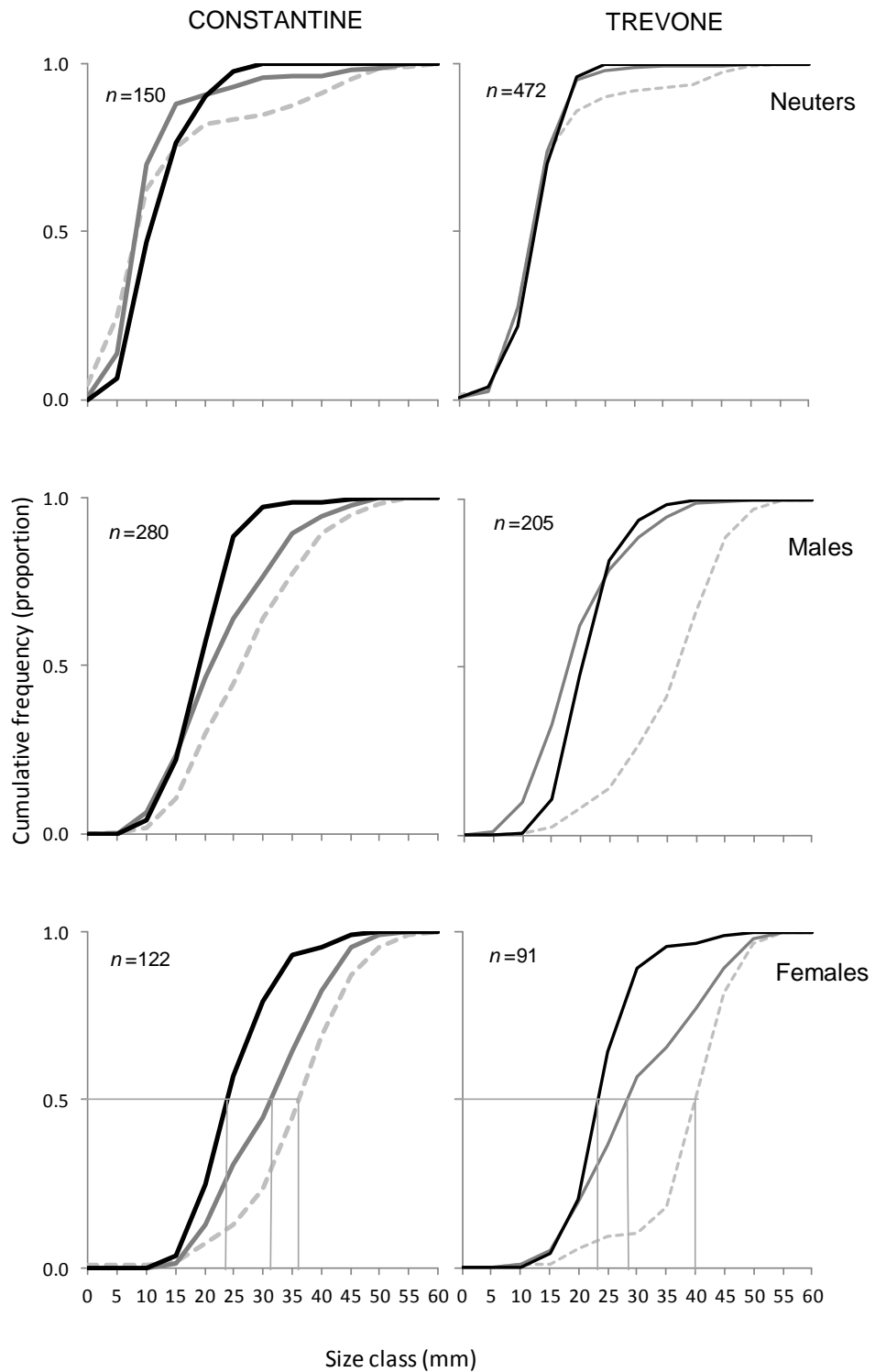
Figure 3.7. Cumulative frequencies per 3 x 3m treatment plot for *P. vulgata* neuters (top row), males (middle row) and females (bottom row) at the end of the experiment by no-exploitation control (grey broken line), low exploitation (grey line) and high exploitation (black line), at Constantine (left) and Trevone (right). *n*= number of individuals per plot. The observed L_{50} size class is the size class with 0.5 males and females.

Kolmogorov-Smirnov tests with Bonferroni correction of α were performed on size distributions of neuters, males and females for each of the three levels of treatment (Figure 3.7). At Constantine both male and female distributions from high treatment plots differed from those on control and low treatment plots ($\alpha < 0.02$). At Trevone both male and female male distributions from control treatment plots differed from those on low and high treatment plots ($\alpha < 0.02$). This suggests that while at Constantine only a high removal of limpets had an impact on the distribution of both males and females compared to the control treatment plots, at Trevone a light removal of limpets at low treatment plots sufficed to change the distribution of males and females compared to the control treatment plots.

The cumulative frequency curves (Figure 3.7) also show a decrease with exploitation of the size class with 50% females. The observed L_{50} size class also decreased with exploitation, due to the size class overlap.

3.4.1.5 Relative size of sex change (L_{50}/L_{max})
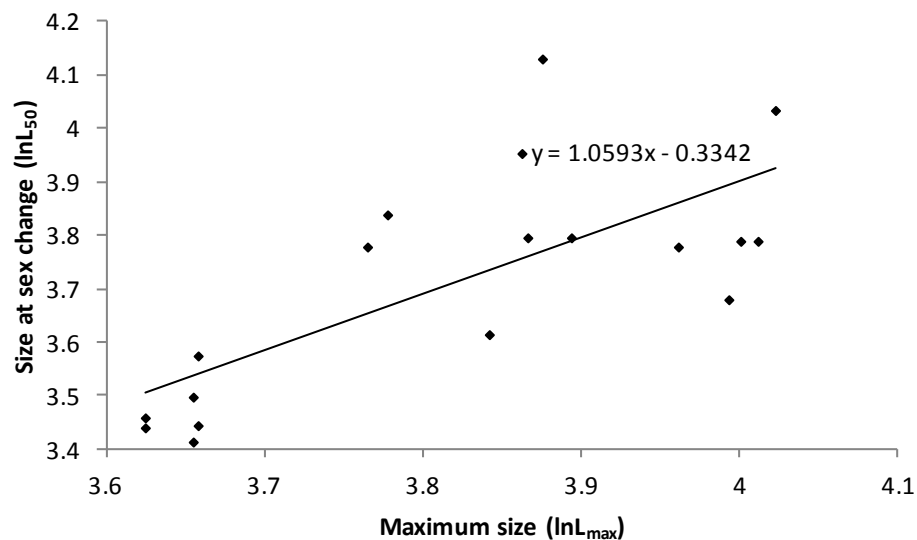
Figure 3.8. Logarithmic plot of size at sex change (L_{50}) versus maximum size (L_{max}) for *P. vulgata* of the treatment plots from Constantine and Trevone.

The relation between L_{50} and L_{max} shows that increments in L_{max} result in increments in the L_{50} with a slope not significantly different from unity (Figure 3.8: $r^2 = 0.50$, slope $= 1.059 \pm 0.012$ confidence intervals at $p = 0.05$). This suggests that the ratio L_{50}/L_{max} is invariant across the two populations of *P. vulgata* and that limpets were changing sex at a constant proportion of their maximum size. The average value across the plots of L_{50}/L_{max} was of 0.90 indicating that *P. vulgata* changed sex when reaching 90% of its maximum size.

3.4.2. Limpet growth and micro-algae film abundance

No differences ($p > 0.05$) were detected in estimated growth of *P. vulgata* from spring to autumn 1999, either under different levels of exploitation or between shores (Figure 3.9). The available microalgal food sampled as chlorophyll abundance in June 2000 did not differ between plots under different levels of exploitation or between shores ($p > 0.05$). Figure 3.10 nevertheless suggests a tendency for increase in microalgal film with increase in exploitation, particularly at Trevone.
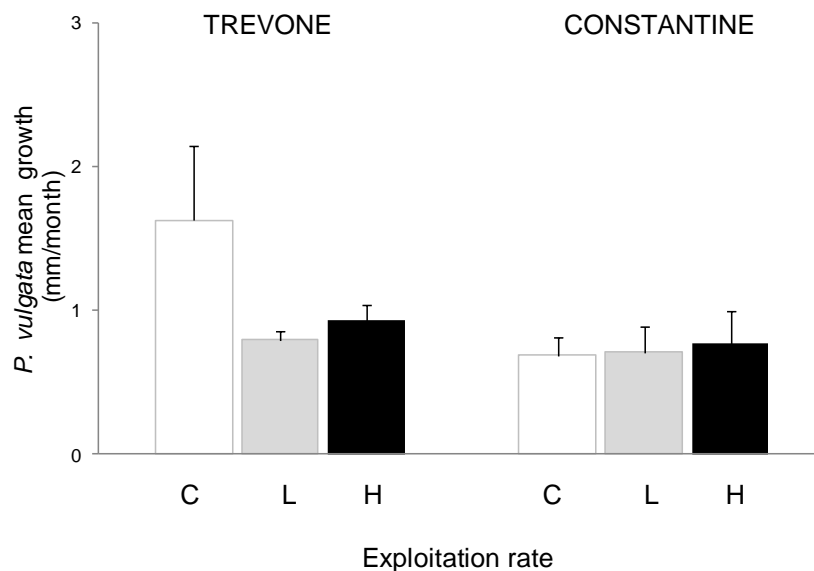
Figure 3.9. Estimated *P. vulgata* mean growth per month (+ SE, *n*= 3 plots per sample) by treatment plots: control (C: clear bars), low exploitation (L: grey bars), high exploitation (H:black bars) at Constantine (left) and Trevone (right) from spring to autumn 1999.
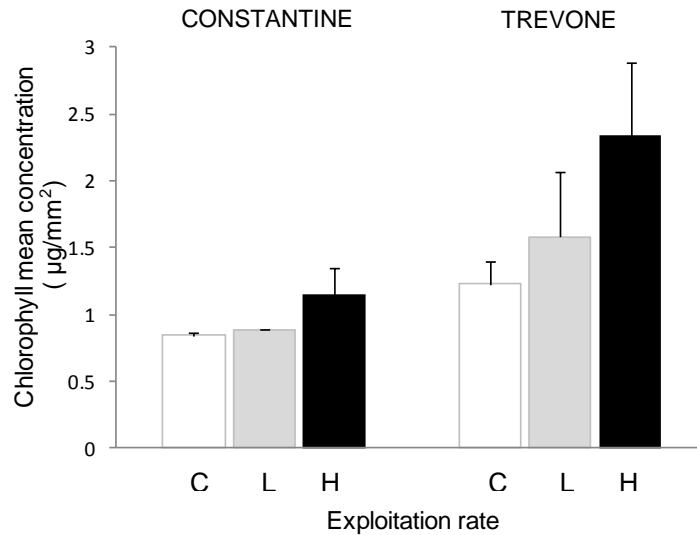
Figure 3.10. Chlorophyll mean abundance (+ SE, n = 3 plots per sample) in treatment plots: control (C: clear bars), low exploitation (L: grey bars), high exploitation (H: black bars) at Constantine (left) and Trevone (right) at June 2000.

Correspondingly, Figure 3.11 shows the microalgal food at the end of the experiment responding to exploitation by location (L*T: $F_{2, 20} = 4.00$, $p = 0.035$; Table 3.8) and a significant interaction of the low vs. high contrast with location and distance (Di*L´*{low vs. high}: $F_{1, 20} = 4.67$, $p = 0.043$; Table 3.8).
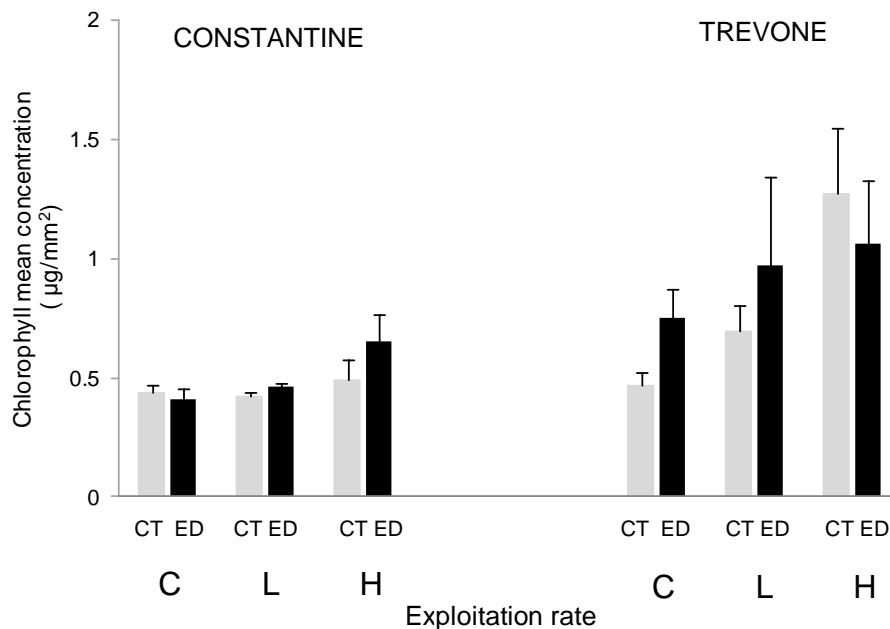
Figure 3.11. Chlorophyll mean abundance (+ SE, n = 3 plots per sample) at the centre (CT:grey bars) and edge (ED:black bars) of plots under treatments of control (C), low exploitation (L) and high exploitation (H), at Constantine (left) and Trevone (right) in June 2000.

Table 3.8. ANOVA on chlorophyll abundance at the end of the experiment, by distance from plot centre, treatment and location (model 3).Orthogonal contrasts are shown partitioning the SS of the exploitation treatment between a contrast of no exploitation (NE: control) to exploitation (EX: low and high pooled) and a contrast between exploitation levels (low vs. high).**Terms with p < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Location = L´	1	1.39	1.39	3.86	0.121
Block = B´(L´)	4	1.44	0.36	-	
Within subjects					
Treatment = T	2	0.77	0.38	3.17	0.240
NE vs. EX	1	0.45	0.45	3.75	0.192
low vs. high	1	0.32	0.32	2.67	0.244
L´*T	2	0.24	0.12	4.00	**0.035**
L´* {NE vs. EX}	1	0.18	0.18	6.00	**0.024**
L´* {low vs. high}	1	0.06	0.06	2.00	0.173
Distance = Di	1	0.06	0.06	6.00	0.247
Di*L´	1	0.01	0.01	0.33	0.572
Di*T	2	0.06	0.03	0.30	0.771
Di*{NE vs. EX}	1	0.01	0.01	0.10	0.782
Di*{low vs. high}	1	0.05	0.05	0.50	0.553
Di*L´*T	2	0.21	0.10	3.33	0.056
Di*L´*{NE vs. EX}	1	0.07	0.07	2.33	0.143
Di*L´*{low vs. high}	1	0.14	0.14	4.67	**0.043**
Error	20	0.58	0.03		

3.4.3. Recruitment and limpet migratory response

No limpet was observed outside its original plot. Recruits (0-15 mm) were considered to have arrived from the pelagic larval pool, subsequently emerging from nursery crevices to take over the space released by the removal of the bigger limpets. No differences were detected ($p > 0.05$) in estimated number of *Patella* spp. (0-15 mm) recruits in summer and autumn 1999, either under different levels of exploitation or between shores. Figure 3.12 nevertheless suggests the possibility of a higher recruitment at Trevone in summer and autumn 1999.
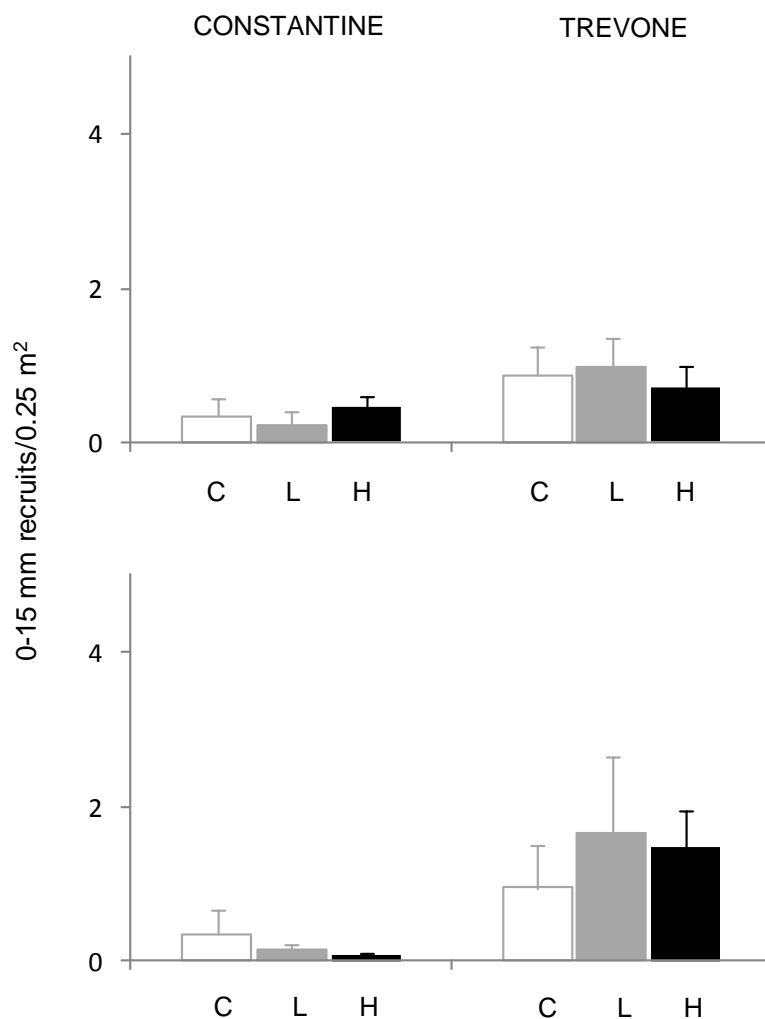
Figure 3.12. Estimated mean numbers (+ SE , *n*= 3 plots per sample) of *Patella* spp. (0-15 mm) recruits scaled to sampled quadrat, at plots with no exploitation control (C: white bar), low (L: grey bar) and high exploitation (H: grey bar) treatments, at Constantine (left) and Trevone (right), in summer (top graph) and autumn (bottom graph) 1999.

At the end of the experiment recruitment was assessed by grouping the removed 0-10 mm and 0-15 mm size classes (Figure 3.13). No differences were detected ($p >$ 0.05) at the end of the experiment in estimated number of *Patella* spp. 0-10 mm recruits either under different levels of exploitation or between shores. Figure 3.13 shows differences by location only for the 0-15 mm recruits ($F_{1,4} = 8.01$, $p = 0.047$; Table 3.9), with no differences detected between treatments. This may reflect the flatter shore at Trevone than Constantine, with smaller crevices allowing refuge for smaller individuals than at Constantine. Differences in recruitment at the end of the experiment are due to the larger animals in the less than 15 mm size class (see Figure 3.6).
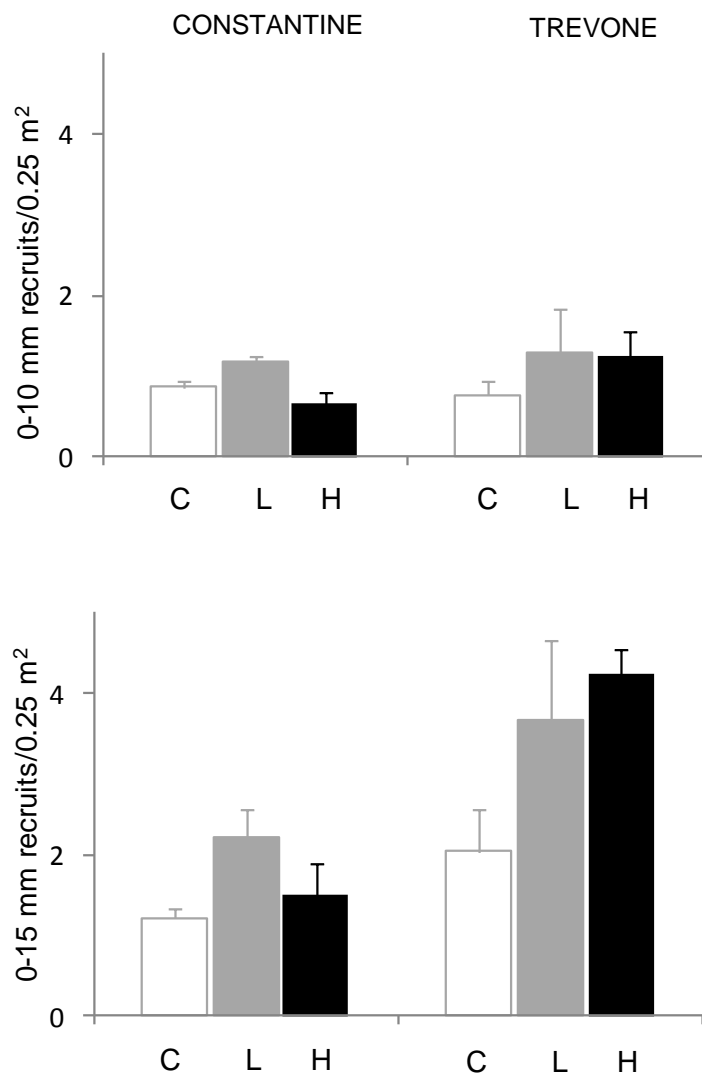
Figure 3.13. Estimated mean numbers (+ SE, *n*= 3 plots per sample) of *Patella* spp. (0-10 mm) and (0-15 mm) recruits at the end of the experiment scaled to sampled quadrat, at plots with no exploitation control (C: white bar), low (L: grey bar) and high exploitation (H: grey bar) treatments, at Constantine (left) and Trevone (right).

Table 3.9. Response of *Patella* spp. (0-15 mm) recruits at the end of the experiment by treatment and location (model 1). **Terms with p < 0.05 are in bold.**

Source	d.f.	MS	F	p
Between subjects				
Location = L´	1	16261.1	8.01	**0.047**
Block = B´(L´)	4	2028.9	-	
Within subjects				
Treatment = T	2	4276.2	2.33	0.300
T*L´	2	1836.2	3.31	0.090
Error	8	555.4		

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3.4.4. Limpet density response

Figure 3.14 shows density fluctuating throughout the course of the experiment ($F_{5, 68} = 5.88$, $p = 0.037$; Table 3.10). The biggest decrease in density occurred at Trevone during spring 2000, possibly in response to gales between autumn and spring inducing limpet mortality.
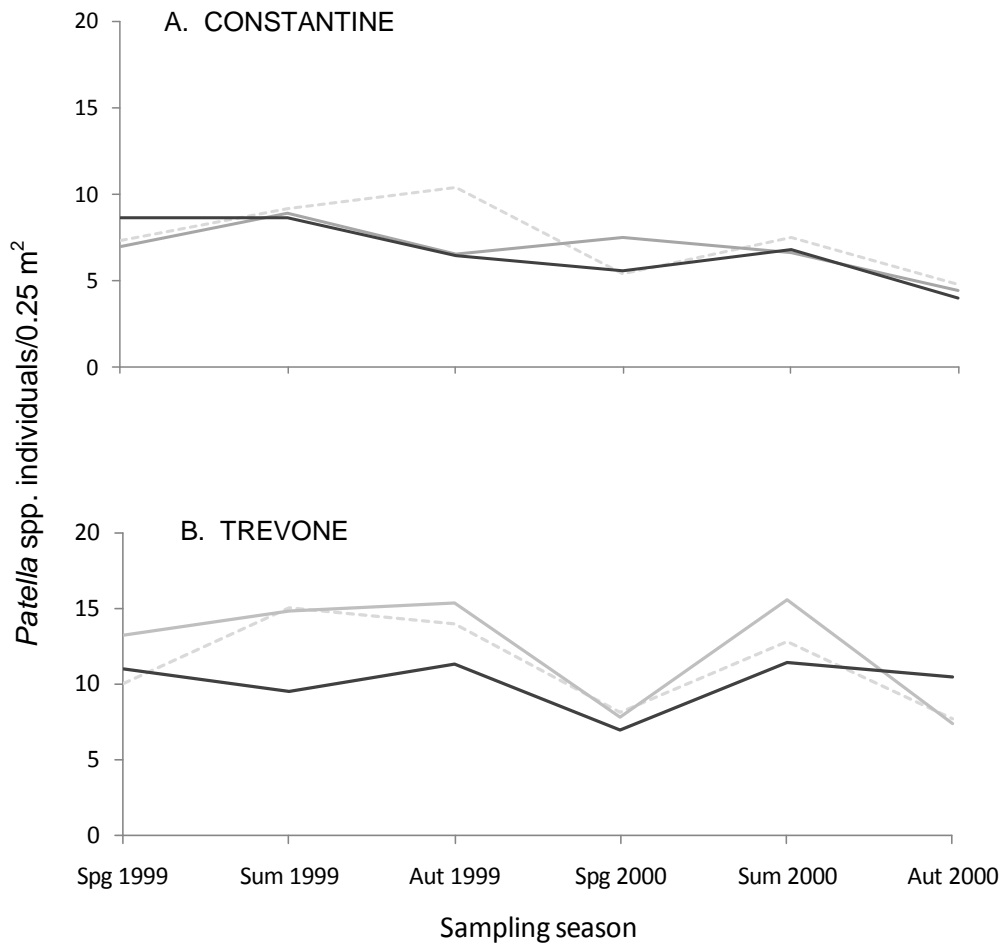
Figure 3.14. Changes over time (Spg- spring; Sum- summer; Aut- autumn) in estimated mean numbers ($n = 3$ plots per sample) of *Patella* spp. individuals scaled to sampled quadrat, in plots under no exploitation control (broken line), low exploitation (grey line) and high exploitation (dark line), at Constantine (A) and Trevone (B).

Table 3.10. Changes over time in the response of *P. vulgata* density (model 4) from spring 1999 to autumn 2000, by exploitation treatment (control; low; high) and location. **Terms with p < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Location = L´	1	457.98	457.98	4.30	0.107
Block = B´(L´)	4	425.69	106.42	-	
Within subjects					
Treatment = T	2	40.97	20.48	1.34	0.427
Season = Se	5	393.55	78.71	5.88	**0.037**
L´*T	2	30.57	15.29	1.45	0.222
L´*Se	5	66.92	13.38	1.27	0.287
T*Se	10	57.99	5.80	1.06	0.466
L´*T*Se	10	54.86	5.49	0.52	0.871
Error	68	714.97	10.51		

3.5.   DISCUSSION

Reduction in mean sizes with increase of exploitation was observed in line with what occurs in most exploited stocks (e.g. Branch 1975; Moreno et al. 1984; Oliva and Castilla 1986; Pombo and Escofet 1996) revealing that the treatments were effective (Figure 3.3 and 3.6). Nevertheless the mean sizes of different sexual stages depend on an interaction with exploitation suggesting that different levels of exploitation will influence differently the mean sizes of the sexual stages or that other variables are determining its variation. Mean sizes of neuters were significantly smaller than mean sizes of males and females, and mean sizes of males were also significantly smaller than those of females. This is in accordance with what happens in natural populations (Orton 1928; Orton et al 1956; Ballantine 1961; Baxter 1983). Therefore despite the exploitation regime and probably due to the occurrence of sex change, limpet populations maintained the natural distribution of sexual stages throughout sizes.

There was strong evidence that the size at sex change decreased in response to exploitation, given by analysis of the averaged L_{50} extracted from the logistic regression on each treatment plot (Figure 3.4). Thus the sex change appears to be a density dependent process.The observed L_{50} in controls (50.5 ± 6.4 mm) suggests that sex change was occurring at a bigger size than expected from the males/females size class range overlap (from 20 to 55 mm size class at Constantine and Trevone). The investigation of the relative size of sex change (L_{50}/L_{max}) indicated that these limpet populations were changing sex at 90% of their maximum size. Indeed Le Quesne and Hawkins (2006) based on the size class overlap indicated 15 to 25 mm as the likely size-range over which sex change might occur for the *P. vulgata* population from their study. Sundelöf et al. (2010) when investigating determinants of reproductive potential in *P. vulgata* considered that the transition from male to females occurred at an age of between three and six years as previously mentioned by Orton et al. (1956) and Ballantine (1961). Determining the age for the 50.5 ± 6.4 mm sex changing limpets would have contributed usefully to the knowledge of sex change, but the individuals' shells were not preserved and data from growth were not sufficient. The global multiple logistic regression (Table 3.6) confirmed my expectation of sex change, revealing that log [P(male)/P(female)] decreases with

increasing size in high exploitation treatment relative to its change in size in the control (pooled across locations). In fact that decrease is generalized when pooling across treatments and locations. These results are consistent with those presented for the protandric *P. ferruginea* where individuals in populations with low density of larger individuals switch to female at smaller sizes (Rivera-Ingraham et al. 2011).

Although the limpet removals were effective, no differences were detected in the different sexual stages densities, suggesting some compensation for the loss of the bigger individuals, which could be recruitment, migration and/or sex change. The significant stage*location interaction and a correspondent significant (NS vs. SE) contrast*location interaction indicates differences in numbers of the not sexed (neuters) and sexed ones by location. Trevone had higher number of neuters than Constantine (Figure 3.5 and 3.6).

No significant differences were detected in *P. vulgata* growth, or in the microalgal food available despite the reduction of grazing pressure in microalgal growth (Thompson et al. 2004). There were, however indications of greater microalgal growth at Trevone. It is known that the mucus from the limpet's trail promotes microalgal growth so the reductions of limpets in numbers could be influencing it (see Davies et al. 1990). The interactions of low vs high exploitation contrasts with location and distance to the microalgal food available at the plot centre and edge, and also of exploitation level with location at the end-of-experiment, suggested that other factors could be determining micro-food abundance (see Thompson et al 2005). These could be grazing activity (due to recruitment or migration), exposure to desiccation of treatment plots (due to height on the shore and shore hydrodynamics). Nevertheless, food availability was probably not a limiting factor inhibiting progression from male to female.

The study sites differed considerably in community structure: Trevone was a moderately exposed shore with barnacles and *Fucus* spp. dominated community, while Constantine can be considered an exposed shore with *Mytilus* spp. dominated shore community (see Chapter 4). These differences in macro-benthic assemblages could be determining the observed differences in recruitment and numbers of neuters individuals conditioning the presented sex change response.

Several confounding effects might have influenced the results. This experiment did not segregate the effects of density and sex ratio which have both been shown as cues for sex change to occur within the order Patellogastropoda (see Wright and

Lindberg 1982; Lindberg and Wright 1985; Wright 1989). Previous experiments (Munday et al. 2006) had confirmed that the timing of sex change is sensitive to:

- Immediate social environment in limpets (Warner et al. 1996; Collin 2005), snails (Chen et al. 2004), shrimp (Baeza and Bauer 2004) and many species of fish (Shapiro 1984; Warner 1984; Ross 1990; Kuwamura and Nakashima 1998).

- Size of an individual relative to others in the social group (Warner and Swearer 1991; Warner et al. 1996; Buston 2003).

- Sex ratio of the social group (Shapiro 1984; Munday 2002; Collin et al. 2005).

- Local density (Wright 1989; Lutenesky 1994).

The apparent overlap of male and female size results from the combination of data from many patches (Collin 1995). To further understand the sex change in *P. vulgata* it is essential to establish experiments that consider differences among patches in size distributions, growth rates, density, mortality rates, sex ratio and mating group opportunities.

P. vulgata reproductive output seems more sensitive to perturbations of the survival of large males and medium and large females than to perturbations of recruitment (Sundelöf et al. 2010). Hence, the removal of the bigger sex changing limpets by collectors seriously compromises the population persistence particularly in a short time scale if recruitment is low. However, in protandric species with sex-change plasticity, the depletion of larger individuals could be partially compensated (Rivera-Ingraham et al. 2011; this chapter). The knowledge of sex change dynamics in such populations would allow management procedures to counter population decline and is essential to the rocky shore conservation and resource management. A precautionary approach would involve having both a minimum size (to ensure breading individuals are present in the population) and a maximum size (to ensure some large females remain).

CHAPTER 4

CONSEQUENCES FOR COMMUNITY STRUCTURE OF SIMULATED HUMAN EXPLOTATION OF A KEYSTONE GRAZER, *PATELLA VULGATA*

4.1. ABSTRACT

Disturbance on rocky shores creates new bare space which, in conjunction with hierarchical species interactions, dictates the pathways of community succession. This chapter describes a field experiment designed to simulate human exploitation of limpets in order to evaluate the effects of this biological disturbance on the composition of the rocky shore community of non-target species. Limpet populations were exploited for 18 months on two shores by systematic removal at two different levels: low and high exploitation. During this period, the abundance, spatial dispersion of key species (*Fucus* spp., *Mytilus* spp., barnacles and limpets), species richness and macrobiotic community structure were recorded. The hypothesis tested was that selective removal through time of the largest limpets would influence the magnitude of these parameters. The studied sites had similar tidal extent and shore profiles but differed in the relative percentages of cover of mussels, fucoids and barnacles. In comparison to control plots with no removal of limpets, limpet exploitation led to establishment of *Fucus* spp. At the end of the experiment, control plots had lower percentage cover of *Fucus* spp. than both low and high exploitation plots. Furthermore, while the variation in estimated percentage cover of *Fucus* spp. decreased over the course of the experiment in control plots, it increased in both low and high exploitation plots. Differences between treatments in the algal, macro-invertebrate and total species richness were detected only about six months after the start of the experiment. For algal and total species richness, low exploitation plots had lower levels of richness than high exploitation plots. Several other measured parameters from univariate and multivariate analyses confirmed that the communities on the two shores responded differently to the same source of disturbance. It is

crucial that resources management strategies assess potential community level impacts of exploitation of key species.

4.2.   INTRODUCTION

As space is probably the most important resource for intertidal organisms, especially for sessile species, processes involved in the creation of free space are of vital importance to the persistence of many species and overall community structure (Connell 1961; Paine 1966, 1969; Dayton 1971; Paine and Levin 1981; Sousa 1984; Blanchette 1996; Raffaelli and Hawkins 1996). Disturbance, the process responsible for the creation of new bare space, provides the room for new colonisers and the hierarchical nature of species interactions dictate the pathway of succession (Sousa 1979b). Disturbance is thus responsible for environmental heterogeneity over time. Predation (Paine 1966; 1971) and grazing (Lubchenco 1978) are the main agents of biological disturbance. According to classical ecological theory, predation on a dominant competitor for primary space (e.g. limpets or mussels, depending on the community structure) leads to increased species diversity in the community (Paine 1966, 1977; Lubchenco 1978; Branch 1984).

Unlike physical disturbance, predation is considered a selective agent since predators feed preferentially on one or fewer types of prey. Castilla and Duran (1985) observed that in sites where human predators were excluded for two years, the keystone muricid predator *C. concholepas* increased in abundance dramatically, leading to a decrease in the cover of the competitively dominant mussel *P. purpuratus* and to an increase in species diversity of the primary space occupiers. This result complied with the "intermediate disturbance hypothesis" (Caswell 1978; Connell 1978), according to which disturbance through selective predation (which in this case coincided with the maximum predatory impact of *C. concholepas* on *P. purpuratus* having the magnitude of an intermediate level), increases species richness. The intermediate disturbance hypothesis proposes that at low levels of disturbance, certain competitive species will predominate as a climax community and hence diversity will be low. At intermediate levels of disturbance, no species will predominate and diversity will be high. This occurs only as long as disturbance renews resources (e.g. space) at a rate that allows continued recruitment and

persistence of species that would otherwise be driven extinct. In this way disturbance must occur with some intermediate frequency and intensity/severity. Under these circumstances, competitive exclusion of species is delayed or never occurs because some areas are routinely set back by "catastrophes" (see Sousa 1979a). As disturbance increases further, only a few highly tolerant or very opportunistic species will occur. Species diversity remained low in sites where there was no predation of *P. purpuratus* by *C. concholepas*; consequently these competitively dominant mussels retained the primary space (Duran and Castilla 1989).

This chapter describes a field experiment designed to simulate human exploitation of limpets in order to evaluate the effects upon the composition of the rocky shore community of other non-target species. Much work has been done on the responses of algal communities to the complete limpet removal (Jones 1948; Southward 1964; Hawkins 1981a), total exclusion (Hawkins 1981b; Jenkins et al. 2005; Coleman et al. 2006) and mass mortality of limpets following oil spill clear up (Southward and Southward 1978; Hawkins et al. 1983; Hawkins and Southward 1992). Nothing has been published, however, on reducing density of limpets experimentally. In the experiment described in this chapter the simulated predation by humans causes biological disturbance (releasing space, reducing grazing activity and increasing trampling) which might increase diversity if limpets were suppressing other species and if that disturbance has an intermediate level. This experiment simulated limpet harvesting around the world, where most shellfish gatherers prefer the bigger limpets. The experiment was carried out in the south-west of England on two replicate shores (Constantine and Trevone) identified as little disturbed by people, having similar tidal range and shore profiles and where, like much of the British coast, limpets were not target species for human consumption.

The specific objectives were firstly to test the effects on key non-target species of removing large limpets at two intensities (low and high) on repeated visits to the shores. The responses of key species were measured using univariate statistics and the whole assemblage using a multivariate approach.

Formally the hypotheses tested were: (1) the selective removal of limpets, the main algae grazers, at different levels will influence the percentages cover of key species and will alter differently the cover of *Fucus* spp., *Mytilus* spp. and barnacles; (2) manipulating the rate of limpet exploitation will reduce the patchiness of the plots

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due to the occupation by the key species of the released bare space; (3) manipulating the rate of limpet exploitation will increase species richness at the low exploitation treatments as long as its exploitation generates an intermediated level of disturbance; (4) the selective removal of limpets, at different levels will cause different changes in ecosystem structure.

4.3.   MATERIAL AND METHODS

4.3.1. Pilot study

An initial pilot study was performed along the south-west coast of England to locate the two rocky shores for the experiment as described in Chapter 3, section 3.3.1. Each plot was sampled with five random quadrats, each 0.5×0.5 m, which sufficed to quantify abundances of macro-algae and macro-benthic invertebrates.

4.3.2. Study sites and organisms

The study was carried out at Constantine (50º31´52.02" N, 5º01´33.32" W) and Trevone (50º32´40.48" N, 4º58´50.08" W) on the west coast of Cornwall, UK (see Figure 3.1). Those sites both have a slate bed rock, although Constantine is more exposed to wave and wind action than Trevone. The shore at Trevone consists mostly of gently sloping bedrock platforms with few boulders and loose rocks. Despite the exposure of this site, its shallow profile causes a reduction in wave energy up the reef.

The limpet *P. vulgata* is the dominant grazer in the mid-intertidal zone of the south-west and is distributed across the wave exposure gradient. *P. depressa* also occurs on open rock and *P. ulyssiponensis* in depressions and rock pools, the latter being dominant on the low shore. These species feed on the microalgal film that coats the rocky intertidal shore as well as on macro-algae (Hawkins et al. 1989; Davies et al. 2007).

Mussels, barnacles and macro-algae are the major space occupiers, and invertebrates find refuge amongst them. At Constantine, in the experimental area,

mussels (a mixture of *M. edulis, M. galloprovincialis* and hybrids) dominated the primary space, covering on average $54.00 \pm 4.00\%$ (SE, $n = 45$), followed by *Fucus vesiculosus* var. *evesiculosus* with cover of $32.00 \pm 3.00\%$ (SE, $n = 45$). At Trevone the presence of mussels was negligible (0.09 ± 0.06 % (SE, $n = 45$)), and the major space occupiers were barnacles with 44.76 ± 7.70 % (SE, $n = 45$) cover and *Fucus* sp. with $8.22 \pm 3.20\%$.

4.3.3. Limpet removal treatments

The exploitation treatment involved removing the biggest limpets at two different rates as described in Chapter 3, section 3.3.3, simulating what happens in most of the exploited limpet populations, where bigger sizes are preferentially removed by collectors (e.g. Branch 1975; Duran and Oliva 1987; Hockey et al. 1988; Espinosa et al. 2009b). Thus on each shore, the three limpet species were targeted as a single resource. The simulated exploitation was performed every three months from March 1999 to October 2000. Before running the experiment, five 0.5×0.5 m quadrats were sampled in each 3×3 m plot of macro-benthic invertebrates, and macro-algal abundances to check for similarity. Data on the abundance of macro-algae and macro-benthic invertebrates were collected four times in spring and summer 1999 and 2000 using five 0.5×0.5 m quadrats in each plot.

4.3.4. Experimental design

At each of the two shores, three adjacent blocks of shore each about 20 m wide were defined at the mean tide level (MTL) as described in Chapter 3, section 3.3.4.

4.3.4.1.   Statistical analysis

A two-factor split-plot design (equivalent to model 5.6 and 6.3 iii in Doncaster and Davey, 2007) was used to test the hypothesis at the end of the experiment, that selective removal through time of the largest limpets would influence responses of percentage cover, spatial distribution and species richness of key species (model 1).

$$Y = T|B'(L') \qquad \text{model 1}$$

This design is described in Chapter 3, section 3.3.4.1 as model 1. A set of planned *a priori* contrasts were used to detect differences between Treatment effects. Treatment contrasts partitioned the sums of squares of the exploitation treatment, with a first contrast of no exploitation (NE: control) versus exploitation (EX: low and high pooled), and a second contrast of exploitation levels (Low vs. High). When non-significant interactions of contrasts with Location were present the interaction between Location and Treatment was pooled with the error term to provide a more powerful test for the contrasts with 10 error degrees of freedom.

For all ANOVAs, visual inspection of the residuals following transformations as necessary indicated normal distributions and homogeneous variances.

4.3.5. Responses of macro-algal and macro-benthic invertebrate abundance

4.3.5.1.   Response of percentage cover and abundance by key species

Data on the abundance of macro-algae and macro-benthic invertebrate were collected four times in spring and summer 1999 and 2000. Percentage cover of macro-algae and abundances of sessile invertebrates (mussels *M. galloprovincialis, M. edulis*, hybrids and barnacles *C. stellatus, C. montagui, S. balanoides*) were recorded in five random 0.5×0.5 m quadrats, subdivided by fishing line into 49 intersection points. Estimation of the percentage cover was made using the point intersect method and presences counted at the intersections. Records were additionally taken of any other species present inside the quadrat that were not under an intersection point. Since shores are three-dimensional structures, whenever large algae (e.g. *Fucus* spp.) were

present, sampling was stratified in different layers and canopy cover was distinguished from substrate cover. The key species considered were *Fucus* spp., *Mytilus* spp. and barnacles. Limpets from the genus *Patella* were counted.

The hypothesised response in percentages cover of key species was that manipulating the rate of exploitation would differentially alter the cover of *Fucus* spp., *Mytilus* spp. and barnacles. The removal of limpets removes grazing pressure freeing space for colonisation by algae and reduces bulldozing of settling barnacles (Hawkins 1983). Colonisation by *Fucus* spp., *Mytilus* spp. and barnacles is expected to be proportional both to the new space created and to the level of exploitation (low to high). The competition for space between the key species is then expected to determine the pathway of space colonisation.The percentage cover of key species was compared over the course of the experiment (final minus first values) and at the end of the experiment.

4.3.5.2. Response of spatial distribution of key species

The "variance-to-mean" index of dispersion (Krebs 1989) was used to quantify the spatial dispersion of key species over the course of the experiment (final minus first values) and at the end of the experiment. The expected values range from 0 (maximum uniformity) to 1 (randomness) and > 1 (clumped pattern). Since the abundance of key species (*Fucus* spp., *Mytilus* spp. and barnacles) was measured as percentage cover, percentages were normalised by arcsine-root transformation. The corresponding "variance-to-mean" index calculated per plot with the transformed data expresses less patchiness in smaller index values.

I hypothesised that manipulating the rate of exploitation would influence the distribution of key species. Created space (with the limpet removal) that is occupied by *Fucus* spp. (assuming its percentage cover will increase with the level of exploitation) can reduce barnacle cover (Southward and Southward 1978; Hawkins 1981b, 1983; Hawkins and Southward 1992; Jenkins et al. 1999b) resulting in greater patchiness in low plots than fully grazed (control) plots. Therefore I expected low levels of exploitation would cause higher patchiness than high levels and control plots.

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4.3.5.3.   Response of species richness

Species richness was measured from counts of the number of macro-algal species, number of macro-benthic invertebrates, and total number of species present in quadrats. No diversity index could be applied because the data obtained were mixed percentages of cover and counts.

I hypothesised that manipulating the rate of exploitation would increase species richness at the low exploitation treatments. The increase of the percentage cover of key algae would increase the refuge for macro-invertebrates. Other algae released from grazing would also increase their percentage cover.

The simulated predation by humans was therefore hypothesised to cause biological disturbance in turn reducing grazing activity which might increase diversity if that disturbance was at an intermediate level. This would be in accordance with the intermediate diversity hypotheses if the low treatments had an intermediate level of disturbance.

The species richness was compared at each sampling date, with results presented for summer 1999 (the sampling season with significant results), during the experiment (final minus start values) and at the end of the experiment.

4.3.6. Response of community structure

Data on macro-algae and macro-benthic invertebrates were collected following the intersection method described in section 4.3.5.1 on March 1999 prior to removals to save background levels before manipulation. Subsequent data collections were made in summer 1999 and spring and summer 2000. Sessile species that were not picked up by an intersection were recorded as present and assigned 0.5% cover in analyses. Limpets < 10 mm long were recorded as "small limpets". Multivariate analyses were performed following the PRIMER package at each shore and during each sampling date.

Non-metric multidimensional scaling (nMDS) was used to assess the impact of the treatments on the overall community structure (excluding limpets > 10 mm). nMDS is an ordination technique based on rank dissimilarity, in this case measured with the Bray-Curtis index on square-root transformed data (Clarke 1993). Permutational

multivariate analysis of variance (PERMANOVA, McArdle and Anderson 2001; Anderson 2005) was used to test the hypotheses of differences in community structure between treatments. For each sampling time at each site a separate analysis was conducted with one factor, "treatment", which was a fixed factor with three levels (Control; Low; High).

4.4. RESULTS

4.4.1. Percentage cover and abundance of key species

Manual removals of limpets were effective in reducing the densities of 25-60 mm *P. vulgata* (target species) and hence grazing pressure at both sites. At Constantine the density reduction per sampling quadrat was from 4.97 ± 0.09 (SE, $n = 30$) to 2.97 ± 0.50 (SE, $n = 10$) at low treatment plots and 2.20 ± 0.66 (SE, $n = 10$) at high treatment plots in summer 2000. Limpet removals at Trevone reduced 25-60 mm *P. vulgata* density per sampling quadrat from 3.26 ± 0.55 (SE, $n = 30$) to 2.60 ± 0.92 (SE, $n = 10$) at low treatment plots and 1.67 ± 0.52 (SE, $n = 10$) at high treatment plots in summer 2000. Reductions in densities of the bigger *P. vulgata* were by approximately 40% and 60% in low- and high-exploitation plots respectively at Constantine, and of 20% and 50% at Trevone.

Figure 4.1 shows *Patella* spp. density fluctuating throughout the course of the experiment. The biggest increase in density occurred at Constantine and Trevone in summer 1999, probably in response to the first removal of limpets in spring 1999 with an influx of juveniles. No differences were detected ($p > 0.05$) at the end of the experiment in the density neither in the density variation over the course of the experiment (end – start values) of the *Patella* spp. by location and treatment (Figure 4.2 and 4.3).

The percentage cover of *Fucus* spp. increased at both Constantine and to a greater extent at Trevone particularly in L and H treatment plots, but decreased in controls (Figure 4.4). *Mytilus* spp. were present at Constantine, where its percentage cover varied during the experiment irrespective of treatment. Barnacles at Trevone varied

during the experiment, but were much less variable at Constantine where they slowly increased over time from a low level.

Figure 4.4 shows differences by location only for *Mytilus* spp. ($F_{1,4} = 116.17$, p <0.001; Table 4.2) and barnacles ($F_{1,4} = 12.68$, $p = 0.024$; Table 4.3) at the end of the experiment, with no differences detected between treatments. Nevertheless for percentage cover of *Fucus* spp., the treatment main effect ($F_{2,2} = 5.60$, $p = 0.152$; Table 4.1) had contrasts revealing that non-exploited plots (17.93 ± 9.44 %) had lower percentage cover than exploited ones (low and high treatment pooled: 58.42 ± 15.92 %, pooled $F_{1,10} = 19.24$, $p = 0.001$). No differences were detected between low- (48.63 ± 16.29 %) and high-exploitation plots (68.20 ± 14.69 %).

Figure 4.5 shows detected differences ($p < 0.05$) in the change in percentage cover over the course of the experiment (end – start values) for *Fucus* spp. The treatment main effect ($F_{2,2} = 6.65$, $p = 0.131$; Table 4.4) had contrasts revealing that non-exploited plots (-9.30 ± 7.59 %) had decreased percentage cover than exploited ones (low and high treatment pooled: 35.23 ± 19.62 %, pooled $F_{1,10} = 20.12$, $p = 0.001$). No differences in the *Fucus* spp. change in percentage cover were detected between low- (25.28 ± 11.88 %) and high-exploitation plots (30.86 ± 14.90 %).
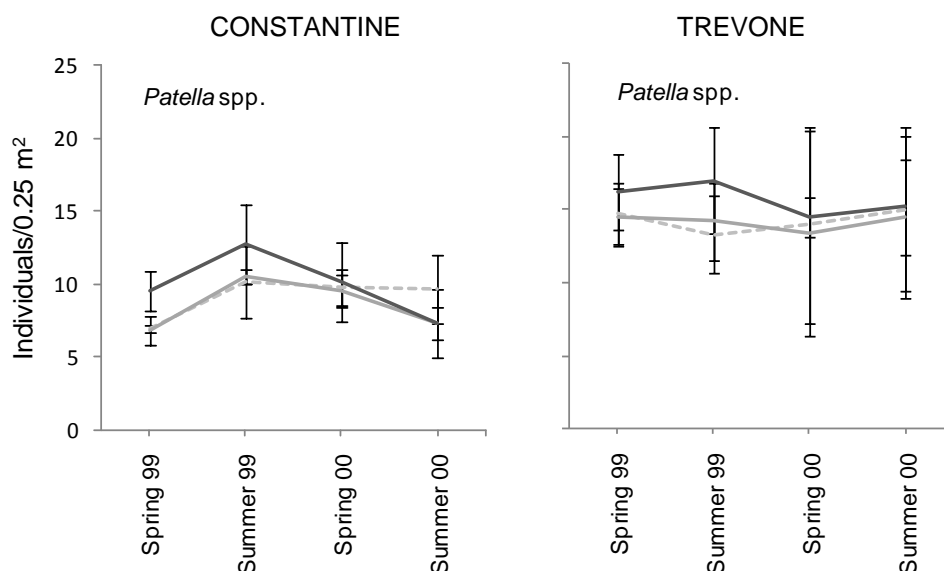
Figure 4.1. Changes over time in estimated mean numbers ± SE (*n* = 3 plots per sample) of *Patella* spp. individuals (scaled to sampling quadrat) in plots under no exploitation control (broken line), low exploitation (grey line) and high exploitation (dark line), at Constantine (left) and Trevone (right).
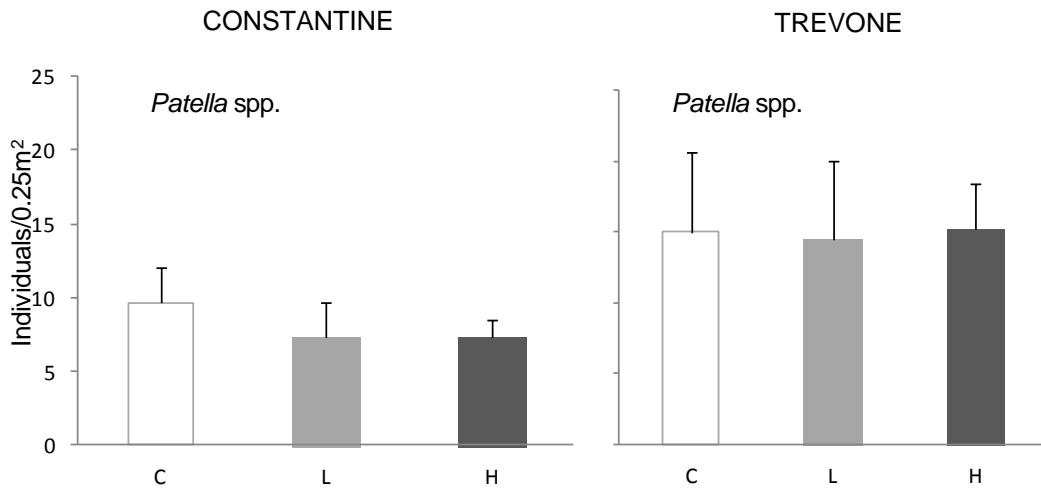
Figure 4.2. Estimated mean numbers with SE (n = 3 plots per sample) of *Patella* spp. (scaled to sampling quadrat) by treatment of control (C) low exploitation (L), high exploitation (H) at Constantine (left) and Trevone (right) in summer 2000 after the experiment had run for 18 months.
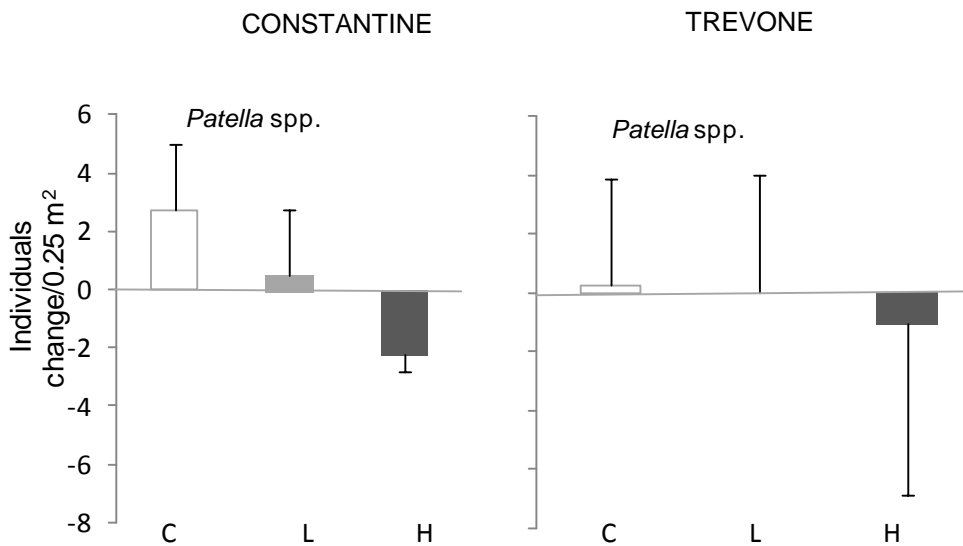
Figure 4.3. Change (end – start values) in the estimated mean numbers ± SE (n = 3 plots per sample) of *Patella* spp. (scaled to sampling quadrat) by treatment of control (C) low exploitation (L), high exploitation (H) at Constantine (left) and Trevone (right) in summer 2000 after the experiment had run for 18 months.

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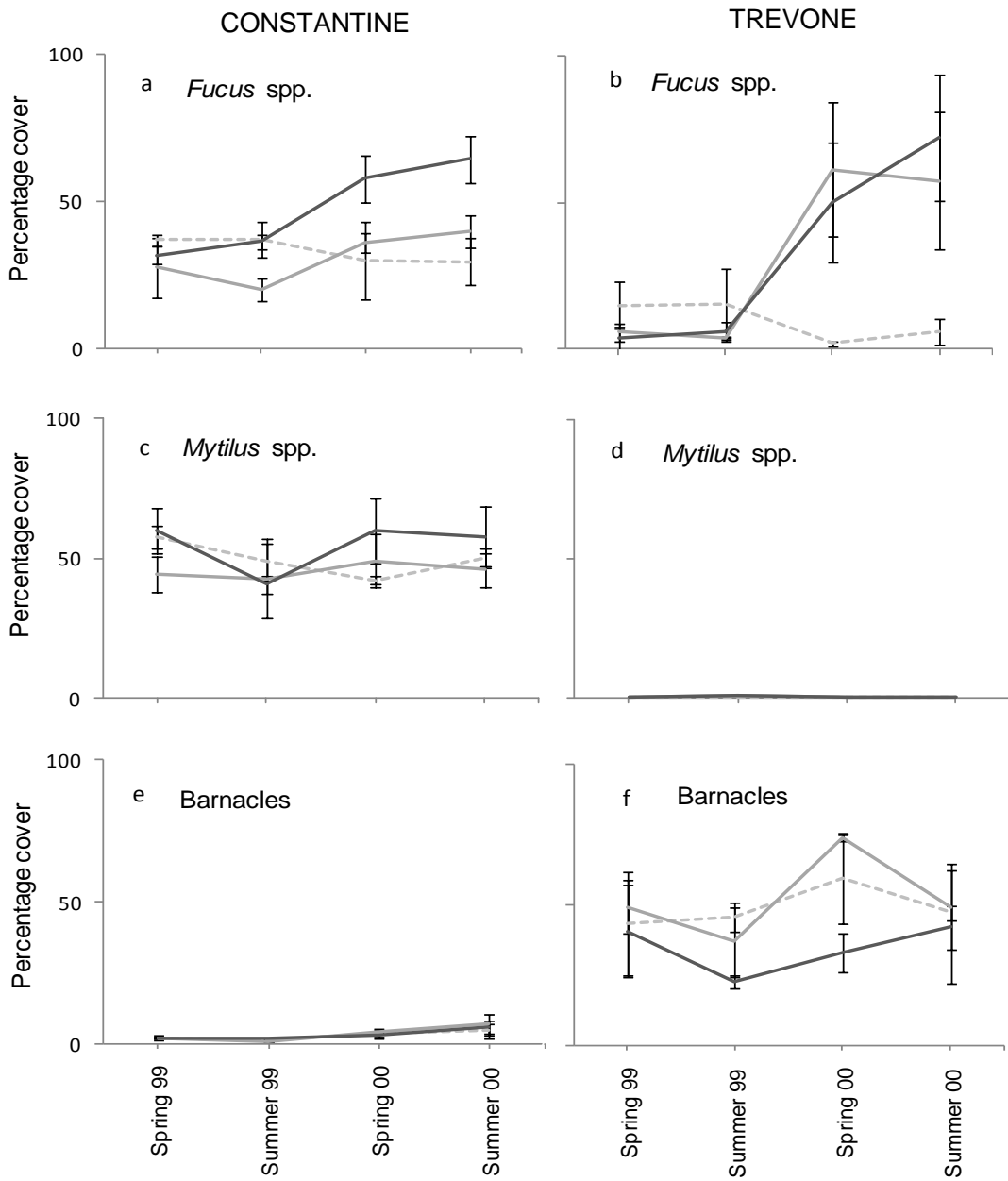
Figure 4.4. Estimated mean percentage cover ± SE (*n* = 3 plots per sample) for *Fucus* spp. (a, b), *Mytilus* spp. (c, d) and barnacles (e, f) at each sampling season by treatment plots: control (broken line), low (grey line) and high (dark line), at Constantine (left) and Trevone (right).

Table 4.1. Response of *Fucus* spp. percentage cover at the end of the experiment by treatment and by location. Orthogonal contrasts are shown partitioning the treatment SS between no exploitation (NE: control) and exploitation (EX: pooled low + high) and between exploitation levels (low vs. high). Orthogonal contrast main effects calculated after *post hoc* pooling the non-significant T*L into the Error term, giving 10 error d.f. **Terms with *p* < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Location = L´	1	0.80	0.80	<0.01	0.981
Block = B´(L´)	4	5142.80	1285.70	-	
Within subjects					
Treatment = T	2	7704.20	3852.10	5.60	0.152
NE vs. EX	1	6555.60	6555.60	19.24	**0.001**
low vs. high	1	1148.60	1148.60	3.37	0.096
T*L´	2	1376.40	688.20	2.71	0.126
{NE vs. EX}*L´	1	1298.40	1298.40	5.11	0.054
{low vs. high}*L´	1	78.00	78.00	0.31	0.593
Error	8	2031.50	253.90		

Table 4.2. Response of *Mytilus* spp. percentage cover at the end of the experiment by treatment and by location. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Location = L´	1	11878.50	11878.50	116.17	**< 0.001**
Block = B´(L´)	4	409.00	102.20	-	
Within subjects					
Treatment = T	2	105.40	52.70	0.73	0.506
NE vs. EX	1	2.20	2.20	0.03	0.866
low vs. high	1	103.30	103.30	1.42	0.261
T*L´	2	105.40	52.70	0.68	0.534
{NE vs. EX}*L´	1	2.20	2.20	0.03	0.867
{low vs. high}*L´	1	103.30	103.30	1.33	0.282
Error	8	620.20	77.50		

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Table 4.3. Response of barnacle percentage cover at the end of the experiment by treatment and by location. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	7385.20	7385.20	12.68	**0.024**
Block = B´(L´)	4	2329.60	582.40	-	
Within subjects					
Treatment = T	2	58.20	29.10	0.17	0.846
NE vs. EX	1	0.10	0.10	<0.01	0.977
low vs. high	1	58.10	58.10	0.33	0.578
T*L´	2	36.90	18.50	0.09	0.918
{NE vs. EX}*L´	1	11.10	11.10	0.05	0.829
{low vs. high}*L´	1	25.80	25.80	0.12	0.738
Error	8	1709.60	213.70		

Table 4.4. Response of *Fucus* spp. percentage cover change (end – start values) by treatment and by location. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	2693.80	2693.80	2.06	0.225
Block = B´(L´)	4	5231.80	1307.90	-	
Within subjects					
Treatment = T	2	10492.70	5246.40	6.65	0.131
NE vs. EX	1	9689.10	9689.10	20.12	**0.001**
low vs. high	1	803.60	803.60	1.67	0.225
T*L´	2	1577.60	788.80	1.95	0.204
{NE vs. EX}*L´	1	1528.80	1528.80	3.78	0.088
{low vs. high}*L´	1	48.80	48.80	0.12	0.738
Error	8	3237.20	404.60		
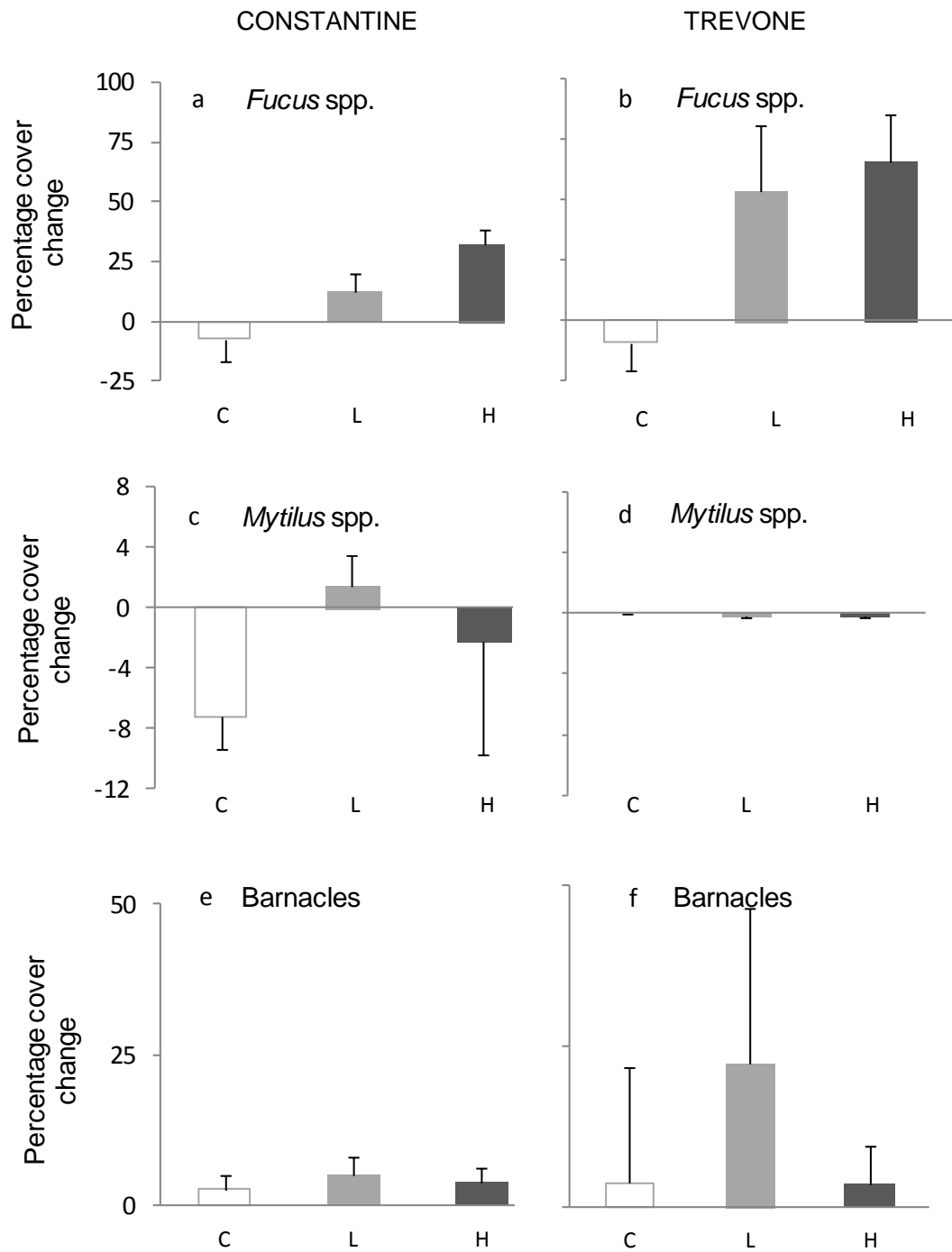
Figure 4.5. Estimated mean percentage cover change (end – start values) ± SE ($n = 3$ plots per sample) for *Fucus* spp.(a, b), *Mytilus* spp. (c, d) and barnacles (e, f) by treatment of control (C), low exploitation (L), high exploitation (H) at Constantine (left) and Trevone (right) in summer 2000 after the experiment had run for 18 months. N.B. Change of scale.

4.4.2. Spatial dispersion of key species

The variance-to-mean index changed from the beginning to the end of the experiment (Figure 4.6). Nevertheless no differences were detected ($p > 0.05$) in the variance-to-mean of *Fucus* spp., *Mytilus* spp. and barnacles at the end of the experiment by location and by treatment (Figure 4.7). *Mytilus* spp. had a change in variance-to-mean index over the course of the experiment (end − start values) that responded to exploitation by treatment after pooling procedures (Figure 4.8 (c, d), $F_{2,\,10} = 5.55$, $p = 0.024$; Table 4.5). The treatment main effect had contrasts revealing that in low-exploitation plots the variance-to-mean index decreased (-0.06 ± 0.04) whereas in high-exploitation plots it increased (0.05 ± 0.06, pooled $F_{1,\,10} = 7.50$, $p = 0.021$). No differences were detected between non-exploited (-0.01 ± 0.01) and exploited plots (0.00 ± 0.06).

Barnacles displayed a change in variance-to-mean index over the course of the experiment (end − start values) with a significant treatment*location interaction (Figure 4.10 (e, f), $F_{2,\,8} = 7.31$, $p = 0.016$; Table 4.6). Table 4.6 reveals that the treatment*location interaction included differences within exploited plots ({low vs. high}*L, $F_{1,\,8} = 13.66$, $p = 0.006$) but not between non-exploited and exploited plots.
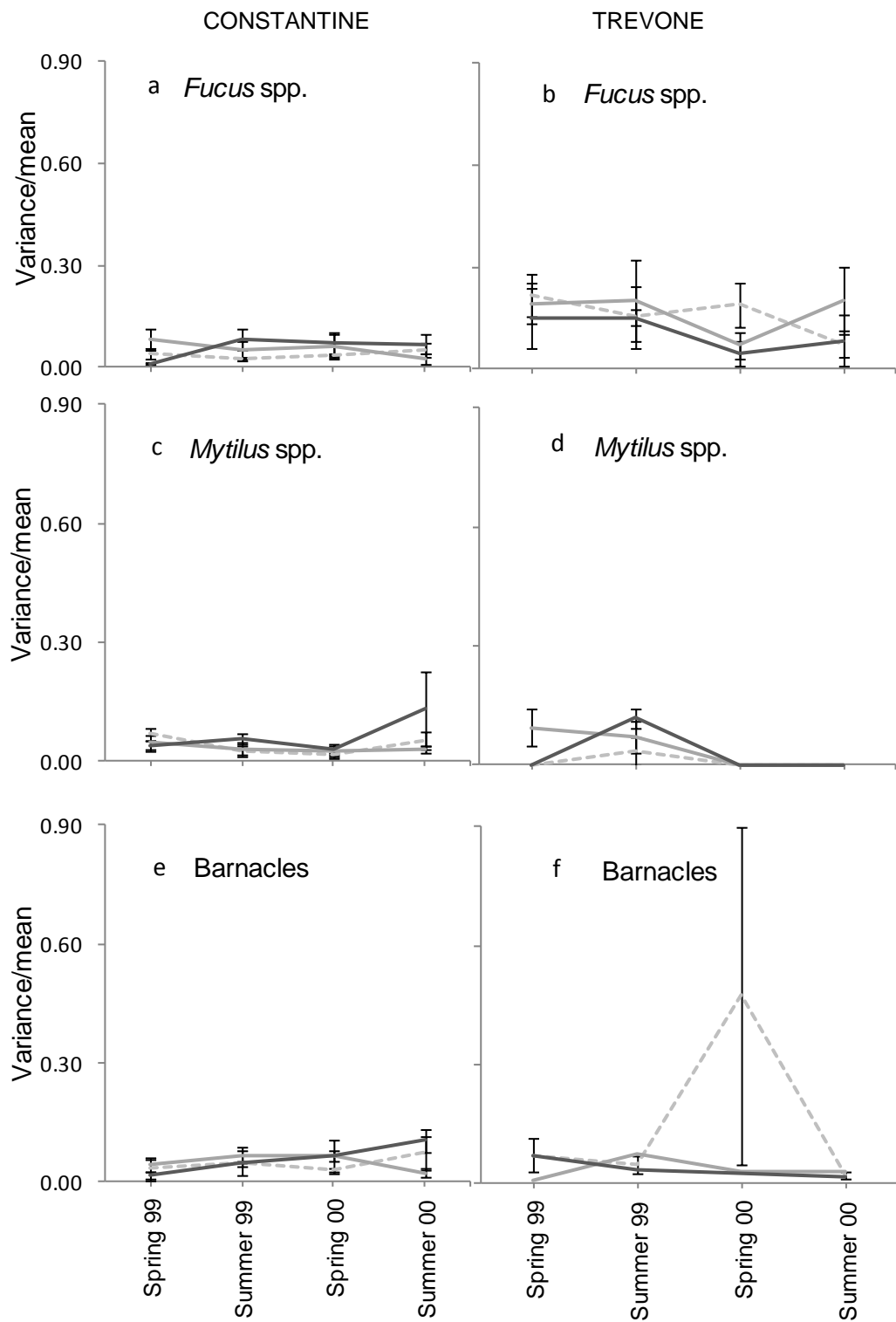
Figure 4.6. Changes during the experiment in estimated mean variance-to-mean ± SE (*n* = 3 plots per sample) for *Fucus* spp. (a, b), *Mytilus* spp. (c, d) and barnacles (e, f) by treatment plots: control (broken line), low (grey line) and high (dark line), at Constantine (left) and Trevone (right).

CONSTANTINE
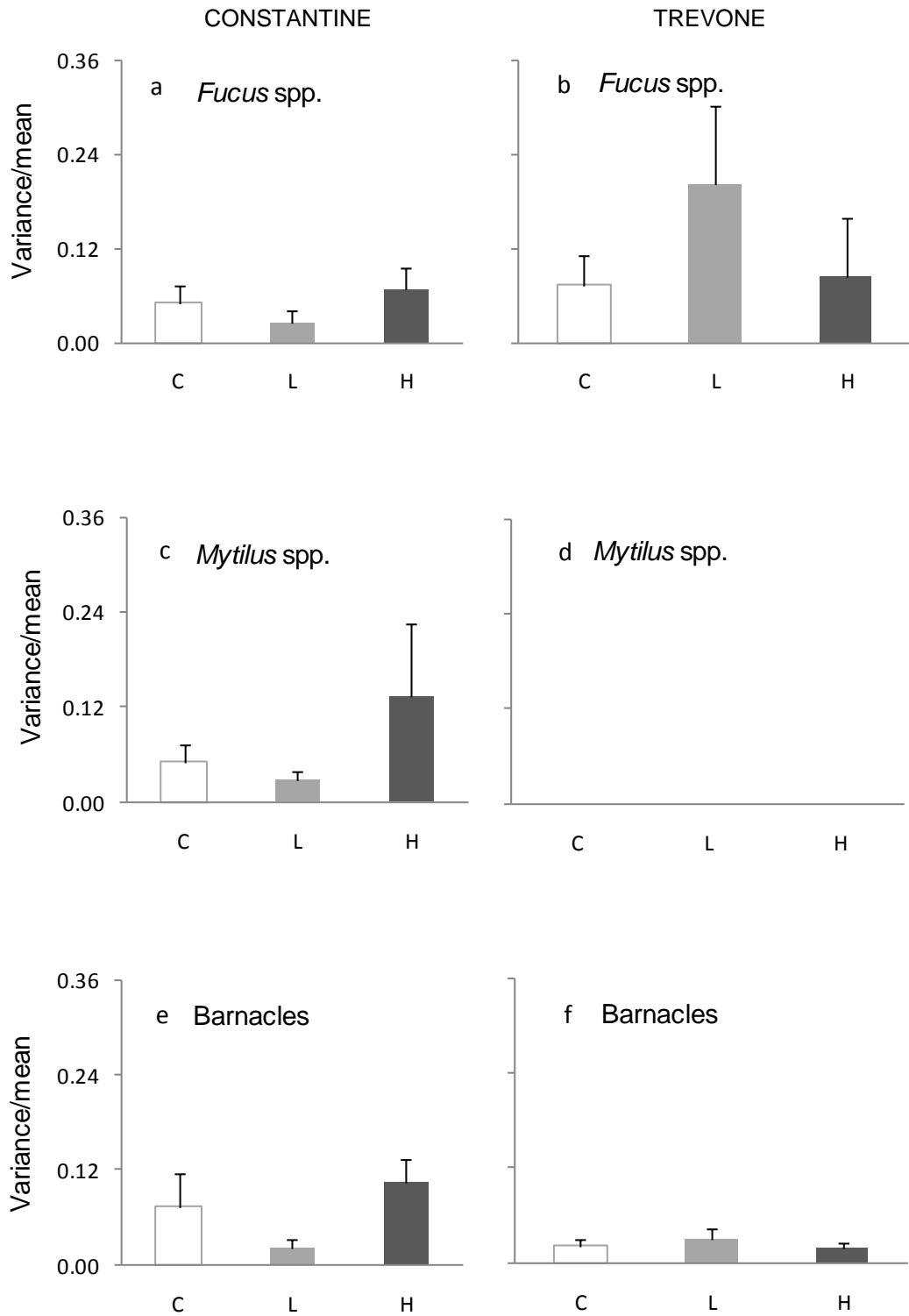
TREVONE

Figure 4.7. Estimated mean variance-to-mean with SE (n = 3 plots per sample) for *Fucus* spp. (a, b), *Mytilus* spp. (c, d) and barnacles (e, f), by treatment of control (C) low exploitation (L), high exploitation (H) at Constantine (left) and Trevone (right) in summer 2000 after the experiment had run for 18 months.
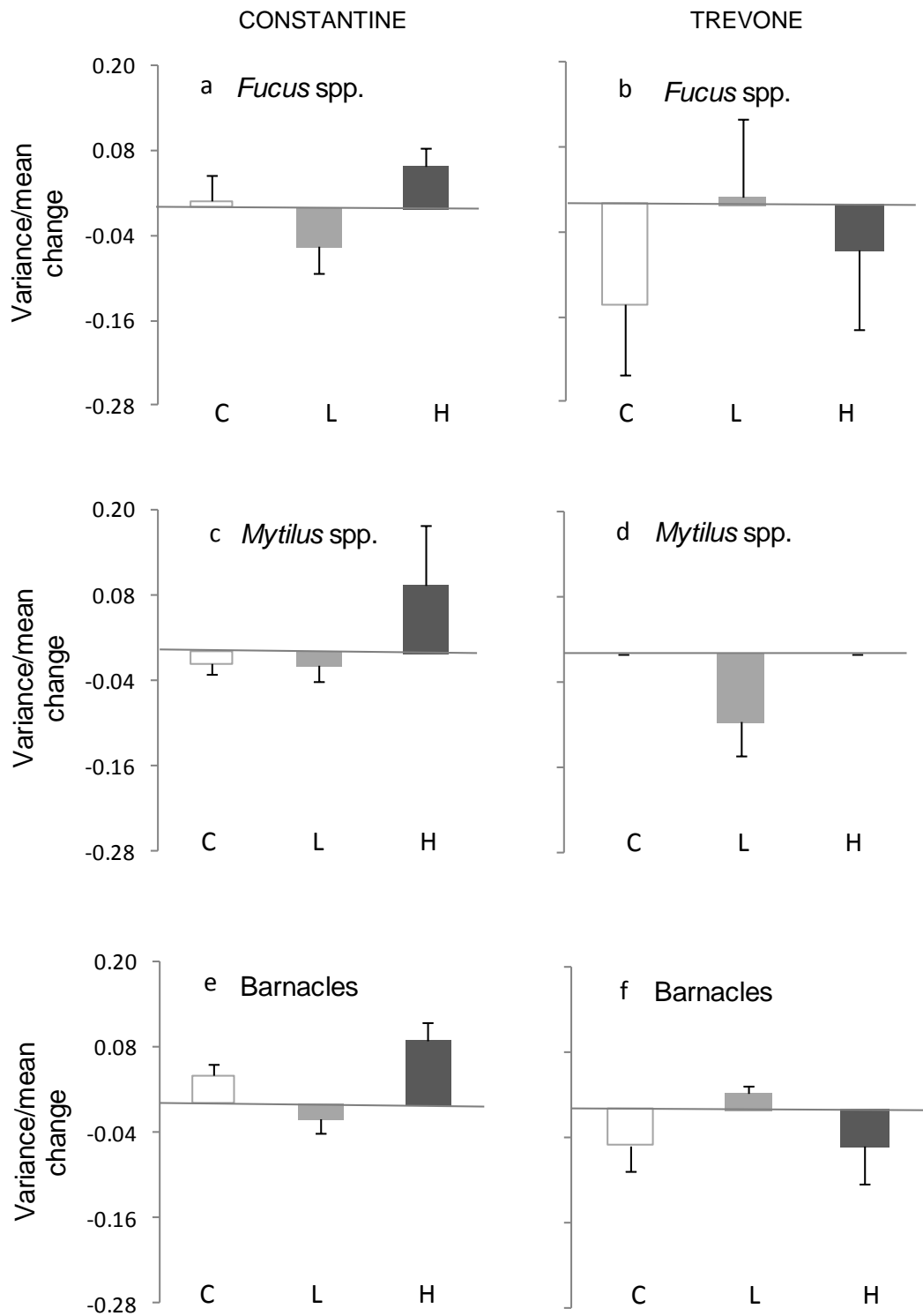
Figure 4.8. Change (end – start values) in the estimated mean variance-to-mean ± SE (n = 3 plots per sample) for *Fucus* spp.(a, b), *Mytilus* spp. (c, d) and barnacles (e, f), by treatment of control (C) low exploitation (L), high exploitation (H) at Constantine (left) and Trevone (right) in summer 2000 after the experiment had run for 18 months.

Table 4.5. Response of *Mytilus* spp. change in variance-to-mean ratio (end – start values) by treatment and by location. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	0.01	0.01	1.49	0.289
Block = B´(L´)	4	0.03	0.01	-	
Within subjects					
Treatment = T	2	0.03	0.02	5.55	**0.024**
NE vs. EX	1	<0.01	<0.01	0.01	0.923
low vs. high	1	0.03	0.03	7.50	**0.021**
T*L´	2	0.01	0.01	1.61	0.258
{NE vs. EX}*L´	1	0.01	0.01	3.33	0.105
{low vs. high}*L´	1	<0.01	<0.01	0.09	0.772
Error	8	0.03	<0.01		

Table 4.6. Response of barnacles change in variance-to-mean ratio (end – start values) by treatment and by location. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	0.02	0.02	3.80	0.123
Block = B´(L´)	4	0.02	<0.01	-	
Within subjects					
Treatment = T	2	<0.01	<0.01	0.07	0.931
NE vs. EX	1	<0.01	<0.01	0.59	0.584
low vs. high	1	<0.01	<0.01	0.04	0.877
T*L´	2	0.03	0.01	7.31	**0.016**
{NE vs. EX}*L´	1	<0.01	<0.01	0.07	0.835
{low vs. high}*L´	1	0.03	0.03	13.66	**0.006**
Error	8	0.01	<0.01		

4.4.3. Species richness

Figure 4.9 shows species richness (algae, macro-invertebrates and total) fluctuating throughout the course of the experiment. The biggest increase in algal, macro-invertebrate and total species richness occurred in summer 1999, at Constantine and Trevone in response to the removal of limpets in spring 1999 and the consequent *Fucus* spp. increase in percentage cover. That was the only sampling date where the treatments had significant effects on the measured parameters (Figure 4.10).

Only after the experiment had run for six months were significant treatment effects detected for algal richness after pooling procedures (Figure 4.10 (a, b), $F_{2, 10} = 4.95$, $p = 0.032$; Table 4.7), macro-invertebrate richness (Figure 4.10 (c, d), $F_{2, 2} = 25.53$, $p = 0.038$; Table 4.8) and total species richness (Figure 4.10 (e, f), $F_{2, 2} = 29.60$, $p = 0.033$; Table 4.9). The treatment main effect on algal richness had contrasts revealing that low-exploitation plots (2.4 ± 0.27) had lower levels of richness than high-exploitation plots (3.00 ± 0.35, pooled $F_{1,10} = 5.87$, $p = 0.036$; Table 4.7). A similar result was observed for the treatment main effect on total species richness where contrasts revealed that low-exploitation plots (7.30 ± 0.43) had lower levels of richness than high-exploitation plots (8.95 ± 0.96, pooled $F_{1,10} = 7.90$, $p = 0.018$; Table 4.9).

No differences were detected ($p > 0.05$) at the end of the experiment in algal, macro-invertebrate and total species richness by location and treatment (Figure 4.11).

Change in species richness over the course of the experiment (end – start values) were detected only on algae and by location (Figure 4.12 (a, b), pooled $F_{1,4} = 10.35$, $p = 0.032$; Table 4.10).
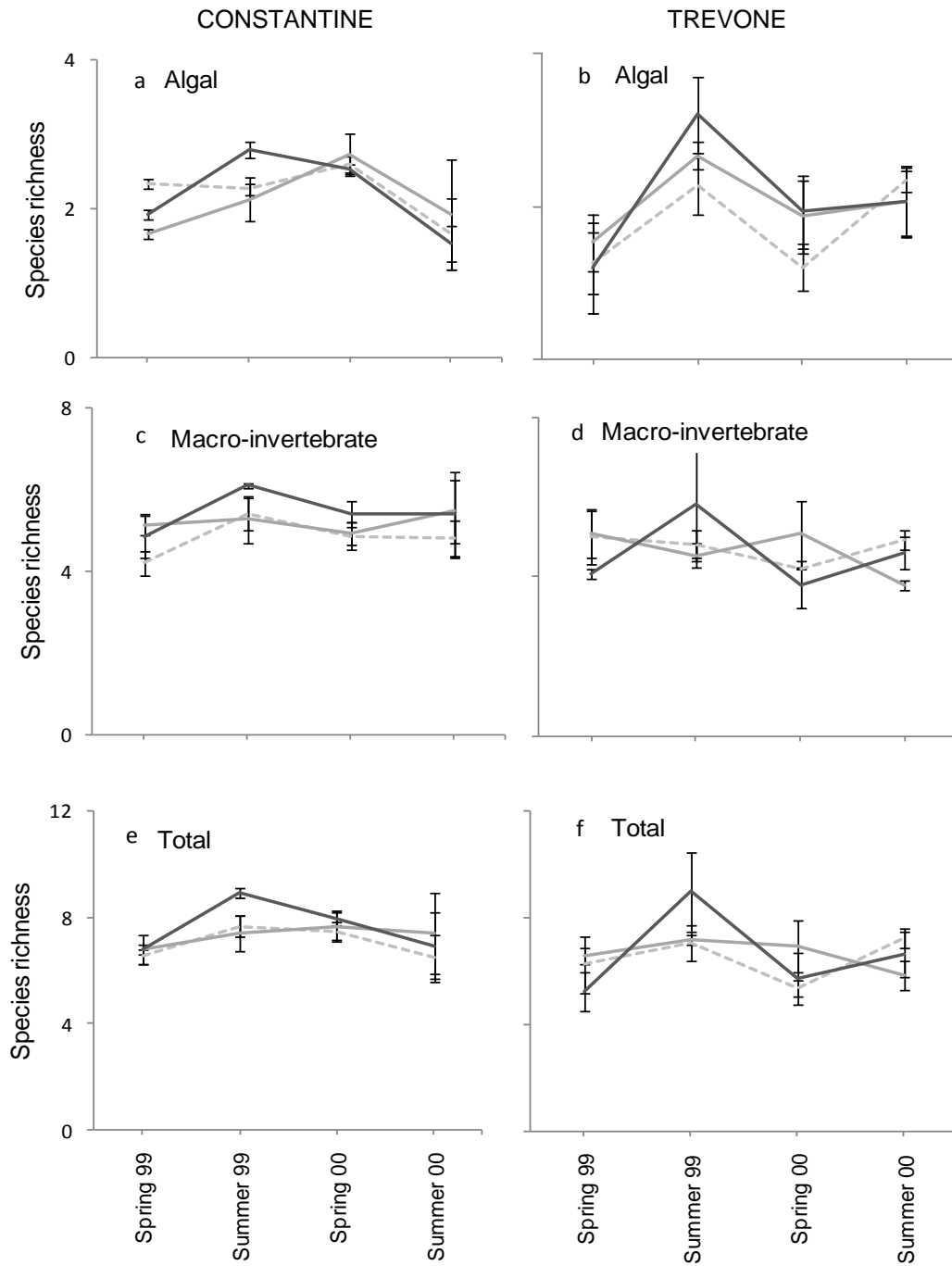
Figure 4.9. Estimated algal (a, b), macro-invertebrate (c, d) and total mean species richness (e, f) ± SE (n = 3 plots per sample) at each sampling season by treatment plots: control (broken line), low (grey line) and high (dark line), at Constantine (left) and Trevone (right).
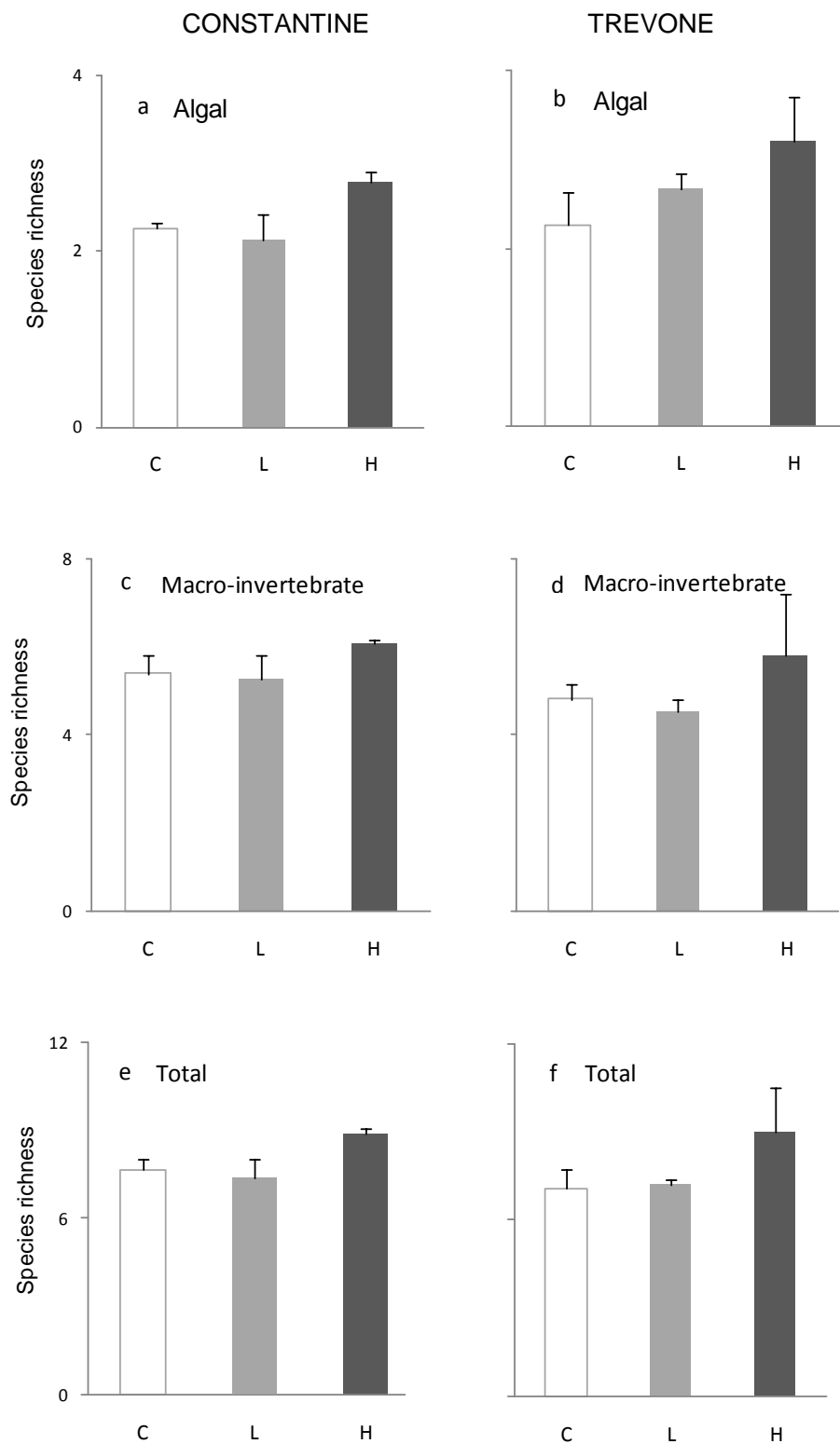
Figure 4.10. Estimated algal (a,b), macro-invertebrate (c, d) and total mean species richness (e, f) with SE ($n = 3$ plots per sample) by treatment plots: control (C: clear bars), low exploitation (L: grey bars), high exploitation (H: black bars) at Constantine (left) and Trevone (right) in summer 1999 after the experiment had run for 6 months.

Table 4.7. Response of algal species richness by treatment and by location in summer 1999. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	0.44	0.44	1.14	0.346
Block = B´(L´)	4	1.52	0.38	-	
Within subjects					
Treatment = T	2	1.83	0.92	4.95	**0.032**
NE vs. EX	1	0.75	0.75	4.08	0.071
low vs. high	1	1.08	1.08	5.87	**0.036**
T*L´	2	0.23	0.12	0.57	0.586
{NE vs. EX}*L´	1	0.22	0.22	1.10	0.325
{low vs. high}*L´	1	0.01	0.01	0.05	0.829
Error	8	1.60	0.20		

Table 4.8. Response of macro-invertebrate species richness by treatment and by location in summer 1999. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	1.31	1.31	0.58	0.487
Block = B´(L´)	4	8.95	2.24	-	
Within subjects					
Treatment = T	2	3.71	1.85	25.53	**0.038**
NE vs. EX	1	0.40	0.40	0.57	0.468
low vs. high	1	3.31	3.31	4.70	0.055
T*L´	2	0.15	0.07	0.08	0.920
{NE vs. EX}*L´	1	<0.01	<0.01	0.01	0.923
{low vs. high}*L´	1	0.14	0.15	0.17	0.691
Error	8	6.90	0.86		

Table 4.9. Response of total species richness by treatment and by location in summer 1999. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	0.23	0.23	0.09	0.774
Block = B´(L´)	4	9.92	2.48	-	
Within subjects					
Treatment = T	2	10.42	5.21	29.60	**0.033**
NE vs. EX	1	2.25	2.25	2.18	0.171
low vs. high	1	8.17	8.17	7.90	**0.018**
T*L´	2	0.35	0.18	0.14	0.871
{NE vs. EX}*L´	1	0.28	0.28	0.22	0.652
{low vs. high}*L´	1	0.07	0.07	0.06	0.813
Error	8	9.99	1.25		

Table 4.10.Response of algal richness change (end – start values) by treatment and by location. Contrasts as for Table 4.1.

Source	d.f.	Seq SS	Seq MS	F	p
Between subjects					
Location = L´	1	5.34	5.34	10.35	**0.032**
Block = B´(L´)	4	2.06	0.52	-	
Within subjects					
Treatment = T	2	0.14	0.07	0.09	0.915
NE vs. EX	1	0.05	0.05	0.07	0.797
low vs. high	1	0.08	0.08	0.10	0.758
T*L´	2	1.68	0.84	1.07	0.388
{NE vs. EX}*L´	1	0.93	0.93	1.18	0.309
{low vs. high}*L´	1	0.75	0.75	0.95	0.358
Error	8	6.31	0.79		
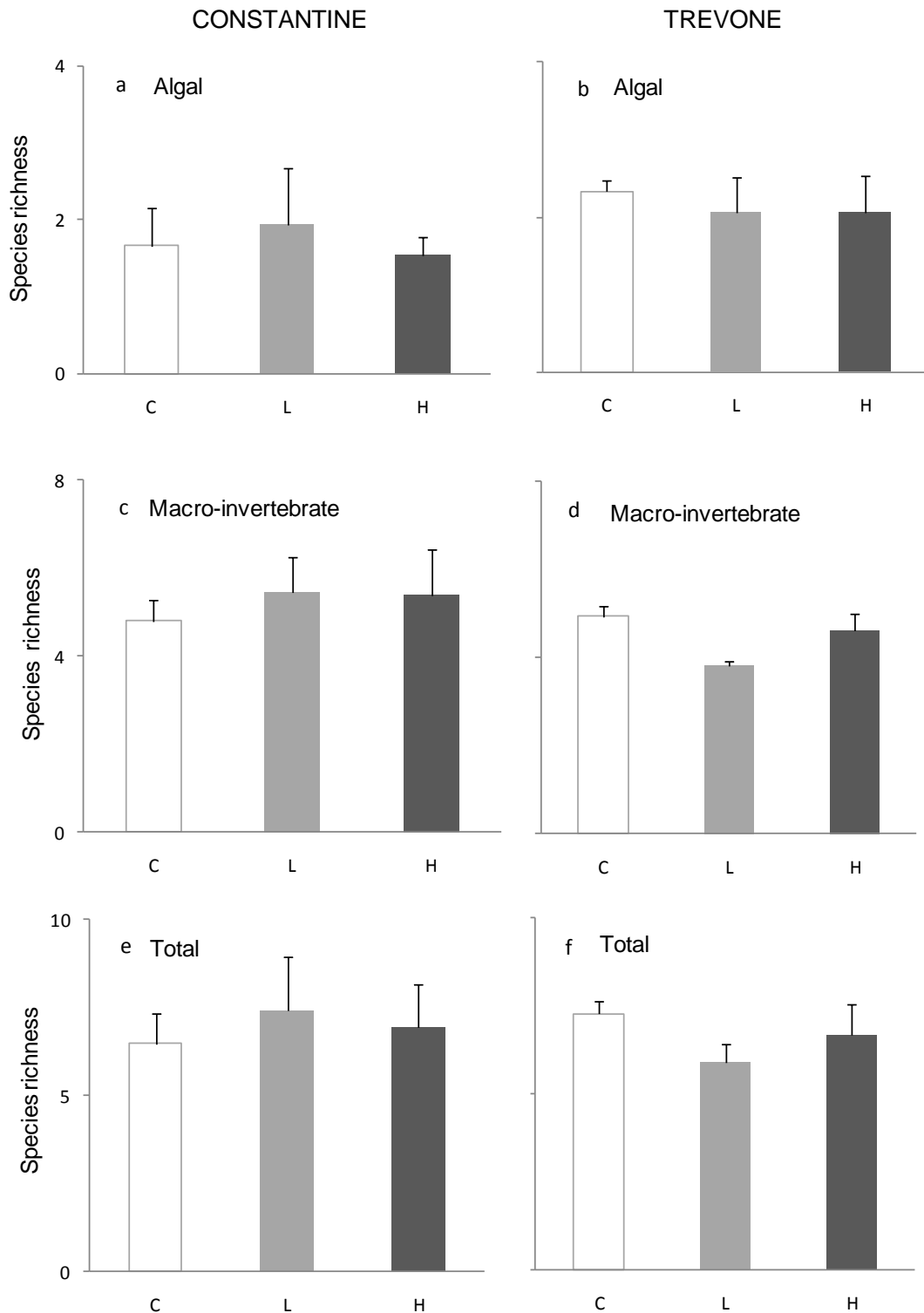
Figure 4.11. Estimated algal (a,b), macro-invertebrate (c, d) and total mean species richness (e, f) with SE (n = 3 plots per sample) by treatment plots: control (C: clear bars), low exploitation (L: grey bars), high exploitation (H: black bars) at Constantine (left) and Trevone (right) in summer 2000 after the experiment had run for 18 months.
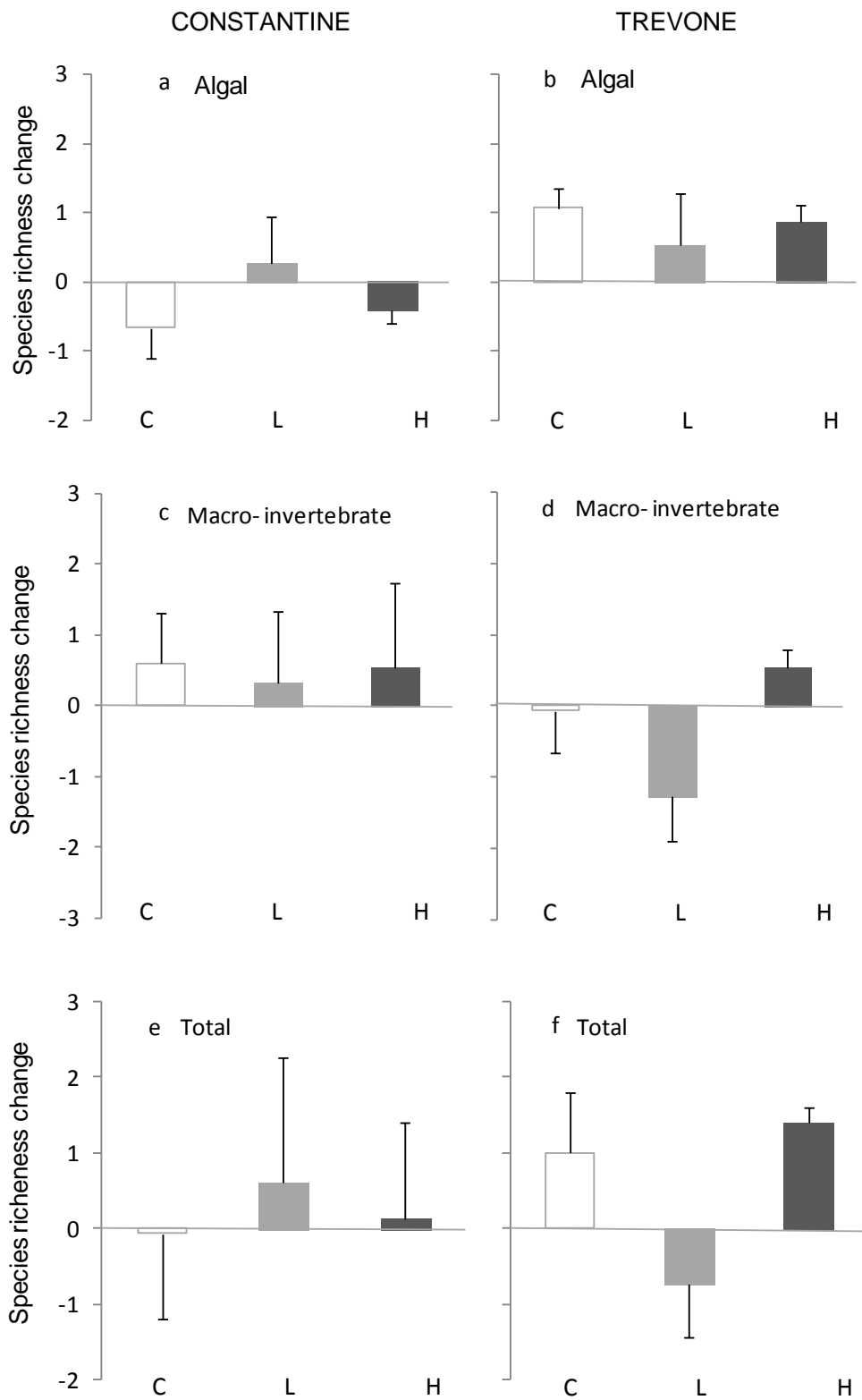
Figure 4.12. Change (end – start values) in the estimated algal (a, b), macro-invertebrate (c, d) and total mean species (e, f) richness ± SE ($n = 3$ plots per sample) by treatment plots: control (C: clear bars), low exploitation (L: grey bars), high exploitation (H: black bars) at Constantine (left) and Trevone (right) in summer 2000 after the experiment had run for 18 months.

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4.4.4. Multivariate analyses of community structure

No differences among treatments were detected on either shore in March 1999, before the experiment was started. Since the treatments had clear effects on both shores only at the last sampling date, in summer 2000, the description of results will focus primarily on that date. Figure 4.14 presents the plots of nMDS ordination for both shores from the beginning of the experiment (March 1999) through to summer 2000. Shifts in community structure were more pronounced from spring 2000 onwards, particularly at Trevone (Figure 4.14 (f, h)).

At Constantine in summer 2000 the removal of all limpets ≥ 25 mm had significant effects on community structure (Figure 4.14 (g), Table 4.11). There were no discernible differences between the control and low-exploitation plots but those treatments were different from the high-exploitation plots (Figure 4.14 (g), Table 4.11, pairwise post hoc comparisons). On the final sampling date, control plots were distinct from high-exploitation plots, due principally to increases in *F. vesiculosus* var. *evesiculosus* and *Mytilus* spp. (Table 4.12). Those species also contributed most to the dissimilarity of low- and high-exploitation plots, with higher removals raising percentage cover but their relative importance was inverted (Table 4.12). In both cases barnacles, *Lithophyllum incrustans* and *Gibbula umbilicalis* were the other species contributing to the dissimilarity between the plots but their influence and impact was minor.

Table 4.11. Results of PERMANOVA analysis for summer 2000 at Constantine. Analyses were based on Bray-Curtis similarities of square-root transformed data. Significant results based on Monte Carlo simulations (p (MC)) are shown in bold.

Source	d.f.	MS	Pseudo-F	p (MC)
Treatment	2	1185.50	2.77	**0.013**
Residual	42	427.62		

Table 4.12. SIMPER analyses for pairs of treatments significantly different from each other in summer 2000 at Constantine. Listed are the 5 species that contributed most to the dissimilarity between pairs of treatments. The SIMPER analyses presented were based on square-root transformed data to correspond with the PERMANOVA presented in Table 4.11. Abundance data shown were untransformed so that abundances/percentage of covers were interpretable. Avg.Abund Treat 1 and Avg. Abund Treat 2: average abundance or cover (untransformed) for the first and second treatments in the pair; Avg.Diss: average dissimilarity among pairs of samples in terms of species in question; Diss/SD: a measure of variation in the contribution of the species to dissimilarities between pairs of samples; Contrib%: percentage contribution of the species to the average overall dissimilarity between groups of treatments; Cum. %:cumulative contribution of the listed species.

Species	Avg. Abund Treat 1	Avg. Abund Treat 2	Avg. Diss	Diss/SD	Contrib %	Cum. %
Control versus High treatment (average dissimilarity 31.29)						
Fucus vesiculosus var. *evesiculosus*	30.34	65.85	8.16	1.46	26.06	26.06
Mytilus spp.	51.43	58.91	6.09	0.99	19.47	45.53
Barnacles	5.03	5.92	4.09	1.34	13.07	58.60
Lithophyllum incrustans	2.24	1.63	2.68	0.85	8.55	67.16
Gibbula umbilicalis	1.80	1.73	2.44	1.23	7.81	74.96
Low versus High treatment (average dissimilarity 28.69)						
Mytilus spp.	46.94	58.91	5.91	1.10	20.59	20.59
Fucus vesiculosus var. *evesiculosus*	40.68	65.85	5.44	1.66	18.96	39.55
Barnacles	7.41	5.92	4.09	1.38	14.27	53.82
Lithophyllum incrustans	1.90	1.63	2.45	0.87	8.53	62.35
Gibbula umbilicalis	2.67	1.73	2.31	1.33	8.04	70.39

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At Trevone in summer 2000 the removal of limpets ≥ 25mm also had significant effects on community structure (Figure 4.14 (h), Table 4.13). There were no discernible differences between the low- and high-exploitation plots but those treatments were both different from the control plots (Figure 4.14 (h), Table 4.13, pairwise post hoc comparisons). As at Constantine, *F. vesiculosus* var. *evesiculosus* was again important in contributing to dissimilarity among treatments and tended to have greater cover where limpets ≥ 25 mm had been removed (Table 4.14). Cyanobacteria *Rivularia bullata* also contributed substantially to the dissimilarity among treatments and tended to have reduced cover where limpets ≥ 25 mm had been removed, probably due to competition with fucoids and other algae (Table 4.14). Barnacles, *G. umbilicalis* and small limpets were the other species contributing to the dissimilarity between the plots but their contribution was minor. In fact, since the beginning of the experiment *G. umbilicalis* density increased particularly in low- and high-exploitation plots at Trevone where at the end of experiment *Fucus* spp. percentage cover was higher than at control plots (Figure 4.13). This increase could be the result of the reduction of competition with the removed limpets or due to the sheltered conditions under the fucoid canopy.

Table 4.13. Results of PERMANOVA analysis for summer 2000 at Trevone. Analyses were based on Bray-Curtis similarities of square-root transformed data. Significant results based on Monte Carlo simulations (*p* (MC)) are shown in bold.

Source	d.f.	MS	Pseudo-*F*	*p* (MC)
Treatment	2	4017.10	6.37	**0.001**
Residual	42	630.36		

Table 4.14. SIMPER analyses for pairs of treatments significantly different from each other in summer 2000 at Trevone. Presentation as for Table 4.12.

Species	Avg. Abund Treat 1	Avg. Abund Treat 2	Avg. Diss	Diss/SD	Contrib %	Cum. %
Control versus Low treatment (average dissimilarity 41.94)						
Fucus vesiculosus var. *evesiculosus*	6.26	58.57	13.39	1.66	31.92	31.92
Rivularia bullata	14.15	3.61	6.17	1.32	14.72	46.64
Barnacles	48.44	50.61	4.83	1.06	11.51	58.15
Gibbula umbilicalis	9.00	7.87	4.42	1.32	10.54	68.69
Small limpets	8.33	8.33	3.88	1.20	9.25	77.94
Control versus High treatment (average dissimilarity 44.14)						
Fucus vesiculosus var. *evesiculosus*	6.26	73.33	15.91	2.11	36.04	36.04
Rivularia bullata	14.15	0.54	6.12	1.34	13.87	49.91
Barnacles	48.44	43.13	5.62	1.70	12.74	62.65
Gibbula umbilicalis	9.00	14.80	5.44	1.31	12.32	74.97
Small limpets	8.33	7.87	3.10	1.25	7.01	81.98
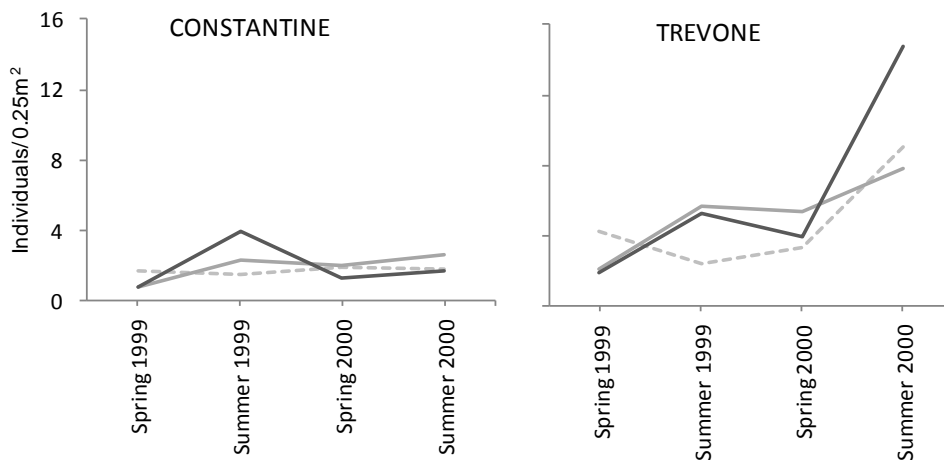
Figure 4.13. Estimated mean numbers ($n = 3$ plots per sample) of *G. umbilicalis* at each sampling season by treatment plots: control (broken line), low (grey line) and high (dark line), at Constantine (left) and Trevone (right).
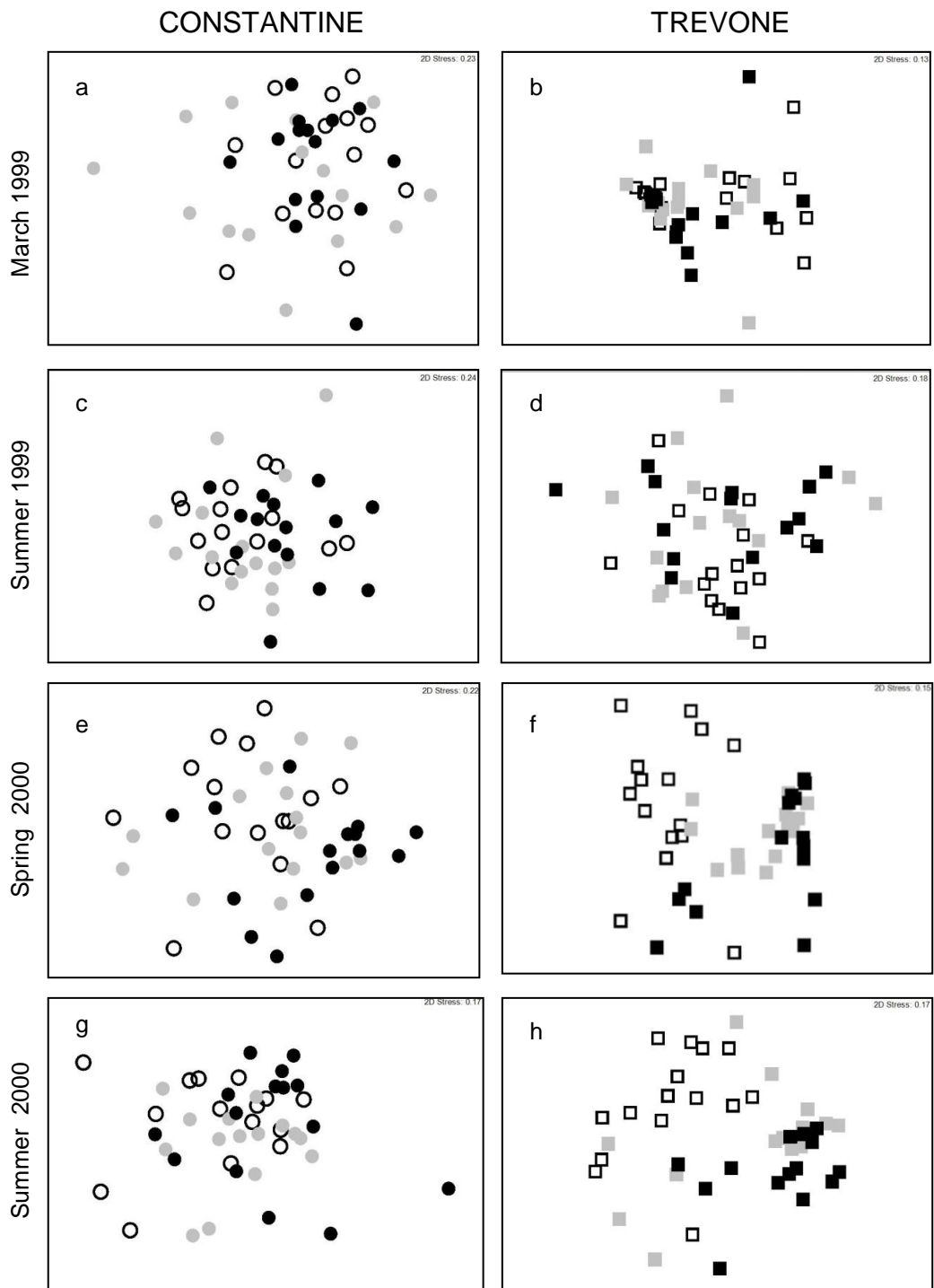
Figure 4.14.Non-metric multi-dimensional scaling (nMDS) representations of community compositions in the experimental plots in (a) March 1999 at Constantine,(b) March 1999 at Trevone,(c) summer 1999 at Constantine, (d) summer 1999 at Trevone, (e) spring 2000 at Constantine, (f) spring 2000 at Trevone, (g) summer 2000 at Constantine and (h) summer 2000 at Trevone. Constantine: circles symbols, Trevone squares symbols; unfilled symbols: control plots, grey symbols: low plots, black symbols: high plots.

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4.5.   DISCUSSION

Limpets regulate algal recruitment by grazing the early stages of macroalgae contained within epilithic microbial films (Hill and Hawkins 1991), and in some cases by direct consumption of mature algae (Davies et al. 2007; Crowe et al. 2011). This has been demonstrated by the establishment of opportunistic and fucoid algae where limpets have been removed or excluded (Jones 1948; Southward 1964; Hawkins 1981b; Jenkins et al. 1999a, 2005, 2008; Coleman et al. 2006; Crowe et al. 2011). My results showed that even a small reduction in limpet density relaxed the growth of fucoids and led to increased cover.

Mussels have been described as foundation species or ecosystem engineers (Jones et al. 1994; Lawton 1994; Crowe et al. 2011) because they modify their environment, changing its suitability for other organisms (Crowe et al. 2011). Intertidal mussel populations provide a biogenic structure for a diverse array of species including annelids, crustacea and other molluscs (Lohse 1993; Seed 1996; Crowe et al. 2004; 2011). Crowe et al. (2011) found that assemblages and cover of algae were positively affected by the presence of mussels.

Barnacles, however, being alternative ecosystem engineers are known for being unable to compensate in functional terms for the absence of mussels as they failed to prevent changes in algal cover and assemblage structure, despite their documented capacity to do so in the NE Pacific (Farrell 1991) and the Mediterranean (Maggi et al. 2009) showing how the influence of ecosystem engineers can vary (Crowe et al. 2011).

The observed differences by location at the end of the experiment on the percentage cover of *Mytilus* spp. (more abundant at Constantine) (Figure 4.4: c, d) and barnacles (more abundant at Trevone) (Figure 4.4: e, f) confirmed the differences in wave and wind exposure of both shores. The limpet-removal treatment was found to result in the establishment of *Fucus* spp. At the end of the experiment non-exploited plots (controls) had lower *Fucus* spp. percentages than exploited plots (low- and high-exploitation plots). Furthermore, while the change in percentage cover of *Fucus* spp. over the course of the experiment (final minus first values) decreased in non-exploited plots (controls) it increased in exploited plots (low- and high-exploitation plots).

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At the end of the experiment no differences were detected in the variance-to-mean of the key species (Figure 4.7), suggesting that either patchiness was equivalent between location and treatments or there was too much variation within plots to make those differences detectable.

Nevertheless, during the experiment there were detectable effects on patchiness in *Mytilus* spp. and barnacles although at different levels. The change in variance-to-mean of *Mytilus* spp. over the course of the experiment (final minus first values) responded to exploitation by treatment, and barnacles had a significant treatment*location interaction suggesting that their response is context dependent. In the case of *Mytilus* spp. in low-exploitation plots the variance-to-mean index decreased (-0.06 ± 0.04), whereas in high-exploitation plots it increased (0.05 ± 0.06), which was opposite to what I expected at the outset. The reduction of patchiness in low-exploitation plots means that the colonisation of the bare space by the mussels was more uniform in low- than high-exploitation plots but the reasons for that are unclear.

The effect of herbivores on diversity of primary producers remains controversial (Olff and Ritchie 1998; Crowe et al. 2011). High grazing pressure seems to reduce algal diversity while moderate grazing pressure can increase it (Paine and Vadas 1969; Lubchenco 1978; Anderson and Underwood 1997; Aguilera and Navarrete 2007; Crowe et al. 2011; Skov et al. 2011). In summer 1999, approximately six months after the experiment started, differences were detected between treatments on the algal, macro-invertebrate and total species richness but not afterwards. The contrast analysis on algal and total species richness for the treatment main effect revealed that low-exploitation plots had lower levels of richness than high-exploitation plots. It could have been the case that during these six months the high-exploitation plots had only reached intermediate levels of disturbance and therefore favoured higher levels of species richness in accordance with Connell's (1978) intermediate disturbance hypothesis. Nevertheless at the end of the experiment no significant differences occurred in species richness. One explanation could be that intermediate levels of disturbance only occurred during early-succession and were no longer apparent by the end of the experiment. Therefore what initially was considered to be set as an intermediate level of disturbance (low-exploitation treatments) probably reached higher levels than expected, although this does not explain the lack of differences to the control plots. The solution could have been to have augmented the resolution of

recording species richness especially of smaller species (see Thompson et al. 1996). The detected differences by location on species richness over the course of the experiment (final minus first values) only emphasise that the communities of both shores respond differently to the same source of disturbance. The collection of key species and microscopic counting of the observed species would certainly have benefited the study. As emphasised by Thompson et al. (1996) experiments where starfish were removed reduced the diversity of larger species (Paine 1969), as space is dominated by mussels rather than a mosaic of less competitive species. However, if the total biota inhabiting a mussel bed is examined in detail, species diversity will be greater because of biological provision of habitat within the mussel matrix (Seed 1996; Thompson et al. 1996).

Similarly, removing grazers can lead to a uni-algal stand of fucoids (Hawkins and Hartnoll 1983), but the species diversity of the whole system may increase due to the provision of habitat by the algae (Thompson et al. 1996). Variations in the patchiness and percentage of cover of mussels, fucoids and barnacles would greatly determine these patterns. Thompson et al. (1996) found that *Fucus* clumps had a greater number of species per number of individuals than were found amongst barnacle covered and open rock, although more individuals were found on barnacle covered and open rock than were found under *Fucus* clumps. Barnacle settlement under *Fucus* is poorer than on open substrate (Menge 1976; Grant 1977; Hawkins 1983). Nevertheless, greater diversity and abundance of macrobiota was found amongst the barnacle mosaic than on surrounding rock (Thompson et al. 1996). This mosaic probably provides a refuge for small invertebrates from dislodgment by wave action and to some extent from desiccation in a similar way to that provided by a matrix of mussel shells (Seed 1996; Thompson et al. 1996).

Rocky shores communities vary considerably both spatially and temporally (Hawkins and Hartnoll 1983; Hartnoll and Hawkins 1985; Hawkins et al. 1992; Thompson et al. 1996) with the proportions of *Fucus,* mussels and barnacles alternating in a cycle over several years on some moderately exposed shores. Therefore biodiversity is expected to co-vary accordingly. There is also evidence that plant diversity may depend more on spatial heterogeneity and variance in grazing pressure than its mean intensity (Olff and Ritchie 1998; Benedetti- Cecchi 2000; Sommer 2000; Crowe et al. 2011). The importance of these links will only be

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understood by examining the whole community at an appropriate range of spatial scales (Thompson et al. 1996).

In the case of limpets, although they were selectively removed at two different effective rates, at the end of the experiment no differences were detected. This is possibly due to the recruitment from crevices and open ocean that was induced by the *Fucus* spp. canopy shelter. Large limpets are superior competitors that may modulate the abundance of small limpets on the shore (Boaventura et al. 2003). Size has been considered as being important in determining competitive superiority, with larger individuals (either from different species, age or size class) usually being superior (Connell 1983; Schoener 1983; Boaventura et al. 2003). In fact, many studies have demonstrated that density-dependent processes can regulate the abundance of limpet populations (see Branch 1981; Hawkins and Hartnoll 1983; Underwood 1992; for a review). A higher requirement for food among large limpets and their greater size could lead to asymmetry in competition for food (Boaventura et al. 2003). Therefore, the removal of the bigger limpets targeted the recruitment and juvenile settlement due to the reduction of competition.

The multivariate analysis confirmed the influence of fucoids when limpets are removed. In both shores *F. vesiculosus* var. *evesiculosus* was the most important species contributing for the dissimilarity between treatment plots. On the other hand it is important to stress that even quite a small reduction in grazer densities can have a profound effect on the rest of the community. Nevertheless differences in both shores community and its response to the limpet removals were also detectable. The other species responsible for the dissimilarity between plots and the community response to the same levels of exploitation were different on each shore. At Constantine, plots where all limpets ≥ 25 mm were removed (high treatment plots) diverged from control and low plots in community structure. Therefore the removal of limpets ≥ 25 mm from half of the plot area (low treatment) had no effect on shifting the community structure. On the other hand at Trevone there was a convergence in community structure between low and high treatment. This suggests that the threshold level of exploitation of limpets causing community level impacts was lower at Trevone than at Constantine implying that decisions about how much exploitation can be allowed need to be site-specific. These results emphasise the idea that different communities (in terms of shore geomorphology, species present and relative abundances) respond differently to

causes of disturbance and management measures should encompass those differences but certainly demand further studies.

CHAPTER 5

COMPARISON OF *PATELLA* SPP. STOCKS ON THE CANARY ISLANDS BETWEEN 1992 AND 1999

5.1. ABSTRACT

Limpets of the genus *Patella* have been harvested for human consumption in Macaronesia for several centuries. In 1999 I repeated an unpublished broad-scale subtidal survey from 1992 by S. J. Hawkins and Norberto Serpa of stocks of limpets (*P. aspera* and *P. c. crenata*) in the Canary Islands, which estimated abundance using catch per unit efforts (timed dives). I quantified spatial variability in limpet catch abundances between and within islands related to human population density and aspect of shores respectively. *P. aspera* capture decreased from 1992 to 1999, while for *P. c. crenata* no differences were detected, suggesting that the former species was under a higher harvest pressure during that period. I detected catches with a significant year*aspect interaction for *P. c. crenata* abundance. This was influenced by the higher catches of *P.c. crenata* in 1999 at the north-facing shores. These differences in catches due to the aspect of the shore could be explained by the preference of this species for exposed shores which prevail in the north mainly due to the effect of the Canary Current and northern winds.This ecological and exploitation regime suggests that in 1999 differences in environmental conditions were influencing *P. c. crenata* abundance leading to differences between the north and south of the islands. In 1999 differences were detected in catches for both species at shore and island level (Tenerife-high exploitation, Fuerteventura and La Palma both lower exploitation). The observed within-islands differences were concordant with later studies in the intertidal zone of the Canary archipelago that detected small-scale, intra-island variation on the abundances of *P. aspera* and *P. c. crenata.* Those studies nevertheless did not report the observed differences between islands since at the time of my survey the overexploitation of this resource across the islands was probably not evident. I review protective measures in the Azores and Canary Islands for

limpets and make some management recommendations for limpet conservation that could be applied to Macaronesia and elsewhere.

5.2. INTRODUCTION

Coastal resources have been subjected to intense human exploitation from the beginning of the 20th century, particularly on islands which have finite resources. The harvest and collection of macro-invertebrates is one of the main human impacts on the littoral environment (Castilla 2000; Thompson et al. 2002). Patellid limpets are heavily collected by both recreational and professional shellfish gatherers throughout Macaronesia, which encompasses the biogeographic region containing the Azores, Madeira, the Savage Islands, the Canary Islands and Cape Verde (Corte-Real et al. 1996; Hawkins et al. 2000; Navarro et al. 2005).

Remains of shellfish collections can be found throughout the Canary Islands archipelago, dating from the Guanche people, the first colonizers of the archipelago prior to the Spanish at the beginning of the 15th century. Some residents consider the arrival of explorers as the reason for the existence of shellfish middens. It is said in folklore that the Guanches used to leave the mountains where they had protection from invaders in order to collect shellfish from the shore, generating the shell piles of which limpets make up the major part. Indirect evidence of human impacts on limpet stocks is suggested by the current distribution of the large intertidal limpet *Patella candei candei* d´Orbigny 1840, now occurring only in Fuerteventura though it has been found in middens and as a fossil in raised beaches from across the archipelago (Núñez et al. 1994; Núñez 1995; Corte-Real et al. 1996; Weber and Hawkins 2002; Moro and Herrera 2000).

Nowadays limpets are a popular gourmet item of many restaurants in the Canary Islands being consumed by both residents and tourists. Pressure on limpet stocks has increased since the tourist boom in the early 1980s and presently they receive protection in law by several regulatory instruments (Boletín Oficial de Canarias (BOC), Ley 17/2003, from 10th April, Fisheries from the Canary Islands; BOC, Orden from 14th April 2008, temporary regulation of professional shellfish harvesting by foot; BOC, Orden from 2nd and 18th May 2011, establishment of several rules for the shellfish harvesting by foot in the Canary Islands). Therefore the

Canary Islands are an excellent location to investigate aspects of human overexploitation of limpet for several reasons: *Patella* spp. have been harvested for several centuries, initially for self-consumption and later also for commercial purposes (trade and restaurant supply). Of the four species occurring throughout the archipelago, *P. aspera* and *P. c. crenata* are present in all islands; these species are highly sought after as a food resource by residents. The human occupation of the islands is not uniform and some islands are more populated than others giving gradients of exploitation.

Human induced processes contribute to the spatial variability that is intrinsic to coastal ecosystems (Menconi et al. 1999; Benedetti-Cecchi 2001; adapted from Navarro et al. 2005). Understanding spatial variation is a key requisite for explaining the distribution and abundance of the populations and understanding the processes operating in the intertidal and subtidal environment (Underwood 1990; Underwood and Chapman 1996; Menconi et al. 1999; adapted from Navarro et al. 2005). Moreover, the temporal variation can contribute to shaping spatial distribution; this relation can be determined by factors such as variation in natural conditions that affect recruitment (winds and currents), exploitation regimes and diseases that can affect the species survival.

The aim of this chapter is to document changes in the status of stocks of the limpets *P. aspera* Röding 1798 and *P. c. crenata* d'Orbigny 1840 in the Canary Islands by repeating in 1999 an unpublished survey made in 1992 by S. J. Hawkins and Norberto Serpa from the University of the Azores, thereby quantifying spatial variability in limpet abundance between and within islands. The two species were studied separately, since they occupy different shore zones and are collected by fishers using different strategies. This chapter tests three hypotheses. Hypothesis (1): Catch per unit effort (CPUE: g/30min) changed from 1992 to 1999 according to exploitation intensity or shore aspect across the islands. I predicted that the most exploited shores would have more reduced catches from 1992-1999 than the least exploited shores. During the field survey I observed that limpet catches were greater on the north-facing and more exposed shores. I therefore predicted that shore aspect would also influence the limpet abundance across the islands. This was also tested for Tenerife Island, as it was the island with most sampled shores. Hypothesis (2): In 1999 there would be differences in the CPUE (g/10 min dive) of both *Patella* species between shores at Tenerife according to aspect. Hypothesis (3): In 1999 CPUE (g/10

min dive) of both *Patella* species for Tenerife, Fuerteventura and La Palma would be explained by variation between island and within island. These islands have different human population density but also have shores with specific geographic and geological features that might be responsible for the variation between and within island. The proportion of females in relation to the abundance was also examined for the two species, to investigate if there were differences between *P. aspera*, possibly a protandric species, and *P. c. crenata* where evidence of protandry is equivocal.

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5.3. MATERIAL AND METHODS

5.3.1. Study sites and organisms

The study was carried out in the Canary Islands, located off the north west African coast, at about 115 km from Cabo Jubi (Figure 5.1). These seven volcanic islands are divided geographically and politically into a western and an eastern group, the former being Tenerife, La Gomera, El Hierro, La Palma, and the latter consisting of the remaining Gran Canaria, Lanzarote and Fuerteventura. According to its geographical location, the Canary Islands belong to the Atlantic-Mediterranean region, which covers the waters from the English Channel until Cabo Blanco.
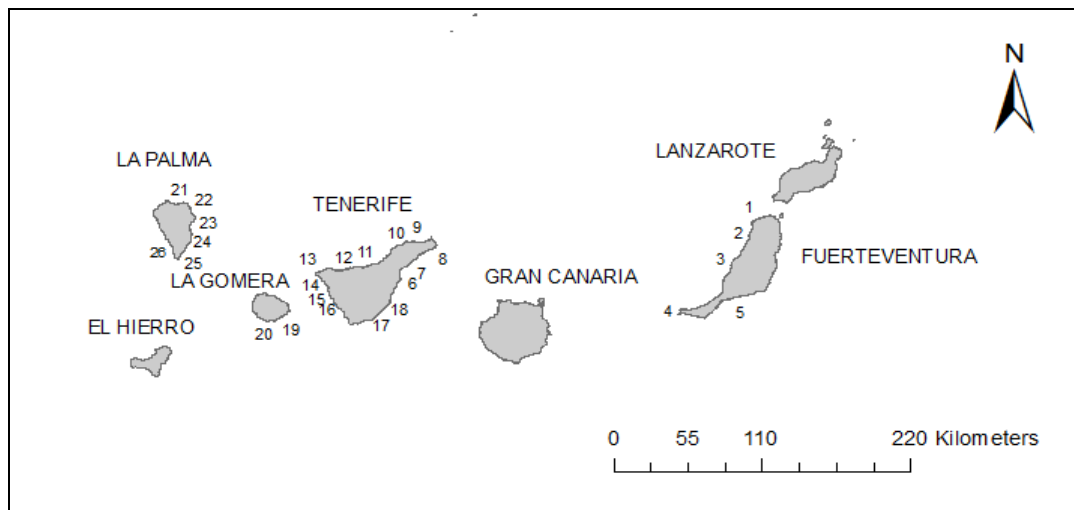
Figure 5.1. Map of the Canarian archipelago showing relative position of sampling shores and those compared in 1992 and 1999 (underlined).Fuerteventura:1, El Cotillo; 2, Los Molinos; 3, Playa de los Muertos; 4, Faro de Jandia; 5, Playa de las Matas Brancas; Tenerife: 6, Candelaria; 7, Las Caletillas; 8, Las Gaviotas; 9, Punta de Hidalgo; 10, Playa del Pris; 11, San Juan de la Rambla; 12, Playa San Marcos; 13, Punta de Teno (exposed); 14, Punta de Teno (sheltered); 15, Los Gigantes; 16, Playa Paraiso; 17, Punta del Sordo; 18, Faro de Abona; La Gomera: 19, San Sebastian; 20,Playa Santiago; la Palma: 21, La Fajana; 22,San Andres; 23, La Palmita; 24, Punta de los Guinchos; 25, Las Cabras; 26, Punta Zamora.

The limpet *P. aspera* occurs in the intertidal zone amongst clearings in the algal turf, and below low-water mark (Hawkins et al. 2000). *P. c. crenata* is mainly intertidal although larger individuals can also be found in shallow subtidal habitats

(Christiaens 1973; Hernández-Dorta 1992). While *P. c. crenata* has a dark foot, *P. aspera* has a foot with light colour, a feature that is used by collectors to distinguish both species. The Macaronesian species are sufficiently genetically different to be considered a separate from the continental specimens (see Weber and Hawkins 2005) and thus the name *P. aspera* is the correct name to use. Unfortunately in the summer both species can enter a resting phase but this is also the best time for diving surveys due to sea conditions. Thus the data on sex should be treated with caution.

Some of the collected specimens of *P. aspera* from Playa de las Matas Brancas at Fuerteventura Island had the boring bivalve *Leiosolenus aristatus* (Dillwyn 1817), previously named *Myoforceps aristatus* (Gofas 2012) and also known as *Lithophaga aristata* incrusted on the shell to a maximum of three bivalves per shell (see appendix 2). It bores into a wide variety of shells including *Spondylus, Chama, Haliotis, Patella, Strombus, Lambis*, and *Ostrea*, as well as calcareous rocks (Turner and Boss 1962). The species is easily identified by pointed tips at the posterior ends of the valves, which cross like fingers (Abbott 1974) and give the common name of the species as "scissor date mussel" (Simone and Gonçalves 2006). *L. aristatus* has a wide distributional range but had never previously been found in the Canary Islands.

5.3.2. Field survey

Data were collected in July 1999. On each shore, three ten-minute snorkel dives were carried out on the same day by Manuel Machado in adjacent areas where at least a few limpets were initially present. The total catches of *P. aspera* and *P. c. crenata* were wet-weighed after removing excess moisture, and each individual was sexed and measured (maximal length). Due to the presence of macro-algae on the shell of *P. aspera* its wet weight was over-estimated and would be a nuisance in an analysis pooling across species. Data were integrated with CPUE data from the same locations collected in June 1992 by Norberto Serpa and S.J. Hawkins and described in Fernandez (1999). The surveys differed in who performed the snorkel dives (and hence also in their collecting abilities), and in methodologies. In the 1992 survey a single 30-minute snorkel dive was performed in each sampled shore, while in 1999 three ten-minute snorkel dives were done at each shore. Some of these analyses combined data from 1992 and 1999 but others such as the investigation of the spatial variability in limpet distributions between and within islands only used the 1999 data

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that I collected and in which I have most confidence. Therefore comparisons of data from these different surveys present some limitations and the lack of replication within shores in 1992 constrains some analyses.

During the 1999 survey, I sought to characterise the foraging behaviour and intensity of collecting activity in the intertidal and subtidal zones by direct observation of collectors, interviewing collectors and restaurant managers. People in general were reticent about collaborating, so this information is scarce and insufficient for quantitative analysis.

To assess the status of the limpet stocks in the Canaries archipelago, replicate shores on each island were assigned with an exploitation index that was defined by two factors: accessibility of limpet stocks (road access conditions to shore and sea state during most of the year) and proximity to urban areas. The exploitation index was calculated by adding the scores of each factor, yielding a total score that could vary between 15 and 50 as shown in Table 5.1.

For each species the arcsine proportion of females ((number of females) / (number of males + number of females)) were plotted against the log of CPUE following the methods and results from Chapter 2.

Table 5.1. Partitions of the exploitation index.

Accessibility (road access and sea state)	Score	Proximity to urban area	Score
Accessible or calm seas	30	Near (0-1km)	20
Some difficulty in access or rough seas	20	Not near (1km-10km)	15
Very inaccessible or strong seas	10	Far away (>10km)	5

5.3.3. Statistical analysis

Several different statistical models were used to test each hypothesis since the shores used in each analysis differed depending on the questions being investigated. This nevertheless presents some limitations to interpretation since different analyses will not reveal potentially confounding differences, for example between exploitation index and aspect of the shores. For all ANOVAs, visual inspection of the residuals

following transformations as necessary indicated normal distributions and homogeneous variances.

5.3.3.1.   Influence of exploitation on CPUE

A two-factor model with repeated measures on one cross factor (equivalent to model 5.6 and 6.3 (i) in Doncaster and Davey, 2007) was used to test the hypothesis that the CPUE for both species would be different from 1992 to 1999 and between the six least and the six most exploited shores (model 1, Table 5.2). The six least exploited shores corresponded to six randomly chosen shores with the lowest exploitation index and the six most exploited shores included six randomly chosen shores with the highest exploitation index. Shores were randomly chosen after been assigned a number that was arbitrarily picked from a choice of many.

This design had two levels of the repeated-measures: fixed factor Year (Y_2), at each of two levels of the fixed factor Exploitation (E_2), with six shores (S_6) nested in each level of exploitation. This design was analysed with the split-plot ANOVA model:

$Y = Y_2 | S_6'(E_2)$ model 1

where a prime represents a random factor, vertical line means 'crossed with levels of', open bracket means 'nested in levels of', and subscript gives number of factor levels. The model was tested with the Minitab statistics package by requesting analysis of terms: $= E|Y + Y|S(E) - Y*S(E)$. It produced an output with the structure described by Table 5.2. This model cannot test the $Y*S'$ interaction, due to lack of replication and the absence of residual error term (bottom line of Table 5.2).

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Table 5.2. ANOVA model 1, degrees of freedom (d.f.), components of variation and MS for F ratios. P´ denotes replicate observations.

Mean square	d.f.	Components of variation estimated in population	F- ratio
Between subjects			
E	1	P´(Y*S´(E)) + S´(E) + E	E/ S´(E)
S´(E)	10	P´(Y*S´(E)) + S´(E)	No test
Within subjects			
Y	1	P´(Y*S´(E)) + Y*S´(E) + Y	Y/ Y* S´(E)
Y*E	1	P´(Y*S´(E)) + Y*S´(E) + Y*E	Y*E/ Y* S´(E)
Y* S´(E)	10	P´(Y*S´(E)) + Y*S´(E)	No test
P´(Y* S´(E))	0	P´(Y*S´(E))	-

5.3.3.2.   Influence of coastal aspect on CPUE

The same model was used to test the hypothesis that the CPUE for both species would be different from 1992 to 1999 and between nine north-facing and nine south-facing shores of the islands (model 2, Table 5.3).

This design had two levels of the repeated-measures fixed factor Year (Y_2), at each of two levels of the fixed factor Aspect (A_2), with nine shores (S_9) nested in each level of aspect. This design was analysed with the ANOVA model:

$Y = Y_2|S_9´(A_2)$ model 2

The model was tested with the Minitab statistics package by requesting analysis of terms: A|Y + Y|S′(A) – Y*S′(A). It produced an output with the structure described by Table 5.3. This model cannot test the Y*S´ interaction, due to lack of replication and the absence of a residual error term.

Table 5.3. ANOVA model 2, degrees of freedom (d.f.), components of variation and MS for *F* ratios. P´ denotes replicate observations.

Mean square	d.f.	Components of variation estimated in population	*F*- ratio
Between subjects			
A	1	P´(Y*S´(A)) + S´(A) + A	A/ S´(A)
S´(A)	16	P´(Y*S´(E)) + S´(E)	No test
Within subjects			
Y	1	P´(Y*S´(A)) + Y*S´(A) + Y	Y/ Y* S´(A)
Y*A	1	P´(Y*S´(A)) + Y*S´(A) + Y*A	Y*E/ Y* S´(A)
Y* S´(A)	16	P´(Y*S´(A)) + Y*S´(A)	No test
P´(Y* S´(A))	0	P´(Y*S´(A))	-

A two-factor nested design (equivalent to model 2.1 (i) in Doncaster and Davey, 2007) was used to test the hypothesis that the CPUE for both species would be different from shores within Tenerife and between five north-facing and five south-facing shores of the island (model 3, Table 5.4).

This design had five levels of the random factor Shore ($S´_5$), nested in each of two levels of a fixed factor Aspect (A_2), with three samples (D_3) in each shore. This design was analysed with the ANOVA model:

$$Y = D_3´(S_5´(A_2)) \qquad \text{model 3}$$

The model was tested with the Minitab statistics package by requesting analysis of terms: = A+ S´(A). It produced an output with the structure described by Table 5.4 where planned *post hoc* pooling is permissible for Aspect if S´(A) has $p > 0.25$ (*post hoc* pooling following Doncaster and Davey, 2007).

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Table 5.4. ANOVA model 3, degrees of freedom (d.f.), components of variation and MS for *F* ratios. "D" denotes within-shore sampling.

Mean square	d.f.	Components of variation estimated in population	F-ratio
Aspect= A	1	D´(S´(A) + S´(A) + A	A/ S´(A)
Shore = S´(A)	8	D´(S´(A)) + S´(A)	S´(A)/ D´(S´(A))
D´(S´(A))	20	D´(S´(A))	-

5.3.3.3.   Comparisons of CPUE between and within islands

A two-factor nested design (equivalent to model 2.1 (i) in Doncaster and Davey, 2007) was used to test the hypothesis that the CPUE for *P. aspera* would be different from five shores within three islands (Tenerife, Fuerteventura and La Palma) and between the islands (model 4, Table 5.5). The same model was used to test the hypothesis that the CPUE for *P. c. crenata* would be different from four shores within three islands (Tenerife, Fuerteventura and La Palma) and between the islands (model 4, Table 5.5).

This design had five levels (four levels for *P. c. crenata*) of the random factor Shore ($S´_5$), nested in each of three levels of a fixed factor Island (I_3), with three samples (D_3) in each shore. This design was analysed with the ANOVA model:

$Y=D_3´(S_5´(I_3))$ model 4

The model was tested with the Minitab statistics package by requesting analysis of terms: = I+ S´(I). It produced an output with the structure described by Table 5.5 where planned *post hoc* pooling is permissible for Island if S´(I) has *p* > 0.25 (*post hoc* pooling following Doncaster and Davey, 2007).

Table 5.5. ANOVA model 4, degrees of freedom (d.f.), components of variation and MS for F ratios. "D" denotes within-shore sampling.

Mean square	d.f.	Components of variation estimated in population	F-ratio
Island= I	2	D´(S´(I) + S´(I) + I	I/ S´(I)
Shore = S´(I)	12	D´(S´(I)) + S´(I)	S´(I)/ D´(S´(I))
D´(S´(I))	30	D´(S´(I))	-

5.4. RESULTS

5.4.1. Influence of exploitation on CPUE

The shores of all islands were ranked in ascendant order of exploitation according to their exploitation index and plotted against the respective CPUE for 1992 and 1999 (Figure 5.2 and 5.3).
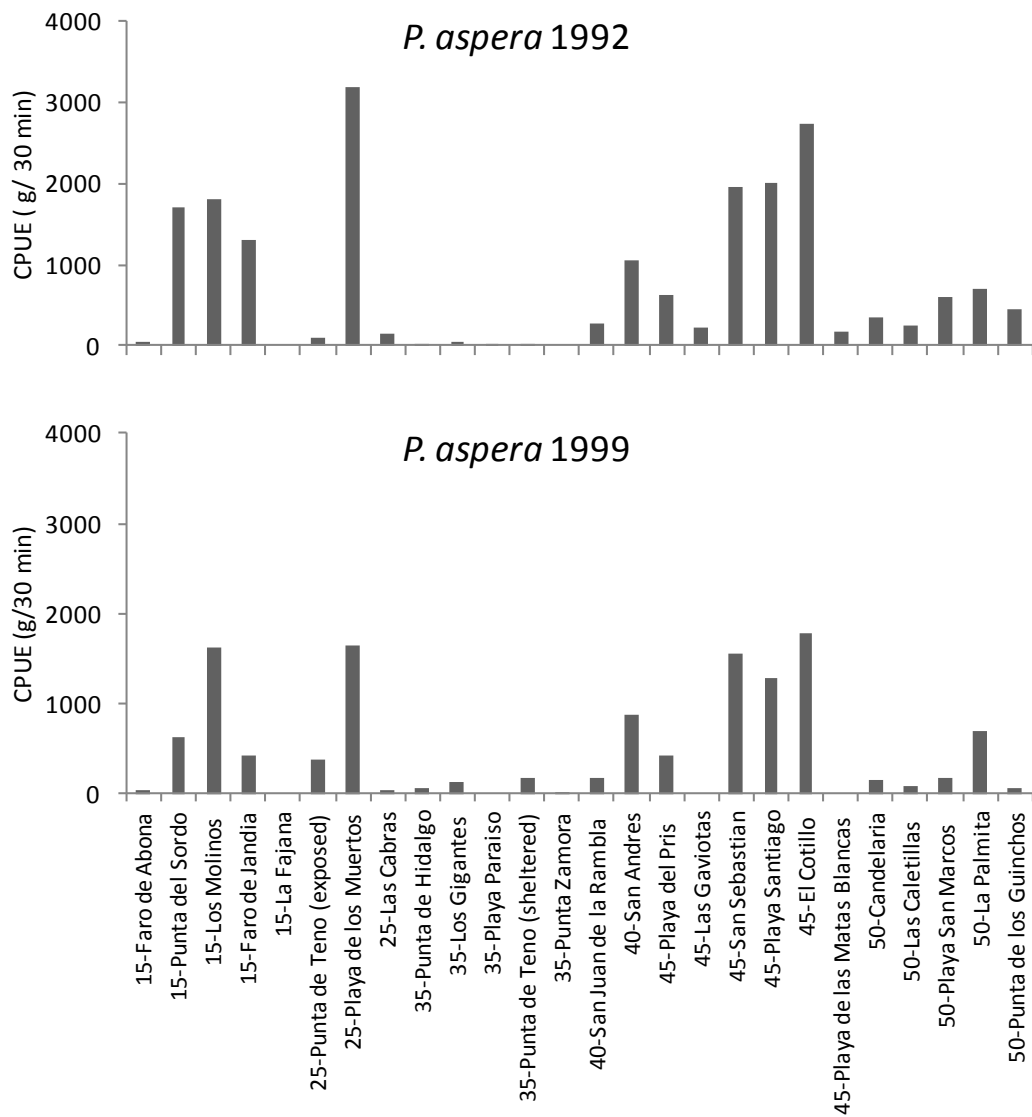
Figure 5.2. Wet weight CPUE (g/30 min dive) for *P. aspera* in 1992 and 1999 on shores from lowest (leftmost) to highest (rightmost) exploitation index.
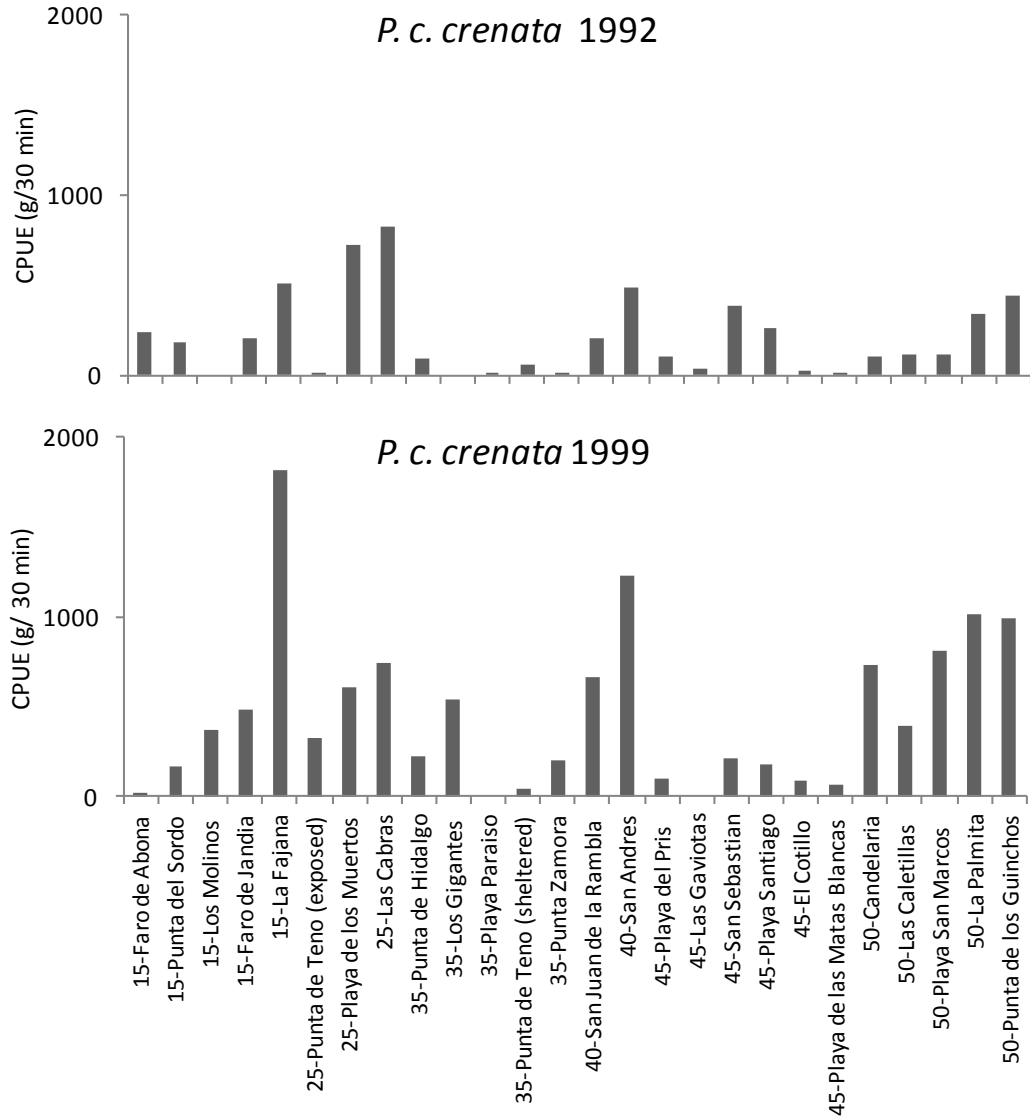
Figure 5.3. Wet weight CPUE (g/30 min dive) for *P. c. crenata* in 1992 and 1999 on shores from lowest (leftmost) to highest (rightmost) exploitation index.

The analysis of variance for Figure 5.4 shows differences by year only for *P. aspera* CPUE ($F_{1, 10} = 10.27$, $p = 0.009$; Table 5.6) with no differences detected between the least and most exploited shores. The catches of *P. aspera* decreased from 1992 to 1999. No differences were detected for *P. c. crenata* CPUE by year or exploitation index ($p > 0.05$, Figure 5.4; Table 5.7).

During the course of the survey more limpets were found on north-facing shores than on south-facing shores. This led to the hypothesis that there were differences in limpet abundance between north-facing and south-facing shores from 1992 and 1999. To test this hypothesis, the CPUE of both species for nine randomly chosen north-

facing shores and nine south-facing shores of 1992 and 1999 were compared in an ANOVA.

The total CPUE (g/30 min dive: from a single dive in 1992 and three ten-minute dives in 1999) by island and shores (and correspondent exploitation index) for both species and years is presented in Table A3.1 located in appendix 3.
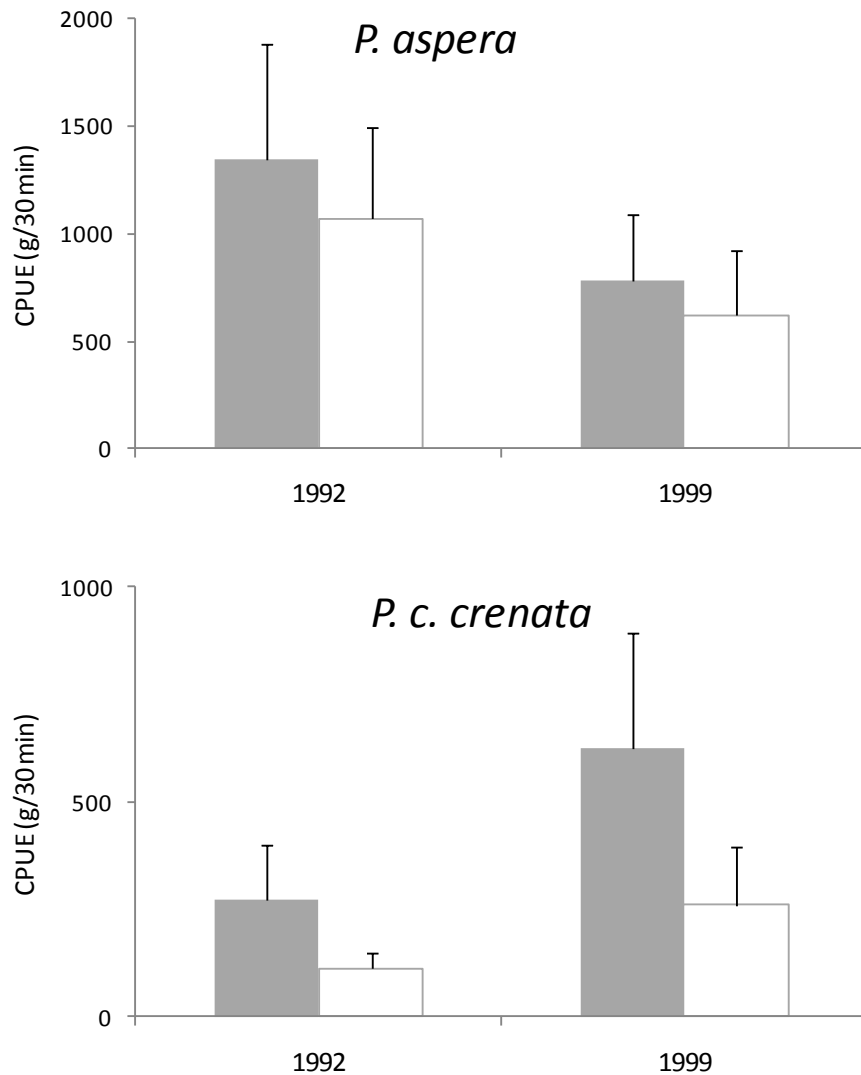
Figure 5.4. Mean wet weight CPUE (g/30 min dive) with SE (*n* = 6 shores) for *P. aspera* and *P. c. crenata* of the least exploited (grey bar) and the most exploited (clear bar) shores of the Canary Islands in 1992 and 1999.

Table 5.6. *P. aspera* ANOVA on CPUE (g/30 min dive) by year and exploitation of shores (model 1). **Terms with *p* < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Exploitation = E	1	287593	287593	0.18	0.682
Shores = S´(E)	10	16167634	1616763	-	
Within subjects					
Year = Y	1	1550747	1550747	10.27	**0.009**
Y*E	1	19311	19311	0.13	0.728
Error	10	1509784	150978		

Table 5.7. *P. c. crenata* ANOVA on CPUE (g/30 min dive) by year and exploitation of shores (model 1).

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Exploitation = E	1	408569	408569	2.20	0.169
Shores = S´(E)	10	1860851	186085	-	
Within subjects					
Year = Y	1	376376	376376	4.48	0.060
Y*E	1	62771	62771	0.75	0.408
Error	10	840849	84085		

The arcsine proportion of females of *P. aspera* decreased with increasing log of CPUE (g/30 min) both in 1992 and 1999 (Figure 5.5: $r^2 = 0.34$, $F_{1, 14} = 7.15$, $p = 0.018$; $r^2 = 0.50$, $F_{1, 18} = 17.75$, $p = 0.001$, respectively).

The arcsine proportion of females of *P. c. crenata* decreased with increasing Log of CPUE in 1992 but did not respond to increments in log of CPUE in 1999 (Figure 5.6: $r^2 = 0.82$, $F_{1,11} = 49.85$ $p < 0.001$; $r^2 = 0.07$, $F_{1, 20} = 1.47$, $p = 0.240$, respectively).

P. aspera 1992
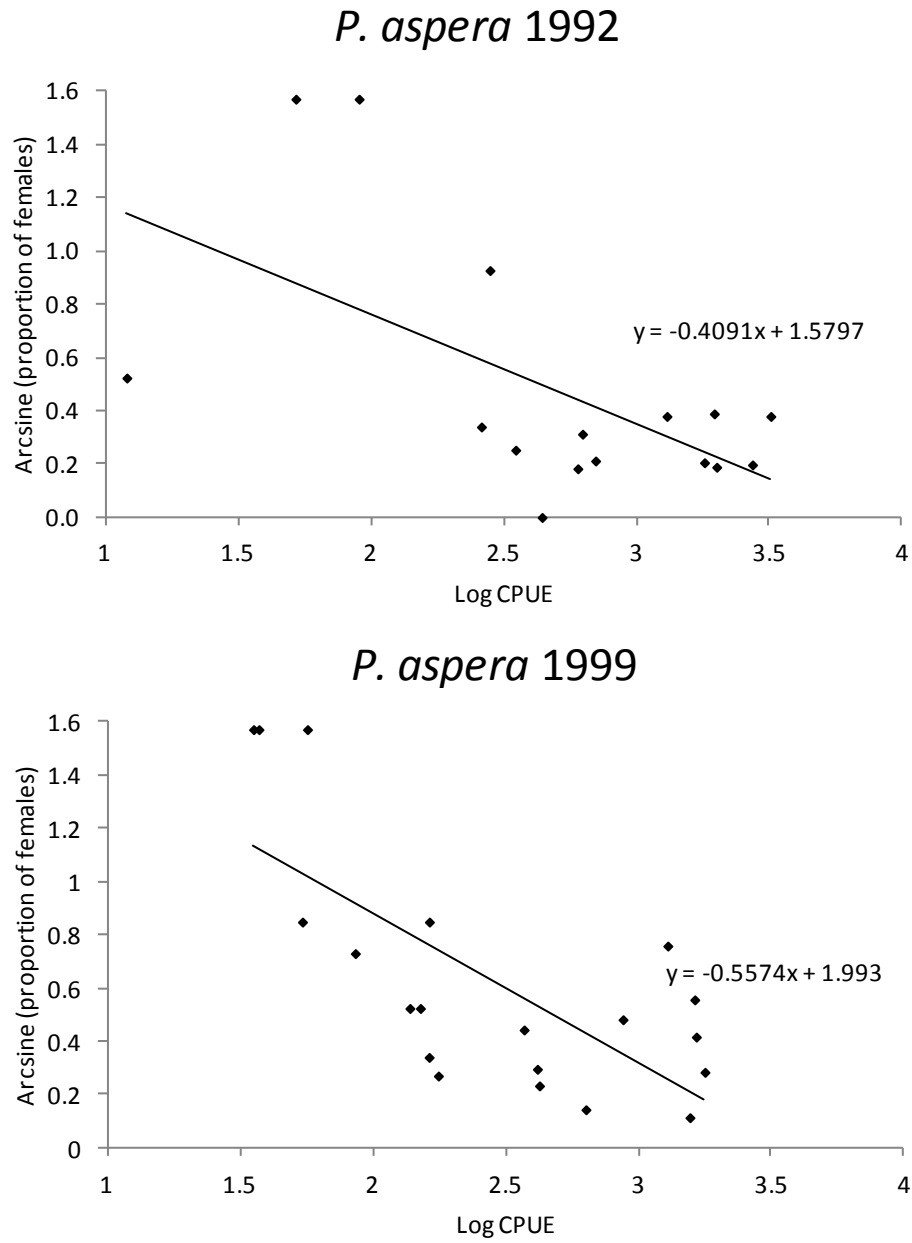
P. aspera 1999

Figure 5.5. *P. aspera* arcsine proportion of females by log of CPUE (g/30 min) for the pooled data of the Canary Islands in 1992 and 1999.

P. c. crenata 1992

Arcsine (proportion of females) — y-axis

Log CPUE — x-axis

y = -0.718x + 2.2866

P. c. crenata 1999

Arcsine (proportion of females) — y-axis

Log CPUE — x-axis
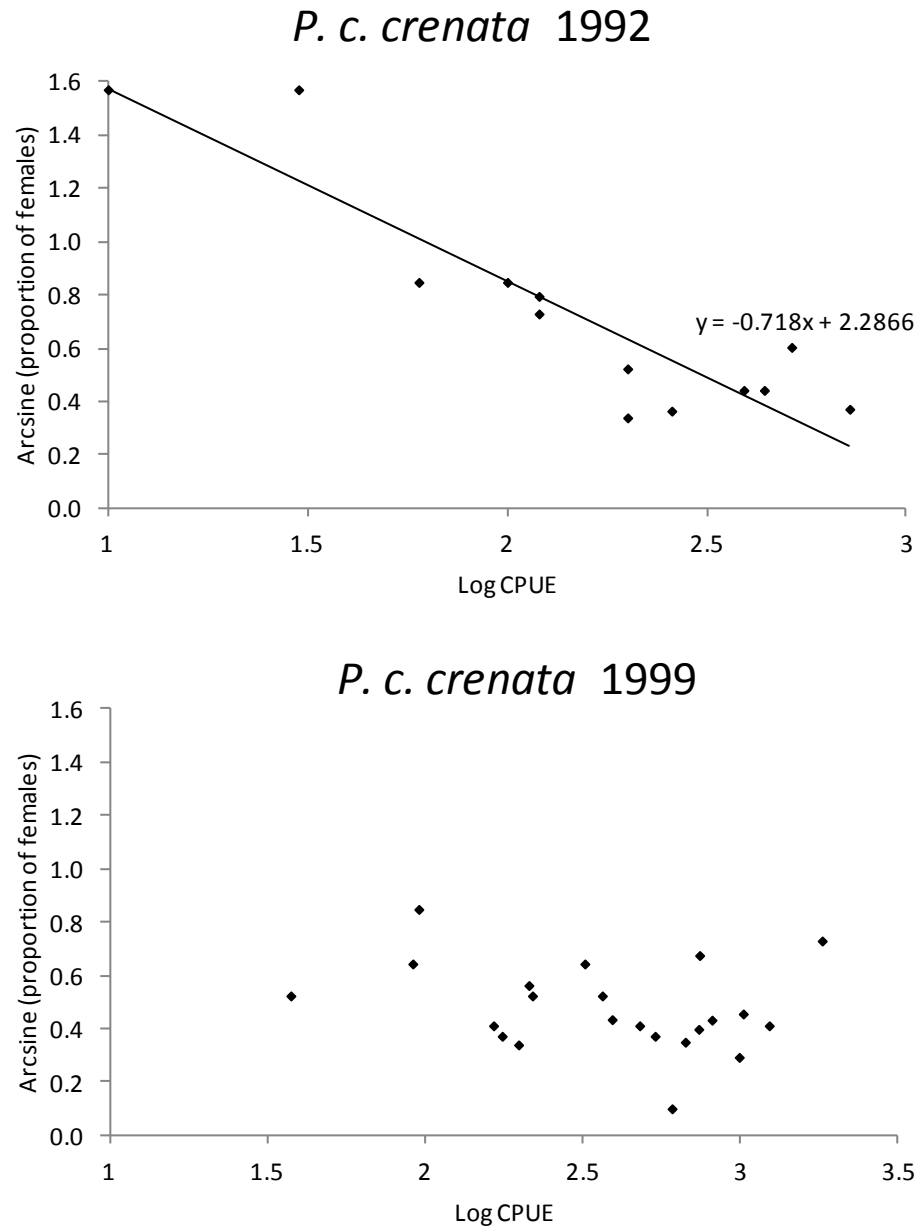
Figure 5.6. *P. c. crenata* arcsine proportion of females by log of CPUE (g/30 min) for the pooled data of the Canary Islands in 1992 and 1999.

5.4.2. Influence of coastal aspect on CPUE

Figure 5.7 shows differences by year only for *P. aspera* CPUE ($F_{1,\,16}$ = 12.75, p = 0.003; Table 5.8) with catches of *P. aspera* decreasing from 1992 to 1999. There were no significant differences between aspect and no interaction with time. In both

years catches were higher on the north-facing shores but these were not significant reflecting considerable among site variation.

P. c. crenata displayed a CPUE with a significant year*aspect interaction (Figure 5.7, $F_{1,\,16} = 4.72$, $p = 0.045$; Table 5.9). Figure 5.7 suggests that differences by aspect were more evident in 1999 than in 1992, with the north-facing shores with higher catches than the south-facing shores.
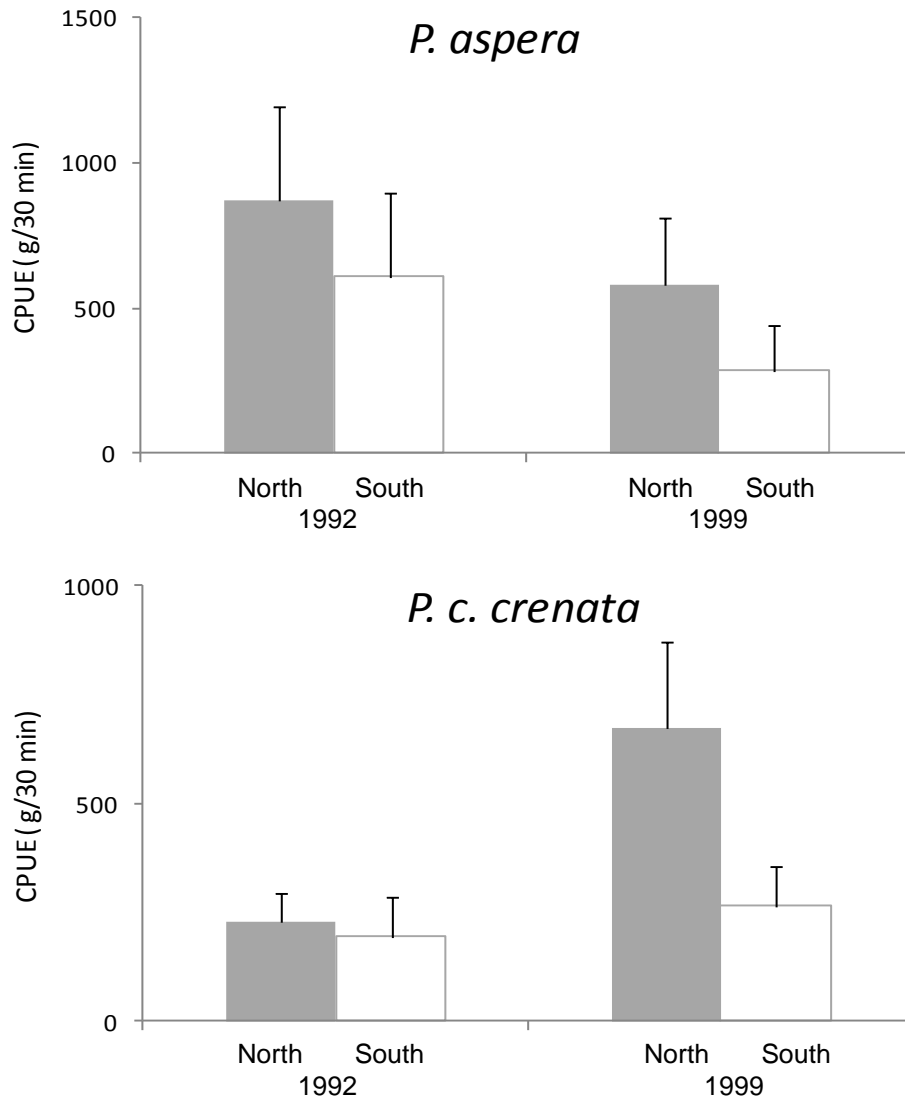
Figure 5.7. Mean wet weight CPUE (g/30 min dive) with SE ($n = 9$ shores) for *P. aspera* and *P. c. crenata* from north-facing shores (grey bar) and south-facing shores (clear bar) of the Canary Islands in 1992 and 1999.

Table 5.8. *P. aspera* ANOVA on CPUE (g/30 min dive) by year and aspect of shores (model 2). **Terms with *p* < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Aspect = A	1	705580	705580	0.71	0.412
Shores = S´(A)	16	15943115	996445	-	
Within subjects					
Year = Y	1	862252	862252	12.75	**0.003**
Y*A	1	1599	1599	0.02	0.880
Error	16	1082326	67645		

Table 5.9. *P. c. crenata* ANOVA on CPUE (g/30 min dive) by year and aspect of shore (model 2). **Terms with *p* < 0.05 are in bold.**

Source	d.f.	Seq SS	Seq MS	*F*	*p*
Between subjects					
Aspect = A	1	443907	443907	2.46	0.136
Shores = S´(A)	16	2883599	180225	-	
Within subjects					
Year = Y	1	588611	588611	8.97	**0.009**
Y*A	1	309800	309800	4.72	**0.045**
Error	16	1049520	65595		

5.4.2.1. Tenerife

For the analysis of Tenerife in 1999, no differences were detected in CPUE (g/10 min) between aspect of the shores (Table 5.10 and 5.11). Although Figure 5.8 suggests differences in catches for *P. c. crenata* according to aspect, for both species differences were detected only between shores within each level of aspect (*P.*

aspera: $F_{8, 20} = 5.13$, $p = 0.001$, Table 5.10; *P. c. crenata*: $F_{8, 20} = 11.00$, $p < 0.001$, Table 5.11). There were the higher catches of *P. aspera* at Punta del Sordo (CPUE: 210 ± 17.7 g/10 min) and the lowest catches at Playa Paraiso (CPUE: 0.0 ± 0.0 g/10 min), both south-facing shores (Figure 5.9). *P. c. crenata* had higher catches at the north-facing shore Playa de San Marcos (CPUE: 270.0 ± 35.1 g/10 min) and the lowest at the south-facing shore Playa Paraiso (CPUE: 0.0 ± 0.0 g/10 min) (Figure 5.9).
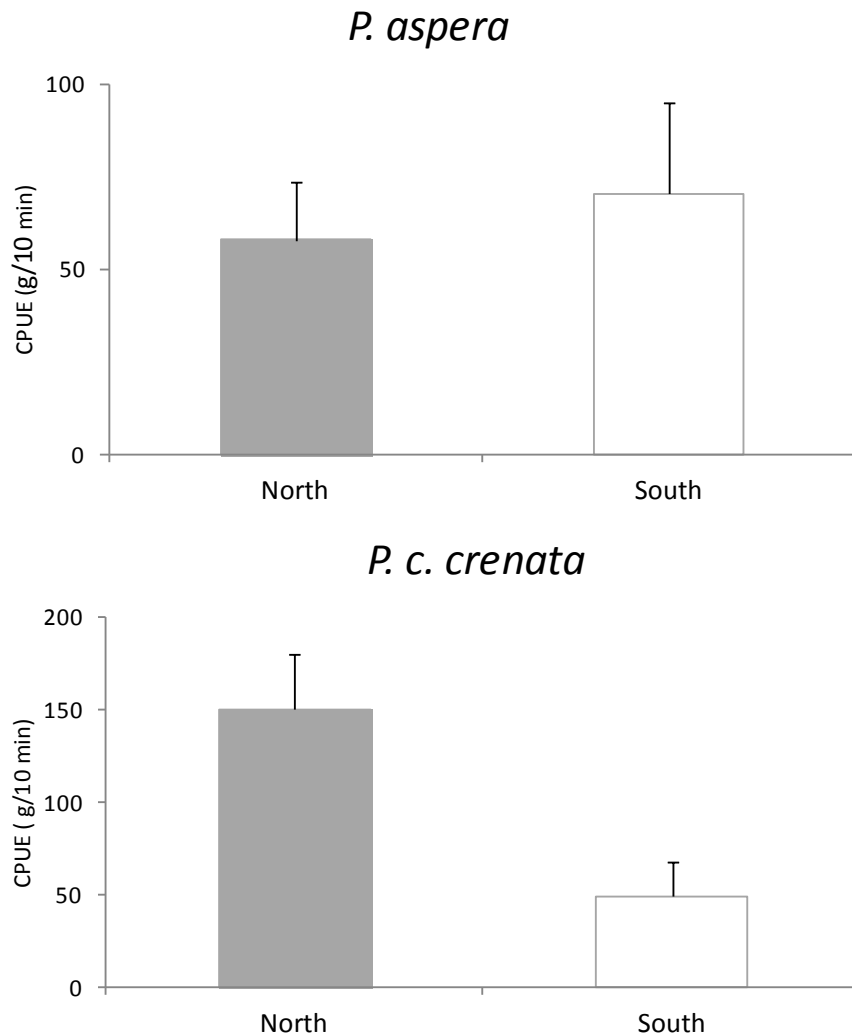
Figure 5.8. Mean wet weight CPUE (g/10 min dive) with SE (*n* = 5 shores) for *P. aspera* and *P. c. crenata* from north-facing and south-facing shores at Tenerife in 1999.

Table 5.10. *P. aspera* ANOVA on Tenerife in 1999 CPUE (g/10 min dive) by aspect and shore (model 3). **Terms with *p* < 0.05 are in bold.**

Source	d.f.	MS	*F*	*p*
Aspect = A	1	1170	0.08	0.781
Shore = S´(A)	8	14212	5.13	**0.001**
Error	20	2773		

Table 5.11. *P. c. crenata* ANOVA on Tenerife in 1999 CPUE (g/10 min dive) by aspect and shore (model 3). **Terms with *p* < 0.05 are in bold.**

Source	d.f.	MS	*F*	*p*
Aspect = A	1	77061	3.08	0.117
Shore = S´(A)	8	25043	11.00	**<0.001**
Error	20	2276		
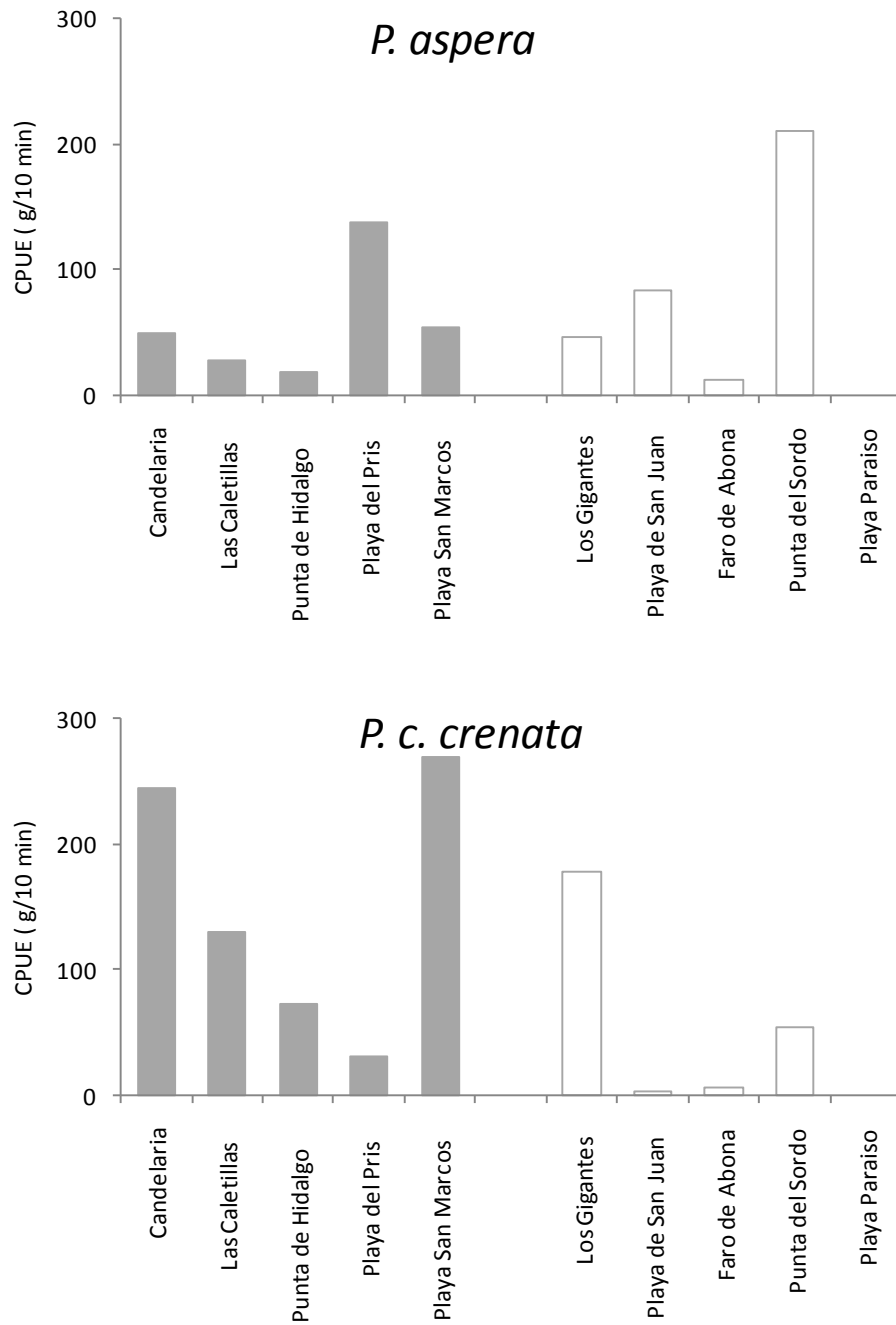
Figure 5.9. Mean wet weight CPUE (g/10 min dive; $n = 3$ samples) for *P. aspera* and *P. c. crenata* by shores (north-facing shores: grey bars and south-facing shores: clear bars) at Tenerife in 1999.

5.4.3. Comparisons of CPUE between and within islands

The CPUE (g/10 min) for both species differed at shore and island level (Figure 5.10, *P. aspera*: respectively $F_{12, 30} = 6.43$, $p < 0.001$ and $F_{2, 12} = 4.95$, $p = 0.027$, Table

5.12; *P. c. crenata*: $F_{9, 24} = 3.72$, $p = 0.005$ and $F_{2, 9} = 11.69$, $p = 0.003$, Table 5.13). Therefore in 1999 CPUE were different among Tenerife, Fuerteventura and La Palma and within the shores of those islands. *P. aspera* catches (Figures 5.10 and 5.11) were higher on Furteventura Island (CPUE: 364.9 ± 80.8 g/10 min) which had the shore with higher CPUE from all the islands at El Cotillo (592.0 ± 64.6 g/10 min), but also the shore with the lowest CPUE from all the islands at Playa de las Matas Brancas (0.0 ± 0.0 g/10 min). *P. c. crenata* catches (Figures 5.10 and 5.12) were higher at La Palma (CPUE: 420.6 ± 37.2 g/10 min), that had the shore with the higher CPUE from all the islands at La Fajana (603.7 ± 29.1 g/10 min). The shore with the lowest CPUE was El Cotillo (30.4 ± 13.0 g/10 min) at Fuerteventura. The encountered discrepancies suggest that either those island and shores were under different exploitation regimes or significant ecological conditions were limiting limpet abundances.
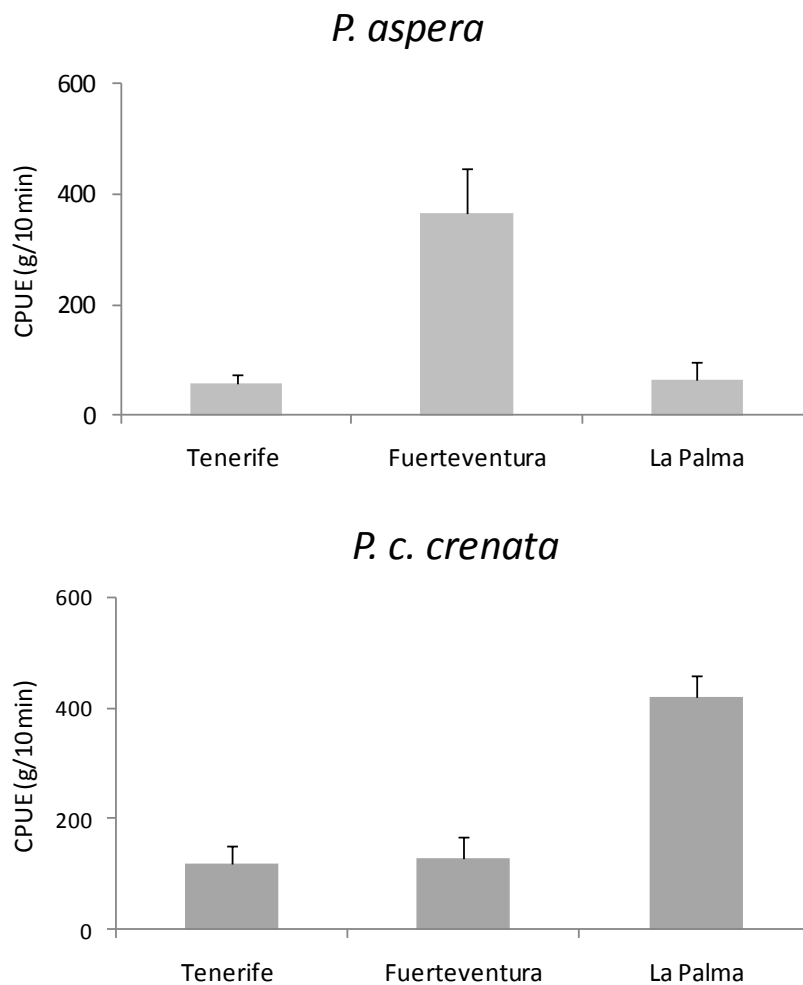
Figure 5.10. Mean wet weight CPUE (g/10 min dive; *n* = 3 samples) with SE for *P. aspera* and *P. c. crenata* at Tenerife, Fuerteventura and La Palma in 1999.

Table 5.12. *P. aspera* ANOVA on 1999 CPUE (g/10 min dive) by island and shore (model 4). **Terms with *p* < 0.05 are in bold.**

Source	d.f.	MS	*F*	*p*
Island = I	2	462366	4.95	**0.027**
Shore = S´(I)	12	93497	6.43	**<0.001**
Error	30	14533		

Table 5.13. *P. c. crenata* ANOVA on 1999 CPUE (g/10 min dive) by island and shore (model 4). **Terms with *p* < 0.05 are in bold.**

Source	d.f.	MS	*F*	*p*
Island = I	2	351903	11.69	**0.003**
Shore = S´(I)	9	30106	3.72	**0.005**
Error	24	8097		
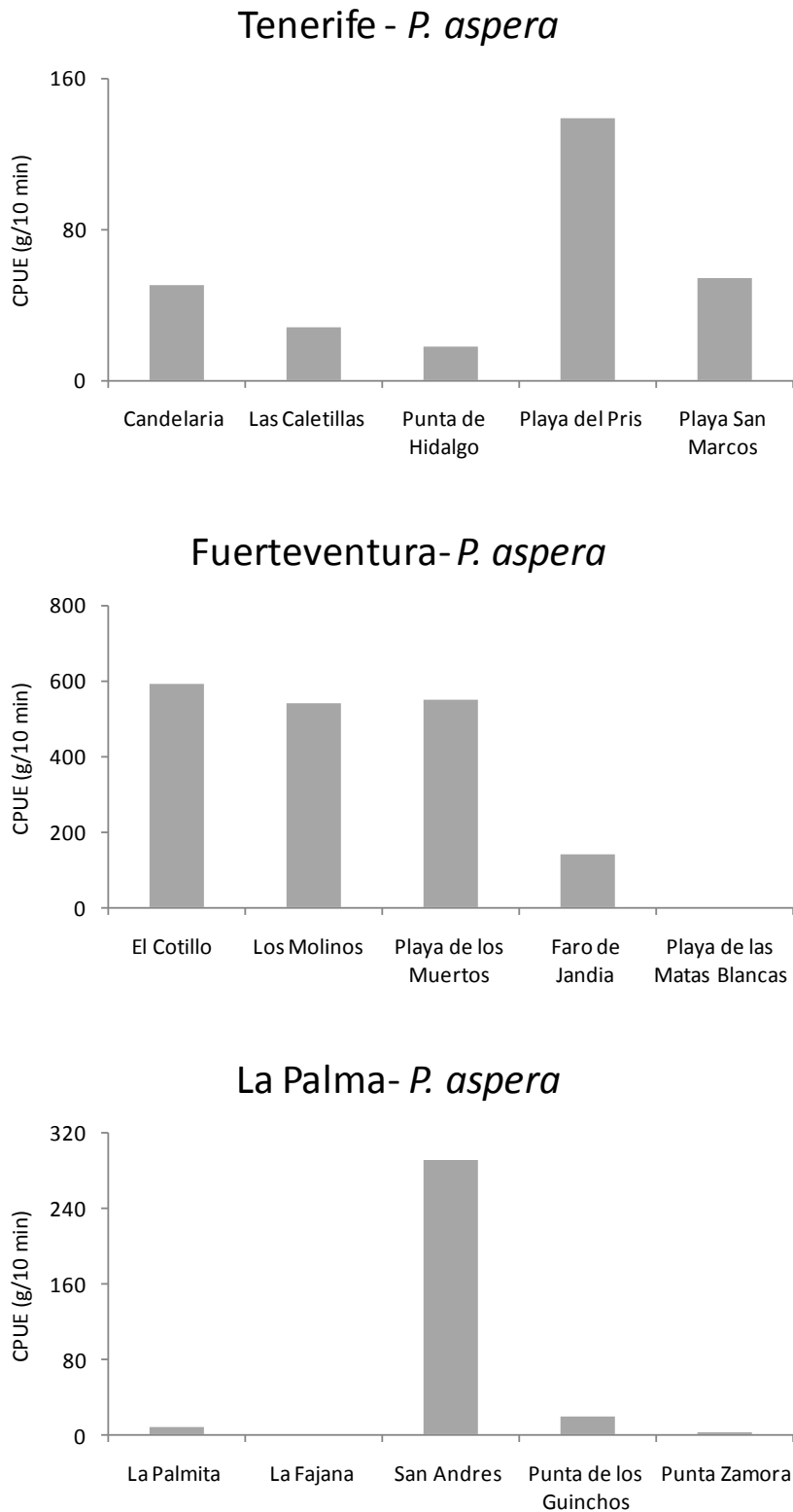
Figure 5.11. Mean wet weight CPUE (g/10 min dive; *n* = 3 samples) for *P. aspera* by shores at Tenerife, Fuerteventura and La Palma in 1999.
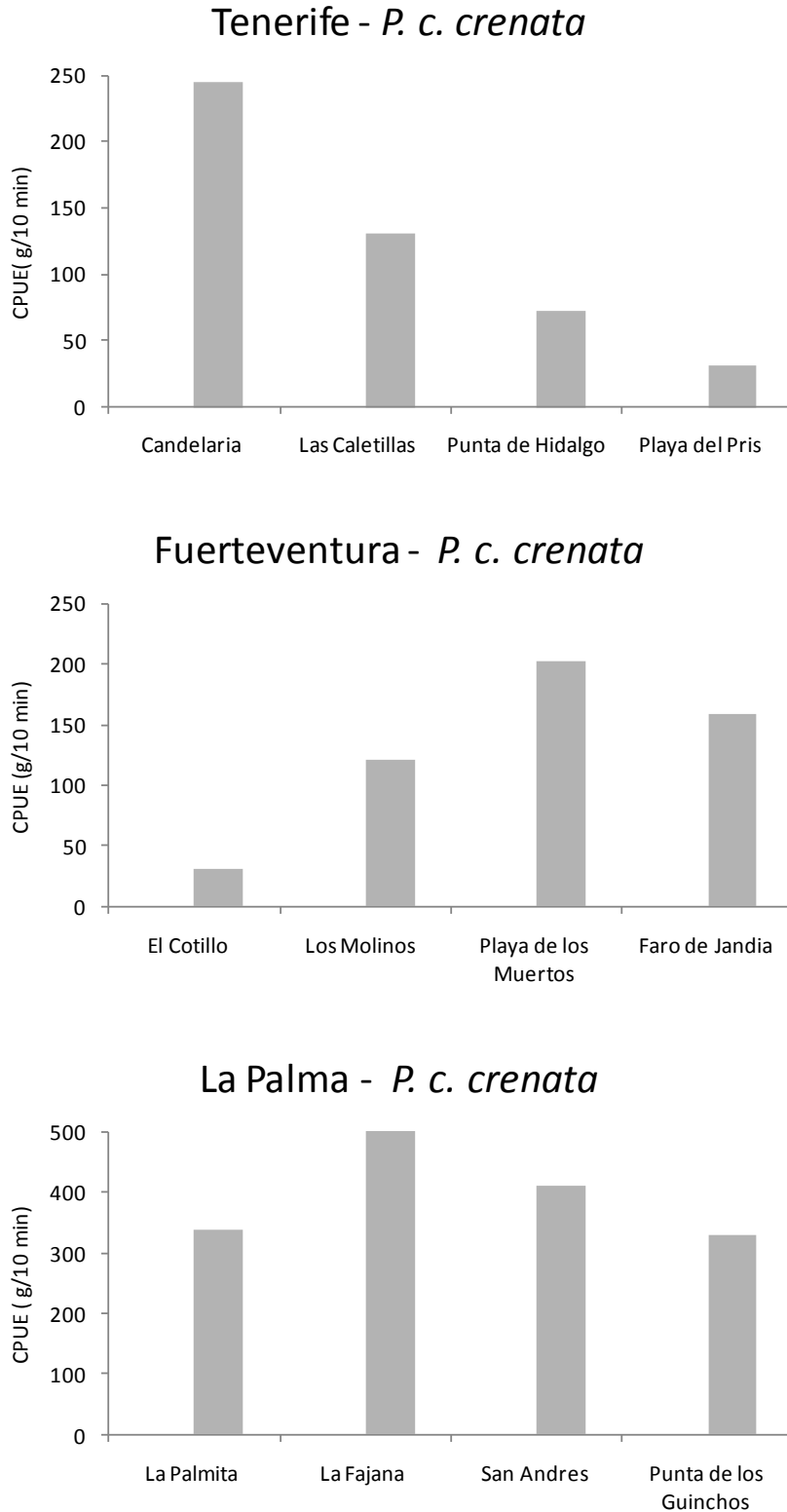
Figure 5.12. Mean wet weight CPUE (g/10 min dive; *n* = 3 samples) for *P. c. crenata* by shores at Tenerife, Fuerteventura and La Palma in 1999.

5.5.   DISCUSSION

In addition to comparing the limpet catches of *P. aspera* and *P. c. crenata* I sought to characterise the human harvesting pressure across the Canary Islands. The absence of an exploitation effect across years suggests that either the degree of exploitation is inaccurately represented by index and/or other factors such as ecological and geological constraints, rather than level of exploitation, influence the availability of limpets on the shore. The exploitation index, however, would have benefited from inclusion of more pertinent variables, such as the human population of the nearest cities or the existence of physical barriers limiting access to the shore. Its components were conditioned on the observed conditions in 1999, inevitably leading to some subjectivity. Therefore it is not surprising that no significant differences were detected for both species in the CPUE of the least and most exploited shores. Ramirez et al. (2009) used a more complex approach where they classified three islands from the Canary Islands (Lanzarote, La Graciosa and Alegranza) with corresponding three levels of human influence: high, medium and low, respectively; which were quantified through three indicators: human pressure (inhabitants (locals and tourists) per km of coast perimeter per day), accessibility to the coast (km of sealed and unsealed roads), and declared volumes of capture (kg). In the Azores, in order to examine if differences among islands could be attributed to the islands' regime of exploitation, the total number of *Patella candei* and the number of larger individuals (>20 mm) at each island were regressed against catch rates and also against the number of islanders per coastal perimeter (Martins et al. 2008). The number of islanders per coastal perimeter was a better predictor of the abundance of *P. candei* across the archipelago than the level of limpet catches that did not include the illegal harvesting (Martins et al. 2008). The use of such indexes and consequent correlative approach provided an indirect estimate of potential exploitation in each island, that may be the only possible way to ascertain the effects of exploitation in areas where suitable control areas (e.g. no-take marine reserves) are lacking (Martins et al. 2008).

The detected differences in CPUE by year should be interpreted with caution since the limpet collection was performed by different snorkel divers with different catching abilities and under different weather and wave conditions. Another

constraint that we faced was the lack of GPS coordinates for the shores in both years (GPS was not available in 1992) that would have made the localisation of the shores more precise. Nevertheless for *P. aspera* the CPUE decreased from 1992 to 1999, while for *P. c. crenata* no differences were detected, suggesting that the former species was under a higher harvest pressure. Since the catches of *P. aspera* decreased from 1992 to 1999 and given the possibility of occurrence of protandry in this species, the lowest densities could have promoted the sex change to occur at high rates resulting in higher proportion of females at lower CPUE. On the other hand, for *P. c. crenata* an intensification of predation on females from 1992 to 1999 could have masked the influence of density on the proportion of females. Even for non sex-changing species lower densities might provide more resources for females to use resulting in higher numbers than in crowded conditions.

The human exploitation of shellfish in the Canary archipelago (limpets included) has generally reduced the species abundance and changed class-size structure due to selection of the biggest specimens (Navarro et al. 2005; Ramírez et al. 2005; Tuya et al. 2006b; Ramirez et al. 2009). This had been demonstrated by other studies elsewhere (e.g. Hockey and Bosman 1986; Keough et al. 1993; Lindberg et al. 1998; Ferraz et al. 2001; Branch and Odendaal 2003; Roy et al. 2003; Sagarin et al. 2007; Martins et al. 2008).The removal of the biggest individuals presents a major risk for *P. aspera* populations if they are protandric, as is thought (Thompson 1979), and the removal of the females occurring in the bigger size classes would compromise its fecundity and stock maintenance (Peña 1996; Hawkins et al. 2000; Delany et al. 2002; Branch and Odendaal 2003). There are some indications of compensation with more females in heavily exploited populations. *P. aspera* has a broad range throughout Macaronesia occurring also in the Azores and Madeira (Hawkins et al. 2000; Weber and Hawkins 2002, 2005). Due to overexploitation *P. aspera* is practically commercially extinct in the Azores and was included in 2003 on the OSPAR list of threatened and/or declining species and habitats (OSPAR agreement 2008-6) since the removal of target larger sizes, which are mainly females has led to biased sex ratio (OSPAR commission 2010).

Endemic to the Canary Islands, *P. c. crenata* populations are probably vulnerable since there are no populations outside the archipelago to act as reservoirs for recruitment during recovery (Hawkins et al. 2000). Fortunately, this species seems to grow faster than *P. aspera* and reach maturity earlier (Hawkins et al. 2000).

Nevertheless as the biggest specimens of the main target species in the Canary Islands (*P. aspera* and *P. c. crenata*) become scarce harvesting pressure will increase on smaller individuals and upon other species such as *P. piperata* (see Moro and Herrera 2000). During the survey I confirmed that intertidal harvesters collected small limpets that would be cooked with rice as in Madeira Island (Meneses et al. (unpublished)).

I expected that for *P. c. crenata* particularly, the differences in the catches due to the aspect of the shore would also be explained by the preference of this species for exposed shores (Hawkins et al. (unpublished)), which prevail in the north mainly due to the effect of the Canary Current and northern winds. This influence of shore aspect on *Patella* spp. abundance was not confirmed by the ANOVA except for *P. c. crenata* in 1999, resulting in a CPUE with a significant year*aspect interaction (Figure 5.7, Table 5.9). Differences in catches by aspect were more evident in 1999 where the north-facing shores had higher catches than the south-facing shores, suggesting that in that year, different biological and ecological conditions were influencing *P. c. crenata* population dynamics from the north and south of the islands. These could be winds and current regimes as well as exploitation status, food supply and growth rates but demand further investigation.

At Tenerife in 1999 the differences in CPUE for both species were only detected at shore level, suggesting that other characteristics within shores determine limpet abundance rather than aspect. Spatial distribution patterns on rocky shores of temperate seas have multiple sources of horizontal and vertical variability (Foster 1990; Archambault and Bourget 1996; Underwood and Chapman 1996; Menconi et al. 1999; Benedetti-Cecchi 2001; Boaventura et al. 2002b,c; Navarro et al. 2005). Causes of horizontal variability could be recruitment variation, behaviour changes, differences in the substratum topography and interaction between biological and physical processes (Navarro et al. 2005). Adding to this, bottom-up processes in coastal populations may be difficult to demonstrate under intense human exploitation (Tuya et al. 2006). In comparisons between islands I found that the CPUE for both species differed at shore and island level. The exploitation index of the shores with higher and lower catches did not reflect those differences, further corroborating its inefficiency. At present, no data are available that would allow to ascertain the causes of this within-shore variability, and since these were considered random factors, comparisons will not be discussed further. These results are in accordance

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with other studies in the intertidal zone of the Canary archipelago that detected small-scale, intra-island variation on the abundances of *P. aspera* and *P. c. crenata* (see Navarro et al. 2005; Ramirez et al. 2009).

Despite the differences at shore level, Fuerteventura had the highest catches for *P. aspera* and La Palma had the highest catches for *P. c. crenata.* These islands are less populated than Tenerife where the pressure on the limpet stocks is greater. In 1999 Tenerife had 340 inhabitants/km^2 while Fuerteventura and La Palma had 32 inhabitants/km^2 and 116 inhabitants/km^2 respectively (Instituto Canario de Estadística 2002). Adding to these factors we should consider the pressure of tourists that increase the demand for shellfish. However differences in limpet CPUE among islands cannot be ascribed to exploitation alone, since these islands may differ in relation to factors other than fishing intensity such as competition, productivity and mortality (see Martins et al. 2008). Important differences exist in the geomorphology of the different islands, and differences are found in oceanographic conditions between the eastern and western islands (Davenport et al. 2002) that may produce changes in the abundance and distribution (Menge et al. 1997; Menge 2000; Benedetti-Cecchi 2001; Nielsen and Navarrete 2004). Nevertheless the observed differences in CPUE between islands for both species is not consistent with other more recent studies of *Patella* spp. abundance in the intertidal zone of the Canary Islands: Navarro et al. (2005) did not detect differences between islands for both species, whereas Ramirez et al. (2009) detected differences between islands for *P. aspera* but not for *P. c. crenata.* Navarro et al. (2005) stated that the absence of detected differences in *Patella* spp. abundances between islands could be the result of overexploitation of this resource across the islands that may not have been evident by the time of the survey.

Adding to human impacts, the isolated nature of island populations, including likely low recruitment due to larval loss, makes many species vulnerable to overexploitation, although in other cases isolated islands with little human impact can become important refuges (Hawkins et al. 2000). Furthermore species that are external fertilizers always face Allee effects when population densities are low, as this reduces the opportunities for sperm and eggs to meet (Jamieson 1993; Levitan and Sewell 1998; Hawkins et al. 2000). Thus, in these highly dispersive insular habitats, a reduction in reproductive output as a consequence of the removal of mature animals is likely to render island populations more susceptible to exploitation

than would be expected elsewhere (Roberts and Hawkins 1999; Martins et al. 2008). This vulnerability of insular limpet populations would be aggravated by diseases or parasitic infestations. I observed some *P. aspera* specimens with the boring bivalve *L. aristatus*, probably originated from the coast of western Africa. It is uncertain if such infestations compromise limpet biological traits (such as growth or reproduction patterns). Further investigations should be prompted to investigate the current occurrence and consequences of this boring bivalve on *Patella* spp. populations from the Canary Islands.

In the Azores since 1993 a number of management measures have been introduced for the limpet fishery since its dramatic collapse in the late 1980's. These comprise, closed seasons, closed areas (limpets MPAs), size limits, licensing of fishermen and endeavours to get better management information (OSPAR Commission 2010). There are currently 34 marine reserves designated strictly to enhance the conservation of the limpets in the archipelago with 10-20% of the shore is closed to exploitation and each island has at least three closed areas (OSPAR Commission 2010). Existing management measures nevertheless are not effective in protecting the limpet population and, consequently, the coastal habitats since one of the main problems is illegal exploitation, with limpets being caught all year round around the islands (OSPAR Commission 2010).

The present legislation applied to limpet conservation in the Canary Islands (BOC 2003; 2008; 2011a, b) establishes several protective measures summarised in Table 5.14. These include measures to allow reproduction of individuals (closed harvesting periods and minimum capture size) and maintenance/recovery of stocks (no-taking limpet zones; maximum limpet intake per day and closure of harvesting in Fuerteventura). Some of these legislative measures were defined in 2011 and the assessment of their effectiveness should be a priority since they will be of no use for conservation if not correctly applied. Previous studies have already questioned the adequacy of surveillance on the MPA from the Canary Islands created in 1995 where limpet stocks had low abundances and reduction of size, as in other non-protected islands (Ramirez et al. 2009). One way to estimate the direct consequences on target species and indirect consequences upon the community of the collection of these natural resources is by comparing protected areas to exploited sites (review by Glasby 1997). MPA and no-take zones can certainly play a major role in improving that understanding but its designations are useless if the applicable legislation is not

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respected (Allison et al. 1998; Boersma and Parrish 1999; Murray et al. 1999; Sagarin et al. 2007; Ramirez et al. 2009).

Table 5.14. Protective measures applied to limpet conservation in the Canary Islands.

Protective measures	
Limpet collection permitted from the 1st December to the 30th April	*P. c. candei,* prohibition of collection since 2003 in the entire archipelago
Prohibition of limpet collection by foot on the subtidal.	*P. aspera* and *P. c. crenata,* prohibition of collection in Fuerteventura since 2004 (permitted temporarily in 2010)
P. aspera, P. c. crenata and *P. piperata* minimum capture size: 45 cm	5-year no-taking limpet zones established during 2011 in the islands of El Hierro, Fuerteventura, Gran Canaria, Lanzarote, La Palma and Tenerife (minimum of three zones per island)
Professional shellfish collectors, maximum limpet intake per day: 10kg	
Recreational collecting, maximum limpet intake per day: 3 kg	

Limpet stocks as most fishery stocks being common-pool resources face the "tragedy of the commons" dilemma that occurs in highly valued, open-access commons where those involved and/or external authorities do not establish an effective governance regime (Hardin 1968; Ostrom 2002). Designing a system in a top-down fashion and imposing it on the harvesters is not as successful as working with the users of a common-pool resource over time to develop a system that is well-matched to the ecological system as well as to the practices, norms, and long-term economic welfare of the participants (Ostrom 2008). Even protected areas, when governed through co-management, can contribute to the problem of overexploitation rather than representing the solution, if they disempower local people and undermine both traditional means of sustainably exploiting nature and the potential for local people to cooperate with conservation restrictions (Hayes and Ostrom 2005; Hayes 2006; Jones et al. 2011). Common-pool resources may be governed and managed by

a wide variety of institutional arrangements that can be roughly grouped as governmental, private, or community ownership (Ostrom 2008).

In the U.S.A. in the state of Maine local fishers and state officials have successfully evolved ways to manage lobster stocks and several rules enabled lobster fishers to monitor each other's harvesting with substantial effectiveness (Acheson 1998, 2003; Ostrom 2008). Indeed, if fishers had not taken substantial responsibility for monitoring each other's harvesting behaviour, the successful fishery would have been overharvested—and potentially may have collapsed like many others around the world (Wilson 2007; Ostrom 2008). Canada, New Zealand, and Iceland have developed individual transferable quota systems that have evolved into a co-management system in which the fishers participate in gathering data and making policies **w**ith substantial reductions on the level of harvesting in key coastal fisheries (Ostrom 2008). In Chile, Territorial User's Rights in Fisheries (TURFs) (Christy 1982; Panayotou 1984); were incorporated in the law in 1991 to enhance self-regulatory practices among artisanal fishermen (Bernal et al. 1999). Among them, the Areas for Management and Exploitation for Benthic Resources (AMEBRs) represented an associative assignment of TURFs (Bernal et al. 1999). AMEBR gave exclusive access to benthic resources in a portion of the coast, to a local organisation of artisanal fishermen (cooperatives, unions and other labour associations), through a payment of an annual fixed fee per hectare, for the purpose of accomplishing a stock enhancement programme and benefiting of the exclusive exploitation of the resources in the area under a technically approved management regime (Bernal et al. 1999). This system generated large expectations among organised fishermen because it was perceived as receiving in permanent ownership the equivalent of a "land grant" (Bernal et al. 1999). Biological data emphasize that AMEBRs have been successful in maintaining target species (Castilla and Gelcich 2008). The number, mean sizes of individuals and CPUE values of *C. concholepas* were significantly higher in an AMEBR (El Quisco) compared to nearby open-access areas (Castilla et al. 1998). Three species of keyhole limpet (*Fissurella* spp.) were larger and mean CPUE higher in El Quisco AMEBR than in open-access fishing grounds (Castilla and Fernández 1998).

In order to protect the biodiversity of the Canary Islands future studies should also encompass the effects of limpet collection upon the shore community, because the viability of other species, many with commercial value, could be compromised by

the removal of limpets. Limpet stocks in the Canary Islands are currently overexploited (Núñez et al. 2003; Navarro et al. 2005; Ramirez et al. 2009) and despite the legal protective measures illegal catches are a major risk to stocks recovery. They are not registered in official records and present an extra pressure on limpet populations not included in management policies. Therefore it is essential to guarantee the application of the current legal protective measures which should be reviewed on a regular basis.

CHAPTER 6

GENERAL DISCUSSION

6.1. INTRODUCTION

The overall theme of this thesis has been the influences of environment and harvesting by humans on limpet populations and assemblages of non-target species.

When investigating the consequences of harvesting for *P. vulgata* individuals and populations, the phenomenon of sex change has emerged as the most interesting and revealing parameter. Any obligate sex changer subject to harvesting must be at risk of suffering substantial biases in population sex ratios, which may influence population viability particularly if females are more heavily harvested than males. Non sex-changers also face the risk of sex-ratio bias from harvesting of bigger specimens, mainly males.

A broad-scale survey of sex ratios of *P. vulgata* and *P. depressa* in the British Isles and Portugal allowed investigation of the occurrence of protandry in the species at the centre of their range, and at range edges towards their northern (*P. depressa*) and southern limits (*P. vulgata*) (Chapter 2). A controlled field experiment was designed to simulate the exploitation of *P. vulgata*, in order to analyse its consequences for the population biology of the species (Chapter 3) and the rocky shore community in which it lives (Chapter 4). Low and high levels of limpet exploitation were simulated, with the biggest specimens as the main target of the harvest, in accordance with harvesting in most parts of the world. To complement the experimental investigations, a field survey was carried out of the population status of exploited limpets *P. aspera* and *P. c. crenata* in the Canary Islands (Chapter 5). In this discussion I present a synthesis of the results before considering the role of ecological experimentation and aquaculture in resource management. I conclude with suggestions for future research.

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6.2.   LIMITATIONS OF THE STUDY

Little empirical information exists in the literature on sex change in patellid limpets and there are some uncertainties. For example, the occurrence of sex change in *P. ulyssiponensis* from the mainland Europe (previously *P. aspera*), which was reported by Thompson (1979) as a possibility, has been referred to by later authors as a certainty (see Ramirez et al. 2009). Therefore further studies should investigate this issue and clarify if sex change does occur in *P. ulyssiponensis* and *P. aspera*, and whether it is obligate or facultative.

The general lack of data on sex change in limpets is due mainly to the difficulty of assessing the sex of an individual without compromising its survival; especially as limpets get damaged when removed and reattach poorly. In the experiment run for this thesis, the sex of individuals could only be confirmed by killing them and the size of sex change inferred from those results. I did not assess the age of the removed limpets during and at the end of the experiment, which could have been done by dating micro-growth bands of the shells (see Ekaratne and Crisp 1982, 1984). Therefore I could not be sure of the age of the removed limpets in order to compare with other studies (Orton et al. 1956; Ballantine 1961; Sundelöf et al. 2010). Although my experiment was preliminary in this respect, it was nevertheless informative about the possibility of plasticity in the sex change of *P. vulgata* as observed in other species (see Rivera-Ingraham et al. 2011). This is a potential compensatory response in exploited protrandic species. Although preliminary, the broad-scale survey in sex ratios also indicated that protandry in *P. vulgata* might be facultative and density dependent. Its major limitation was the impossibility of unequivocally observing the occurrence of sex change, because individuals were not followed from early maturation stages.

The investigation of the consequences of limpet harvesting upon the rocky shore community considered only species observed with a naked eye on the shore. It would have benefitted the study, and therefore the evaluation of the intermediate disturbance hypotheses (Caswell 1978; Connell 1978), if samples had been taken for subsequent sorting and identification microscopically. Not having done this meant that smaller species were excluded from the analysis, underestimating overall diversity.

The major difficulty in the survey of limpet stocks across the Canary Islands was to identify the exact location and sites for the sampling in order to match previous surveys. We had no GPS coordinates (not available in 1992) but only the names of the localities and their relative positions on a map. Additionally, as the snorkel dives were performed by different operatives from those on previous surveys, the comparisons are conditioned on their different abilities to catch the limpets under water.

6.3.   PRINCIPAL FINDINGS AND NEW OPPORTUNITIES FOR RESEARCH

The four sections of the thesis, concerning respectively broad-scale survey of sex ratios in the British Isles and Portugal, the simulated limpet species harvesting, the community-wide consequences of its exploitation, and the field survey of *Patella* spp. stocks in the Canary Islands, provide complementary information on the implications of limpet harvesting. The broad-scale survey in the south of England and Portugal showed the context dependency of skewed size by sex population structure indicative of protandry.

The experimentally simulated limpet exploitation provided insights into sex change constraints and dynamics in *P. vulgata* that will be useful for the study of this phenomenon in other exploited sex-changers. These experiments also demonstrated the major implication of limpet harvesting upon the rocky shore community as being the subsequent overgrowth by macro-algae. The survey of the limpet stocks in the Canary Island demonstrated continuity in past and present susceptibility of the stocks to human harvest pressure. Here I discuss the main findings from each study, preparatory to a discussion of the implications and wider perspectives of the work.

The broad-scale survey investigated the occurrence of protandry in *Patella* spp. in the British Isles and Portugal. It confirmed the likelihood of protandry in *P. vulgata* populations from the south of England: females predominated in larger size classes; cumulative frequency distributions of males and females were different; sex ratios were biased towards the first sex and smallest sizes of males were smaller than the smallest sizes of females. In contrast in Portugal, such population characteristics were not apparent. This could be due to lack of protandry in these populations or

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perhaps due to low intraspecific competition (see Chapter 2) leading to early promotion of males to females. Alternatively, this could just be a result of low recruitment leading to fewer small males. Distorted sex ratios of *P. vulgata* could be involved in setting southern range limit of the species, since the likelihood of being male increased with latitude from Alentejo (Portugal) through Plymouth (England). Thus sperm could be limiting in low recruitment range edge populations.

P .depressa from England and Portugal showed contrasting results more typical of gonochorist species. Interestingly populations from Portugal also showed some patterns indicating the possibility of slight protandry.

The body size at sex change for *P. vulgata* was addressed by the experimental simulation of two levels of limpet exploitation (Chapter 3). A global multiple logistic regression revealed that log [P(male)/P(female)] decreased with increasing body size in high exploitation treatment relative to its change in body size in the control (pooled across locations); this confirmed that transition to female occurred at smaller body size. This decrease was generally apparent when pooling across treatments and locations. These results accord with those presented for the protandric *P. ferruginea* where individuals in populations with a low density of larger individuals switch to female at smaller sizes (Rivera-Ingraham et al. 2011). As for *P. ferruginea*, *P. vulgata* seems to have sex change plasticity evidenced in an apparent capacity to respond to density reductions with an earlier sex change (assuming a relationship between age and body size). This plasticity is probably context dependent, since I observed a significant stage-by-location interaction and visual differences between locations in numbers of the neuters and sexual individuals. The body size at sex change (specifically, L_{50}, the body size at which an individual is as likely to be male as female) in control plots, which mimicked the population under natural conditions, was larger than expected from the male/female size class overlap. The dependence of sex change on numerous factors, including the immediate social environment (Warner 1984; Shapiro 1987; Ross 1990; Warner at al. 1996; Kuwamura and Nakashima 1998; Baeza and Bauer 2004; Chen et al. 2004; Collin et al. 2005), the size of the individual relative to others in the social group (e.g. Warner and Swearer 1991; Warner at al. 1996; Buston 2003; Colin et. al 2005), the sex ratio of the social group (Shapiro 1984; Munday 2002; Colin et. al 2005), and local density (Wright 1989; Lutenesky 1994), has been signalled for other species of limpets (e.g. Warner at al. 1996; Collin et al. 2005), snails (Chen et al. 2004), shrimp (Baeza and Bauer

2004) and fish (e.g. Warner 1984; Shapiro 1987; Ross 1990; Kuwamura and Nakashima 1998), but to my knowledge has never been reported in *P. vulgata* (see Munday et al. 2006).

The experimentally simulated exploitation of limpets leading to reduced grazing pressure resulted in the establishment of *Fucus* spp. at higher density than in the unexploited control plots, in accordance with other studies of complete limpet removal or exclusion (e.g. Jones 1948; Southward 1964; Hawkins 1981b; Jenkins et al. 1999a, 2008; Coleman et.al. 2006). Furthermore, while the variation in estimated percentage cover of *Fucus* spp. decreased over the course of the experiment in control plots, it increased in both low and high exploitation plots. Moreover, the observed differences by location at the end of the experiment on the percentage cover of *Mytilus* spp. (more abundant at Constantine) and barnacles (more abundant at Trevone) reflected the differences in wave and wind exposure of both shores. This provides an explanation for the observed differences by location both at species and macro-structure level (see also Crowe et al. 2011).

I found evidence for the intermediate disturbance hypothesis, although only during early succession when the experiment had run for just six months. The analysis of contrasts on algal and total species richness for the treatment main effect revealed that low-exploitation plots had lower levels of richness than high-exploitation plots. Total limpet exclusion would have been necessary to reveal an unequivocal intermediate disturbance event. During the first six months the high-exploitation plots had probably represented intermediate levels of disturbance, therefore favouring higher levels of species richness, in accordance with Connell's (1978) intermediate disturbance hypothesis. Levels of disturbance probably surpassed intermediate levels after those six months resulting in no differences in species richness between the different treatments. The detected differences by location in species richness over the course of the experiment (final minus first values) only emphasise that the communities of both shores respond differently to the same source of disturbance. This was confirmed by the multivariate analysis where convergence between the control and low treatments occurred at Constantine while at Trevone the convergence occurred between the low and high treatments. The multivariate analysis also confirmed the importance of fucoids when limpets are

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removed. In both shores *F. vesiculosus* var. *evesiculosus* was the most important
species contributing for the dissimilarity between treatment plots.

Both sets of results on the sex change phenomenon and on the community
diversity emphasise that the same species (*P. vulgata*) responds differently when
present in communities with different structures and that different communities
respond differently to the same levels of limpet exploitation.

The repeat survey of limpet stocks in the Canary Islands (1992 *versus* 1999)
allowed validation of some of the anthropogenic consequences of limpet harvesting
presented in the introduction and Chapters 3 and 4. Despite the inherent limitations
of the survey, I was able to show both within-island and between-island variability in
catch per unit effort (CPUE) for both *Patella* species in 1999. More recent studies in
the intertidal have not found such between-islands variability in abundances,
suggesting that by the time of my survey the resources were not yet overexploited
(see Navarro et al. 2005; Ramirez et al. 2009). Nevertheless for *P. aspera* the CPUE
decreased from 1992 to 1999, while for *P. c. crenata* no differences were detected,
suggesting that *P. aspera* was under a higher harvest pressure. In the Canary Islands,
as in others parts of the world, human exploitation of limpets has reduced species
abundance and changed the structure of size classes due to selection for the biggest
specimens (Navarro et al. 2005; Ramírez et al. 2005; Tuya et al. 2006b; Ramirez et
al. 2009). Although this can present a particular threat to protandric species, since the
females occur mainly in the bigger size classes, the finding of possible sex change
plasticity in harvested species could be a mechanism by which the species avoids a
compromise to its fecundity and consequently mitigates damage to stock sizes. If the
size at which sex change occurs responds to the removal of bigger limpets and
reductions of density, some smaller females may escape exploitation to fertilize and
maintain recruitment levels. This seems to be the mechanism that prevented *P.
ferruginea* from becoming extinct despite intense harvesting that targeted large
individuals in Ceuta (Rivera-Ingraham et al. 2011). However, I found that in the
Canary Islands not only the biggest specimens were preferred by collectors. Smaller
individuals were also harvested by intertidal collectors for different cooking
purposes, presenting an additional pressure on the limpet stocks. The influence of
shore aspect on *Patella* spp. abundance was only evident for *P.c. crenata* in 1999,
resulting in a CPUE with a significant year-by-aspect interaction where the north-
facing shores had higher catches than the south-facing shores. This suggested that

different biological and ecological conditions were influencing *P. c. crenata* population dynamics from the north and south of the islands in 1999. Therefore besides its intrinsic characteristics that determined the observed within-shores variability, shores and its limpet stocks are vulnerable to other constraints that conditioned the observed CPUE. These findings accord with results from Chapters 2 and 3 where site specific characteristics such as community structure and foraging strategies were identified as key limpet population responses.

6.3.1. Conservation measures

Experimental evidence has shown that limpets, and particularly patellid limpets in the North-east Atlantic, have a strong top-down influence on the structure of the rocky intertidal (e.g. Jones 1948; Southward 1964; Southward and Southward 1978; Hawkins and Hartnoll 1983; Hawkins et al. 1992; Jenkins et al. 2005; Coleman et al. 2006), suggesting that a reduction in limpet abundance as a consequence of overexploitation is likely to have strong community level effects through direct and indirect interactions (e.g. Van Tamelen 1987; Martins et al. 2008). Overexploitation could have irreversible impacts such as the local extinction of target species and a substantial decrease in, or even a total elimination of the role in ecosystem functioning provided by the exploited species, that in an extreme scenario could lead to the disruption of a system by altering the food chain length (Martins et al. 2008). Studies in the Azores have shown a clear decrease in algal cover with increasing limpet abundance as well as a positive relationship between the abundance of limpets and barnacle cover (see Hawkins 1983), indicating the importance of limpets in structuring the rocky intertidal does not differ between Macaronesian and continental habitats (Martins et al. 2008).

Scientific studies (e.g. long-term monitoring programmes assessing abundances, size-frequencies, recruitment, larval dispersal, sex ratios and effectiveness of MPA and no-take limpet zones) should be encouraged to evaluate the status of limpet stocks, including potential recovery, and to validate future adjustments to established policies. Local authorities should be encouraged to take action to protect limpet populations, given that they are disappearing and that their protection is not only of economic importance, but also of environmental importance (OSPAR commission

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2010). Environmental policies are defined by people and to be implemented by people. Therefore complementary education and outreach programmes should be developed and directed at the local population and tourists (OSPAR cCmmission 2010). These should be implemented for several years and include explanations of what is happening with the stocks and the importance of protecting the limpets in the breeding period, as well as providing information to locals and visitors about what they can and cannot eat in each season (OSPAR Commission 2010). Complete closure of the limpet fishery has been instituted in the Azores in the past, and could be an option if required to stabilise stocks, aid recovery and/or prevent further decline (OSPAR Commission 2010).

In order to improve exploited stocks, alternative and more proactive measures have increased in recent years such as the deliberate release of animals grown in culture (Booth and Cox 2003). Despite some concern that this measure may have negative effects such as changing the genetic diversity of wild populations or introducing diseases (Bartley et al. 2006; Bell et al. 2006) it is intended to be implemented in the Mediterranean Sea for *P. ferruginea* in pursuance of the conservation strategy for this species by the Spanish government. Alternatively, modifications to existing and proposed coastal engineering structures offer an opportunity for integrative management between the need to protect coastal areas or generate energy and the enhancement of biodiversity or stocks of exploited species (Moschella et al. 2005). Modifications made to coastal structures deployed for coastline protection in the Azores demonstrated that *P. candei* benefits from the protection provided by microhabitats, as in many other upper shore intertidal gastropods (Catesby and McKillup 1998; Gray and Hodgson 1998; Martins et al. 2010). The experimental provision of pits to a featureless artificial structure resulted in a five-fold increase on average in the abundance of this intertidal limpet compared to control areas (Martins et al. 2010). Compared to natural shores (Martins et al. 2008a), enhanced areas of the seawall also supported four times greater abundance of limpets (Martins et al. 2010). If the changes made to the seawall are applied to larger areas or integrated to future coastal engineering, the resulting increase in limpet abundance could have a significant contribution for increasing reproductive output and thereby promote the sustainability of overexploited populations (Martins et al. 2010). Increasing habitat heterogeneity - the number of different components of habitats - will diversify the set of niches that can be exploited by different species, or

within species, by different life-history stages, minimizing the effects of key species reductions (Matias et al. 2007; Martins et al. 2010). The best strategy for mitigating the impacts of these structures on natural systems, already modified by loss of limpets through exploitation, would be to mimic local habitats as closely as possible (Moschella et al. 2005).

A complementary strategy could be to reduce the demand for specimens from natural populations by farming individuals that could be sold frozen, canned or dried. Limpet aquaculture presents several challenges, not least the provision of suitable food for limpets. It is a virtually unexplored field, since few studies exist on rearing limpets of the genus *Patella*. In contrast, abalone species (*Haliotis* spp.) aquaculture is well developed worldwide and useful lessons can certainly be applied to *Patella* spp. farming. Farming infrastructures could encompass an integrated strategy where limpet water flow residues could be the substratum for algal culture minimising ecological impacts. These algae could be used as natural limpet food (e.g. kelp) or incorporated in assembled micro- and macro-algal processed limpet food. Other algal species could be sold as human food, soil fertilizers or directed to cosmetic, pharmaceutical and biotech companies. Limpet shells could also be of use to calcium extraction or ornamental purposes. As an example farmed *P. ulyssiponensis* from the mainland could be sold in the Azores and Canary Islands where demand for *P. aspera* is high. The knowledge of plasticity in the sex change dynamics could then be used to enhance commercial profits. One could envisage delaying sex change to female if it is established that males grow faster than females. Studies of the economic viability of bigger males and females could also determine the best sex to be sold. In this sense it would be preferable to farm fast-growing species such as *P. c. crenata* in place of *P. aspera.* To implement the best limpet farming conditions in sex changing species will require further clarification of sex changing plasticity constraints, optimal males/females ratios, densities, food availability and physical conditions determining growth. These unknowns warrant innovative laboratory and field experiments in areas isolated from human activities, and that is where MPAs and no-take zones can have a major role by providing exceptional conditions for experimental manipulations and for stock recovery.

APPENDICES

APPENDIX 1: GEOGRAPHICAL COORDINATES OF SAMPLED SHORES

Table A1.1. Location names of sampled shores and geographical coordinates.

Location	Coordinates (latitude/longitude)
England	
Jennycliff Bay	50° 21' N / 04° 07' W
Heybrook Bay	50° 19' N / 04° 07' W
Sidmouth	50° 40'N / 03° 17' W
Highcliff	50° 44'N / 01° 41' W
Portugal	
Cabo Mundo	41° 13' N / 08° 43' W
Foz	41° 09' N / 08° 40' W
Cabo Raso	38° 42' N/ 09° 29' W
Avencas	38° 41' N / 09° 21' W
Almograve	37° 38' N / 08° 48' W
Zambujeira do Mar	37° 31' N / 08° 47' W

APPENDIX 2: LIMPET BORING BIVALVE PHOTOGRAPHS
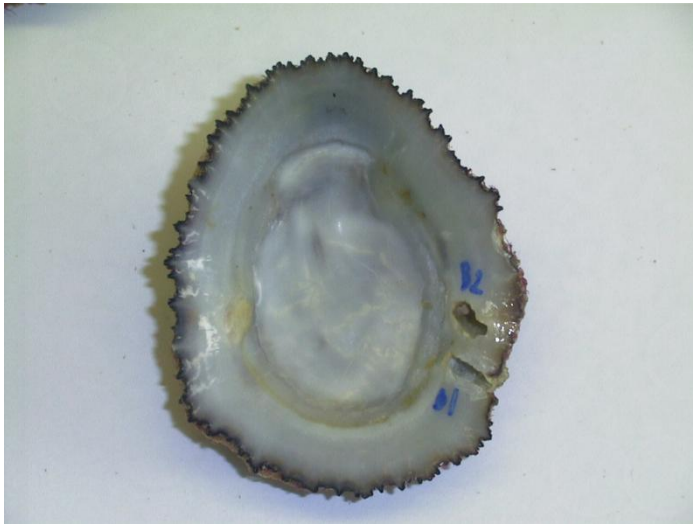
Figure A2.1. Internal view of *P. aspera* shell (length of 60 mm) presenting the cavities where the boring bivalve *L. aristatus* (Dillwyn 1817) were incrusted.
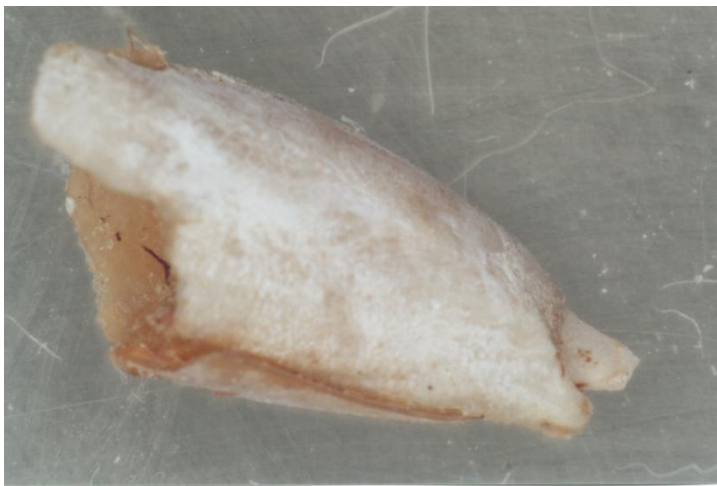
Figure A2.2. *L. aristatus* (Dillwyn 1817) specimen (broken during the extraction with about 7 mm long) collected from *P. aspera* showing the pointed tips at the posterior ends of the valves.

APPENDIX 3: *PATELLA* SPP. CATCHES IN 1992 AND 1999

Table A3.1. Sample shores ordered by region, exploitation index and its partitions (Ac- Accessibility; Pr- Proximity to urban area), and total CPUE (g per 30min. dive) for *P. aspera* and *P. c. crenata* in 1992 and 1999.

SHORES	Exploitation Index (Ac+Pr)	*P. aspera*		*P. c. crenata*	
		1992	1999	1992	1999
Tenerife					
Faro de Abona	15 (10+5)	60.0	35.4	240.0	19.9
Punta del Sordo	15 (10+5)	1700.0	632.6	180.0	164.3
Punta de Teno (exposed)	25 (20+5)	90.0	370.0	10.0	320.0
Punta de Hidalgo	35 (20+15)	35.0	54.1	95.0	218.3
Los Gigantes	35 (20+15)	52.0	137.4	0.0	534.6
Playa Paraiso	35 (20+15)	30.0	0.0	18.0	0.0
Punta de Teno (sheltered)	35 (30+5)	12.0	162.7	60.0	37.4
San Juan de la Rambla	40 (20+20)	280.0	175.9	200.0	666.5
Las Gaviotas	45 (30+15)	220.0	0.0	40.0	0.0
Playa del Pris	45 (30+15)	625.0	415.2	105.0	95.2
Playa San Marcos	50 (30+20)	600.0	162.3	120.0	810.0
Candelaria	50 (30+20)	350.0	150.5	100.0	735.3
Las Caletillas	50 (30+20)	260.0	85.6	120.0	390.7
La Gomera					
San Sebastian	45 (30+15)	1960.0	1559.0	391.0	212.7
Playa Santiago	45 (30+15)	2001.0	1287.0	258.0	175.0
Fuerteventura					
Faro de Jandia	15 (10+5)	1300.0	422.8	200.0	477.6
Los Molinos	15 (10+5)	1800.0	1625.6	0.0	363.6

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Playa de los Muertos	25 (20+5)	3200.0	1648.3	720.0	605.0
El Cotillo	45 (30+15)	2730.0	1776.0	30.0	91.1
Playa de las Matas Blancas	45 (30+15)	180.0	0.0	16.0	65.3
La Palma					
La Fajana	15 (10+5)	0.0	0.0	515.0	1811.0
Las Cabras	25 (20+5)	140.0	37.1	820.0	740.0
Punta Zamora	35 (30+5)	0.0	5.0	10.0	197.3
San Andres	40 (20+20)	1058.0	872.7	490.1	1230.0
La Palmita	50 (30+20)	700.0	22.0	340.0	1017.7
Punta de los Guinchos	50 (30+20)	440.0	56.5	440.0	988.1

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