

Cristina Isabel Oliveira Espírito Santo

**INSIDE A MARINE HARBOUR: PATTERNS OF DISTRIBUTION AND ABUNDANCE
OF *Mytilus galloprovincialis* IN THE PORT OF SINES**



Dissertação apresentada à Universidade de Évora para o cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia e Ecologia do Litoral Marinho. Esta dissertação não inclui as críticas e sugestões feitas pelo júri.

Orientadora: Professora Doutora Teresa Paula Gonçalves Cruz

**Departamento de Biologia
Laboratório de Ciências do Mar (CIEMAR)
Universidade de Évora**



Outubro de 2007

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ciemar



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**As I turn to sand, you took me by the hand,
and declared that love prevails over all...**

**Aos meus pais Luisa e Manuel,
Always...**

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ABSTRACT

Inside the Port of Sines, mussels are abundant in vertical seawalls and buoys, and absent in horizontal breakwaters; outside the port, mussels form patchy monocultures in rocky shores. As a first objective, the patterns of distribution, abundance and dimensional structure of mussels were studied, and three processes that generate patterns: predation, thermal stress, and recruitment. Differences were apparent between orientations inside the port (vertical vs. horizontal) and among areas inside vs. outside, with significant effects of predation and thermal stress. Recruits were present in all areas; however, the three processes didn't explain the distribution and abundance of mussels inside the port. The second objective consisted in a preliminary study of the morphology of mussels. Qualitative and morphometric characters were analysed in three environments, being useful to significantly separate mussels from each area inside and outside the port. Results were insufficient to discriminate similarities or differences meaningful of one or two species.

RESUMO

Dentro do Porto de Sines, mexilhões são abundantes em paredes verticias e bóias, e ausentes em molhes horizontais; fora do porto, são comuns monoculturas de mexilhões em praias rochosas. Como primeiro objectivo, foram estudados os padrões de distribuição, abundância e estrutura dimensional de mexilhões, e três factores que geram padrões: predação, stress térmico e recrutamento. Foram obtidas diferenças significativas entre orientações dentro do porto (verticais vs. horizontais) e entre áreas dentro vs. fora, com efeitos significativos de predação e stress térmico. Registou-se recrutamento em todas as áreas; porém, os três factores não definiram um padrão de distribuição e abundância de mexilhões dentro do porto. O segundo objectivo consistiu num estudo preliminar da morfologia de mexilhões. Foram analisados caracteres qualitativos e morfométricos em três ambientes, sendo significativamente úteis para separar mexilhões de cada área dentro e fora do porto. Os resultados foram insuficientes para discriminar semelhanças ou diferenças características de uma ou duas espécies.

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CHAPTER 1. General introduction

1.1 Human-made constructions in coastal areas

Coastal areas play a crucial role in the economical, social and political development of most countries, and their economic importance is set to grow considerably due to concentration of populations, industries and recreational activities. In recent times, although the tendency is a more residential development in the coastal zone (Gray, 1997; Bulleri & Chapman, 2004), the effects of urbanisation on marine environments have received little attention in comparison to terrestrial counterparts (reviewed in Pickett et al., 2001; but see Chapman & Bulleri, 2003), particularly those fragmented by the deployment of hard man-made structures such as port installations and coastal defences. In Europe, these structures have proliferated and lead to a severe artificialization of coastal areas; for example, in the Italian coasts of the Adriatic Sea, they cover over half of the shoreline, resulting in dramatic changes to coastal landscapes and environments (Bacchiocchi & Airoidi, 2003).

Hard-substrate defence structures of different materials (wood, concrete, limestone, sandstone) are among the most common human-made constructions in coastal areas, and have been built since the 1960s (Glasby, 2000; Bacchiocchi & Airoidi, 2003; Chapman, 2003). The most frequent typologies are longitudinal structures, such as walls, quays, pontoons, pier-pilings and breakwaters, and transversal structures, like groynes and dikes (Connell & Glasby, 1999; Glasby, 1999a; Bulleri et al., 2000; Connell, 2001; Glasby & Connell, 2001; Davis et al., 2002; Bacchiocchi & Airoidi, 2003; Chapman, 2003; Chapman & Bulleri, 2003; Bulleri & Chapman, 2004; Airoidi et al., 2005; Blockley & Chapman, 2006). As documented by Glasby (2000), Glasby & Connell (2001) and Airoidi et al. (2005), the primary purposes of defence structures are to prevent or reduce erosion and flooding of high value coastlines, to stabilize and retain beaches and reclaimed land, and to increase the amenity value of the coast (e.g. beach use, surfing). Therefore, different design criteria result in different hydrodynamic and physical conditions around the structures, with possible important effects on the distribution of epibiota.

Once it has been decided to build a structure on rational grounds to protect an area of coastline there will inevitably be consequences for the environment. These are well

described in Airoldi et al. (2005), who summarized those regional effects: increased abundance and incidence of rocky shore species; decreased abundance and incidence of soft sediment species; changed composition or structure; increased gene flow; and increase of non-native species. As these authors suggest, high number of nearby artificial structures can act as stepping stones, disrupting natural barriers and facilitating the dispersal of rocky coast species across habitats and regions that naturally would be poorly connected. The type and magnitude of the changes induced can vary considerably depending on the environmental setting where the structures are built.

Overall, the construction of hard defence structures always results in a local loss of soft-bottom habitats and associated assemblages of animals and plants (Connell & Glasby, 1999; Glasby, 1999a; Chapman & Bulleri, 2003; Bulleri & Chapman, 2004; Airoldi et al., 2005). Therefore, as reported by Bulleri (2005a), whenever artificial structures are going to be introduced in shallow coastal waters, mitigating the changes to natural assemblages of organisms should be a priority. According to this author, understanding the mechanisms that determine the establishment of different assemblages on natural and artificial habitats might enable a better designing of artificial structures as surrogates for the natural habitats they may replace. This would improve our ability to manage transformations of coastal landscapes in urban areas, contributing to the conservation of marine biodiversity.

1.2 The rocky intertidal habitats

For several purposes, intertidal communities are an attractive system to examine the role played by direct positive and negative interactions and habitat modification in natural communities (Paine, 1966; Seed & Suchanek, 1992; Bertness & Leonard, 1997; Bertness et al., 1999, 2002). There are many reasons why rocky intertidal habitats have been a model system for examining how natural communities are structured, studying the processes that generate patterns and structure. First, they are relatively simple assemblages that are accessible and dominated by small and easily manipulated sessile plants and invertebrates, as well as slow moving consumers that are often readily removed manually or effectively manipulated in the field with cages. Second, one of the most valuable attributes is that they occur across very compact and severe gradients in physical stress (Carroll & Highsmith, 1996; Martin et al., 2005). This makes it relatively easy to experimentally evaluate the impact of variation in physical

stress on organism interactions. It could be argued, in fact, that modern experimental marine community ecology developed as a reaction to the emphasis of ecologists on physical stress explanations of intertidal community patterns (Bertness & Leonard, 1997).

In intertidal rocky systems, causes underlying the distributional patterns of organisms have been approached by many authors. Examples include the role of competition (e.g. Griffiths & Hockey, 1987; Wootton, 1993; Underwood, 2000), herbivory and predation (e.g. Hawkins & Hartnoll, 1983; Griffiths & Hockey, 1987; Petraitis, 1990; Bulleri et al., 2000; Harley, 2003), settlement and recruitment (e.g. Connell, 1985; Porri et al., 2006), height above chart datum (e.g. Underwood, 1978; Griffiths & Hockey, 1987; McQuaid et al., 2000; Harley & Helmuth, 2003; Davenport & Davenport, 2005; McQuaid & Lindsay, 2005), and gradient of wave exposure (e.g. Underwood, 1981; McQuaid & Lindsay, 2000; McQuaid et al., 2000; Harley & Helmuth, 2003; Fitzhenry et al., 2004; Hammond & Griffiths, 2004; Davenport & Davenport, 2005; McQuaid & Lindsay, 2005; Westerborn & Jattu, 2006; McQuaid & Lindsay, 2007).

Mussels are one of the dominant competitors for space in this mechanically stressful rocky intertidal environment (Witman & Suchanek, 1984; Seed & Suchanek, 1992), and one key aspect of their success is their ability to maintain a secure attachment to the substrate (Bell & Gosline, 1997). Attachment is achieved by means of a byssus, which is an extracellular, collagenous secretion of the foot (Carrington, 2002a,b). Other important features of mytilids are their rapid growth rate at differing water temperatures, high fecundity, and resistance to desiccation, salinity and parasites (Griffiths & Hockey, 1987; Van Erkom Schurink & Griffiths, 1990; Seed & Suchanek, 1992; Petraitis, 1995). In the subtidal environment, however, they are limited by high predation pressure and competition (Wootton, 1993).

At levels on the shore above the physiological limits of their major predators, and in the absence of physical disturbance, mussels can form a virtual patchy monocultures in areas more exposed (Carrington, 2002a). In sheltered environments, mussel usually form highly dense, overcrowded and multilayered matrices or beds (reported as thick as ca. 120 cm), where self-thinning processes are expected to occur (Newell, 1989; Seed & Suchanek, 1992; Alvarado & Castilla, 1996). These beds allow colonization by infaunal organisms in the sediment trapped in the interstices among shells – organisms which otherwise cannot live in rocky habitats (e.g. Tokeshi & Romero, 1995). Hence,

as a dominant competitor for space, mussels have the potential to reduce the diversity of primary space-occupying species on the shore, and to control species richness (Levin & Paine, 1974; Newell, 1989; Seed & Suchanek, 1992; Carrington, 2002a; Hammond & Griffiths, 2004). Sometimes gaps can be formed between the beds, which may be initiated by either physical factors (e.g. Levin & Paine, 1974; Sousa, 1984; Denny, 1987), or biological processes (e.g. Paine, 1966; Witman & Suchanek, 1984), so processes contributing to variation in its abundance can have cascading influences throughout the community (Blanchette et al., 2007). As they live in conspecific or heterospecific groups, the individual's risk of being damaged and killed is reduced because neighbours directly or indirectly buffers environmental extremes (Bell & Gosline, 1997; Bertness & Leonard, 1997).

1.3 The genus *Mytilus*

1.3.1 Taxonomy

Marine mussels of the genus *Mytilus* (Class: Bivalvia, Pelecypoda; Order: Mytiloidea; Family: Mytilidea) are present at higher latitudes in all oceans and major seas of the world (Newell, 1989; McDonald et al., 1991), being one of the most studied genera in the marine environment. The systematic status of *Mytilus* species have been subject of considerable discussion since the 1860s (reviewed in Gosling, 1984, 1992a). In an extensive review of the genus, Gosling (1992a,b) summarized the recognition of about nine distinct species of *Mytilus* based on studies prior to the use of electrophoresis: *M. edulis* Linnaeus, 1758 from northern temperate latitudes; *M. galloprovincialis* Lamarck, 1819 from the Mediterranean Sea; *M. trossulus* Gould, 1850 and *M. californianus* Conrad, 1837 from the Pacific coast of North America; *M. chilensis* Hupe, 1854 from Chile; *M. platensis* Orbigny, 1846 from Argentina; *M. planulatus* Lamarck, 1819 from Australia; *M. desolationis* Lamy, 1936 from the Kerguelen Islands; and *M. coruscus* Gould, 1861 (= *M. crassitesta* Lischke, 1868) from Japan and China. Nevertheless, there was by no means a general concensus on this.

In some areas, namely the coasts of England, Ireland, France and Spain, separation of two different forms of mussels (*M. edulis* and *M. galloprovincialis*) have proved to be exceedingly difficult due to a considerable degree of overlap in morphological characteristics (Gosling & Wilkins, 1981; Skibinski et al., 1983; Fish & Fish, 1996). This, together with the large number of truly intermediate forms observed (e.g.

Skibinski et al., 1978; Gosling & Wilkins, 1981; Skibinski et al., 1983; Gosling, 1984; Blot et al., 1988; Gardner & Skibinski, 1990; Kautsky et al., 1990; Lobel et al., 1990; Tedengren et al., 1990; Väinölä, 1990; Gardner & Skibinski, 1991; McDonald et al., 1991; Skibinski & Roderick, 1991; but for reviews see Gosling, 1992a,b), was the first indication that hybridization and introgression might be occurring between species. While some earlier authors regarded the genetic differences large enough to warrant the discrimination of both as distinct species, others consider it merely as a variety of the larger *M. edulis* superspecies or complex (see Skibinski et al., 1983; Gosling, 1984; Blot et al., 1988; Johannesson et al., 1990; Tedengren et al., 1990; McDonald et al., 1991; Beaumont et al., 1993).

Earlier classifications based only on external shell morphology showed to be complex and confusing (Gosling, 1992a). More recently, multidisciplinary studies have shed new light on the previously uncertain taxonomy of the *Mytilus* species complex which can now be considered to consist of three distinct evolutionary lineages, *M. edulis*, *M. galloprovincialis* and *M. trossulus* (McDonald et al., 1991; Beaumont et al., 1993; Sanjuan et al., 1994; Rawson & Hilbish, 1995; Suchanek et al., 1997; Comesaña et al., 1998; Innes & Bates, 1999; Rawson et al., 1999; Hummel et al., 2001; Secor et al., 2001; Hilbish et al., 2002; Wood et al., 2003a; Ridgway & Nævdal, 2004; Wonham, 2004; Toro et al., 2005; Beaumont et al., 2006). Whether they are regarded as separate species or not, they do differ biochemically at a number of loci (Skibinski et al., 1978), maintain unique genetic cohesiveness throughout much of their ranges, and have distinct evolutionary histories (Riginos & Cunningham, 2005). The debate is still opened. Therefore, as suggest by Gosling (1992a,b), 'in order to avoid unnecessary confusion, the most prudent scenario would seem to be to continue referring to the taxa as: *M. edulis*, *M. galloprovincialis*, *M. trossulus*, while at the same time recognizing – with the morphological and genetic information we have at present – that there is a considerable lack of agreement on their exact taxonomic status'.

Genetics have been extremely useful in helping to resolve the systematics of the genus, as well as to map the global distribution of *Mytilus* and their hybridization. From 1970s to the 1990s allozymes (electrophoretic analysis) were the main genetic markers used (e.g. Skibinski et al., 1983; Gosling, 1984; Johannesson et al., 1990; Väinölä, 1990; McDonald et al., 1991; Sanjuan et al., 1994; Quesada et al., 1995). Nowadays, the advent of PCR-based techniques has opened up new possibilities for finding diagnostic markers, but it was only very recently that microsatellite loci have been

isolated for mussels and employed in an extensive population study (e.g. Inoue et al., 1995; Rawson & Hilbish, 1995; Rawson et al., 1996; Inoue et al., 1997; Suchanek et al., 1997; Comesaña et al., 1999; Daguin & Borsa, 1999; Rawson et al., 1999; Daguin & Borsa, 2000; Hilbish et al., 2000; Daguin et al., 2001; Skurikhina et al., 2001; Bierne et al., 2002; Hilbish et al., 2002; Bierne et al., 2003; Hilbish et al., 2003; Wood et al., 2003a,b; Śmietanka et al., 2004; Toro et al., 2004, 2005).

1.3.2 Distribution

In terms of geographical distribution, all three species are now considered as globally widespread, dominating exposed or moderately exposed rocky shore communities where they overlap (reviewed by Seed & Suchanek, 1992). *Mytilus edulis* is recognized along the Atlantic coast of Europe, from the northern White Sea, Norway and Iceland, down to England and north of France in the western European coast, and is common in Canada, eastern North America and South America (Chile, Argentina, and the Falkland and Kerguelen Islands); *M. galloprovincialis* has been unambiguously identified on the Mediterranean coast and Black Sea, as well as in north-west Africa and South Africa, being common in countries like Mauritania, Portugal, Spain, France and Italy, as well as in eastern Asia, Australia, Tasmania, New Zealand, southern California, Chile, and the south and west coasts of Ireland, Wales and England; finally, *M. trossulus* apparently occurs only in the northern hemisphere, namely in the Baltic Sea, eastern Canada, Alaska, Siberia, and western North America (California) (Johannesson et al., 1990; McDonald et al., 1991; Gosling, 1992a; Sanjuan et al., 1994; Ardizzone et al., 1996; Fish & Fish, 1996; Suchanek et al., 1997; Comesaña et al., 1998; Daguin & Borsa, 1999, 2000; Hilbish et al., 2000; Hummel et al., 2001; Ridgway & Nævdal, 2004; Śmietanka et al., 2004; Wonham, 2004; Toro et al., 2005).

In Portugal, mussels have been recognized as *M. galloprovincialis* (e.g. Saldanha, 1974; Santos, 2000; Rius & Cabral, 2004). However, some genetic studies (McDonald et al., 1991; Sanjuan et al., 1994; Quesada et al., 1995; Daguin et al., 2001; Bierne et al., 2002, 2003; Śmietanka et al., 2004) reported the occurrence of *M. edulis*-like alleles. As suggest by Daguin et al. (2001), this occurrence can be explained by past introgression by *M. edulis*. Other important conclusion from these population genetic studies is that *M. galloprovincialis* is genetically subdivided into an Atlantic group and a Mediterranean group, with a break point at the well defined Almeria-Oran oceanographic front (Quesada et al., 1995; Daguin et al., 2001). This geographic

isolation can be also the cause of the genetic differences exhibited by north-western African *M. galloprovincialis* with the Mediterranean group, but not with the Atlantic mussels from Portugal (Daguin & Borsa, 1999). Therefore, instead of a single genetic gradient from *M. galloprovincialis* of the Iberian Peninsula to *M. edulis* populations in the North Sea, several successive transitions are observed delineating patches of populations characterised by high frequencies of parental alleles (Bierne et al., 2002). As stated by Beaumont et al. (2006), it seems that *M. galloprovincialis* is slowly spreading northwards invading territory once exclusive to *M. edulis* and that this may be partly a result of global warming. Whether or not it has been involved so far, it is likely that climate change will increase the rate of this. In fact, the precise distributions of the two species, and the extent of their hybridisation, remain to be characterised for most areas of the European Atlantic coast, being the coast of Iberian Peninsula a key area for understanding the distribution of the genus *Mytilus* (Sanjuan et al., 1994; Beaumont et al., 2006).

1.3.3 Hybride zones

Mussel hybrid zones have long attracted attention in evolutionary biology as they present the opportunity to examine the genetics of differentiation among taxa and the process of speciation (Hilbish et al., 2003). In the last years, the most intensely studied marine hybrid zone is of *Mytilus edulis* × *M. galloprovincialis* species, which stands on the coasts of England, France and Spain, with a considerable extension up to Ireland and Scotland (see Gardner et al., 1993; Sanjuan et al., 1994; Comesaña & Sanjuán, 1996; Wilhelm & Hilbish, 1998; Gilg & Hilbish, 2000; Daguin et al., 2001; Secor et al., 2001; Bierne et al., 2002; Hilbish et al., 2002; Bierne et al., 2003; Wood et al., 2003b; Coghlan & Gosling, 2007). Another well-known hybrid zones are the one for *M. edulis* × *M. trossulus* species in Atlantic Canada and northern Europe, namely in the Baltic and North Seas (see Saavedra et al., 1996; Comesaña et al., 1999; Innes & Bates, 1999; Toro, 1999; Gardner & Thompson, 2001; Toro et al., 2002; Śmietanka et al., 2004; Toro et al., 2004; Riginos & Cunningham, 2005), and for *M. galloprovincialis* × *M. trossulus* in the Pacific Coast of North America and Japan (see Inoue et al., 1997; Suchanek et al., 1997; Rawson et al., 1999; Skurikhina et al., 2001).

The main feature of these zones is its mosaic structure, in which populations of pure genotypes alternate with hybrid populations by differential adaptation to patchy environments (reviewed in Daguin et al., 2001 and Bierne et al., 2002, 2003). These

are thought to reflect geographical variation in the opportunities for interbreeding, enhanced by geographically variable levels of selection on recruits (Skibinski et al., 1983).

Despite the fact that *Mytilus* species have a prolonged larval stage enabling dispersal over large distances, little is known about how these hybrid zones are maintained and no diagnostic criteria exist at the species level for early-stage *Mytilus* larvae (Wood et al., 2003a). Therefore, the availability of a rapid method for identifying larvae of these three species and their hybrids would facilitate studies of hybridization, and would aid the study of larval dispersal and gene flow. Large scale studies have yet to been carried out to characterise the mosaic of populations of *M. edulis*, *M. galloprovincialis* and their hybrids.

1.3.4 Reproduction

The settlement and recruitment processes of mytilid bivalves have received considerable attention, but because of the ongoing confusion in the literature with respect to the use of the terms settlement versus recruitment (Connell, 1985; Seed & Suchanek, 1992), it is important to ascertain its definitions. As suggested in Lasiak & Barnard (1995) and Porri et al. (2006), settlement is the permanent, reversible or irreversible contact that planktonic larvae establish with the substratum. As this contact is made, the larvae may or may not go through a phase of metamorphosis, so we can consider settlement to be the transition from the planktonic larval stage to life in the benthos (Seed & Suchanek, 1992; Alfaro, 2006). Recruitment is less easy to define, being more than a simple arrival of new individuals on the shore; is essentially the number of individuals that have survived for a certain period after settlement, during which time post-settlement mortality may have occurred (Connell, 1985; Seed & Suchanek, 1992; Lasiak & Barnard, 1995; McQuaid & Lindsay, 2005; Porri et al., 2006). Rrecruitment rate can be defined as the rate at which juveniles join the population, and is usually only possible to measure some time after settlement (Connell, 1985).

As broadcast spawners with external fertilisation (Gardner & Skibinski, 1990; Fish & Fish, 1996; Helson & Gardner, 2004), each individual produces more than a million eggs, of which ultimately only a very few will settle and survive per m² (Harris et al., 1998; Brinkman et al., 2002). Larvae are pelagic (planktotrophic) and distributed by

wind and tidally driven currents (Cáceres-Martínez et al., 1994; Bertness et al., 1996; de Vooy, 1999; McQuaid & Phillips, 2000; Branch & Steffani, 2004; Beaumont et al., 2006). Initially, they settle preferentially on filamentous substrata (hydroids and filamentous algae such as *Polysiphonia* and *Ceramium*) with proteinaceous threads (byssus or byssal threads), and after a period of growth they detach and enter into a secondary pelagic phase, the so-called bysso-pelagic migration phase (Dare et al., 1983; Barkati, 1989; King et al., 1989; Newell, 1989; Lutz & Kennish, 1992; Lasiak & Barnard, 1995; Fish & Fish, 1996; Pulfrich, 1996).

Newly settled mussels are known as early plantigrades and have <0.5 mm of shell length, but after 4 to 8 weeks, late plantigrades up to a size of 2 mm drift to new sites until they reach adult mussel beds (Sprung, 1984; King et al., 1989; Lutz & Kennish, 1992; Seed & Suchanek, 1992; Cáceres-Martínez et al., 1993, 1994; Fish & Fish, 1996; Pulfrich, 1996; Suchanek et al., 1997; Chícharo & Chícharo, 2000; Dobretsov & Wahl, 2001). This distinction may be important also for the study of selection between *Mytilus* species among spat (Gilg & Hilbish, 2000). All this behaviour pattern at settlement is believed to be an adaptation to reduce competition between the newly settled and adult mussels, but there is growing evidence that, in some mytilid populations, early plantigrades forgo this initial growth phase on filamentous substrata and settle directly onto adult mussel beds (Fell & Balsamo, 1985; McGrath et al., 1988; Seed & Suchanek, 1992; Cáceres-Martínez et al., 1993, 1994; Fish & Fish, 1996; Alfaro, 2006). However, some authors focus the little direct evidence for this (King et al., 1989; Lasiak & Barnard, 1995; Gilg & Hilbish, 2000). In general, exact predictions of recruitment to any given mussel population are difficult, becoming apparent that it can occur at almost any time of the year (Newell, 1989; Brinkman et al., 2002).

Filamentous algae, together with other algae such as *Corallina* and *Gigartina*, appeared to provide an extensive pool of young mussels. Many of which could be migrating onto the adult beds more or less at any time of the year, accounting for the sporadic and often unpredictable pulses of recruitment that characterize many *Mytilus* populations (review in Seed & Suchanek, 1992). As these authors document, while migration from primary attachment sites to the adult habitat appears to be due to changes in the ecological requirements of the plantigrades, many mussels will also be liberated involuntarily by the seasonal die-back of their host algae, or through the action of winter storms. The suitability of the substratum seem to be related to its general surface texture and retention of water, such as roughened or discontinuous

surfaces rather than to any chemical attraction (Dare et al., 1983; Petraitis, 1990, 1991; Seed & Suchanek, 1992). As a consequence, plantigrades usually attach and detach themselves many times before finally settling under more favourable substrate conditions on established mussel beds (Lutz & Kennish, 1992; Cáceres-Martínez et al., 1994).

In terms of spawning, Southern Hemisphere species usually reproduce later in the year and have a progressively restricted season further north; Northern species exhibit the reverse trend, spawning earlier and with a more extended season further south (e.g. Curiel-Ramírez & Cáceres-Martínez, 2004). In northwestern Europe, namely in England, *M. edulis* spawns generally during spring and early summer (King et al., 1989; de Vooy, 1999), but at a more eastern place, in the Wadden Sea, it extends throughout the year with peaks in early summer and autumn (Pulfrich, 1996). In Mediterranean, *M. galloprovincialis* has a much longer spawning period, covering a large part of autumn, winter and spring seasons with a resting stage in summer (Ceccherelli & Rossi, 1984; Ardizzone et al., 1996; de Vooy, 1999). However, in the Ria de Vigo (north-west Spain), two peaks of recruitment were recorded for this species, one in spring and other in summer (Molares & Fuentes, 1995; Cáceres-Martínez & Figueras, 1998). In Portugal, the only two recruitment studies of Saldanha (1974) and Santos (2000), based in length frequencies, suggest the existence of recruitment all the year. The generality is that the recruitment patterns of most sessile benthic macro-invertebrates, especially barnacles and mussels, follow a bimodal pattern, with two peaks during a year that seem to be related to temperature increase of seawater (e.g. Seed & Suchanek, 1992; de Vooy, 1999; Buck, 2007), salinity (e.g. Fell & Balsamo, 1985) and/or food availability (e.g. Menge et al., 2004; Beaumont et al., 2006).

1.3.5 Economical importance

Mussels have received much attention as marine foulers as well as indicators of marine environmental quality in recent years (Widdows & Donkin, 1992). In addition, they are important as food in many countries (e.g. Inoue et al., 1997). As a consequence, they represent an economically important resource for human harvesting (e.g. Rius & Cabral, 2004) and for aquaculture (e.g. Hickman, 1992). There is a very extensive mariculture of mussels almost throughout their distribution (Beaumont et al., 2006). In Europe, Spain is by far the greatest producer of mussels by aquaculture (300 000

tonnes annually), higher than the combined total of other important mussel producing countries, such as Netherlands, France, Italy, Ireland and UK (Cáceres-Martínez et al., 1993; Beaumont et al., 2006). As an example, European countries produced 38% of world production in 2003 (Beaumont et al., 2006), being China, however, the largest producer by far (Buck, 2007). With the worldwide increasing development of marine aquaculture the necessity of evaluate the genetic impact of its escapees is extremely important.

With the increasing rates of accidental or deliberate introduction of alien ('exotic' or 'non-native') species in coastal marine habitats, considering the global rise in shipping and aquaculture activities over the last century (Carlton, 1992; Branch & Steffani, 2004; Wonham, 2004; Minchin, 2007), mussels have become invasive in many parts of the world (Grant & Cherry, 1985; McDonald et al., 1991; Hilbish et al., 2000). This can be one reason for the similarity between northern and southern *Mytilus* spp. (McDonald et al., 1991; Hilbish et al., 2000). While the majority of these transplantations remain restricted to harbours and sheltered lagoons or estuaries, in other places, like the South African coast (Branch & Steffani, 2004; Rius & McQuaid, 2006; Zardi et al., 2006a,b), one single species spread extensively. Hence, this phenomenon can have major consequences for community structure, including the elimination of indigenous species by competitive advantage for the same resources (Branch & Steffani, 2004; Bownes & McQuaid, 2006).

1.4 Environmental monitoring in the Port of Sines

Among the many industrial activities of the Port of Sines, stands out the charge and discharge of crude oil, liquefied petroleum gas and refined products on the Liquid Bulks Terminal, ethylene and propylene on the Petrochemical Terminal, and coal in the Multipurpose Terminal. With economical importance at regional and national levels, this port is one of the principal places that supply the oil-bearing and electrical producing industries in Portugal (Figure 1). At local level, there also the movement of chemical materials from the Service Harbour, and activities in the Leisure and Fishing Harbours. All together, these industrial and recreational movements can have potential environmental impacts, along with the continuous discharge of raw domestic sewage from the town of Sines. Hence, the release of pollutants in the marine environment can influence, direct or indirectly, the environmental quality of the Port of Sines, as well as adjacent areas. Considering this, and taking into account the tendency of intensification

in the port activities, only a continuous and profound knowledge of its environmental impact will allow us to intervene and manage its environments.

With the overall aim of assessing the impact of the Port of Sines in the marine environment, several projects funded by the Administration of the Port of Sines were conducted from 1996 to 2006 under the coordination of CIEMAR (University of Évora; e.g. CIEMAR, 2004).



Figure 1. The geographical distribution of Sines in the Iberian Peninsula (*in* Google Earth).

This thesis was integrated in one of those projects (“Monitorization of Marine Environments of the Port of Sines – MAPSi 2004/2006”). Therefore, earlier studies in the rocky shores detected an effect of the Port of Sines, with the number of taxa being lower inside the port than outside, and the dissimilarity of assemblages higher between inside and outside areas than among inside or between outside areas (Cruz & Castro, 2002). As these authors suggest, one main explanation for this intertidal pattern can be the differences in the type (artificial substrata inside the port, natural substrata outside),

age and disturbances past (outside substrata are older and with a longer history of disturbances) of substrata. Another impact that can be frequent but spatially restricted is the effect of the Liquid Bulks and Petrochemical Terminals in variables as total hydrocarbons in sediments and mussels, and polycyclic aromatic hydrocarbons (PAH) in mussels (higher values were recorded in areas close to these terminals; Cruz & Castro, 2002). Finally, these authors report also an important environmental problem in the Fishing Harbour, probably due to the discharge of raw sewage in this area and close to it, which effects can be magnified by its high closure, and diverse industrial activities (boat painting and repair, fish landing, etc.).

1.5 Objectives of the thesis

In the Port of Sines, mussels (*Mytilus galloprovincialis*) are extremely abundant in the intertidal and subtidal environments of vertical seawalls, as well as attached to floating buoys, and are absent in the horizontal breakwaters adjacent to them (preliminary observations). Outside the port, at northern and southern natural areas, mussels are abundant in exposed shores. In this study, the main purpose is to characterize the spatial distribution of mussels inside and outside the Port of Sines, taking mostly into account its total absence in horizontal breakwaters. Hence, the models analyzed were:

- a different spatial pattern of distribution in areas inside and outside the Port of Sines;
- a different dimensional structure of the mussels surviving inside and outside the port, as an adaptation to the different environments;
- an heavier predation pressure in the breakwaters comparing with vertical seawalls;
- a greater thermal stress in the breakwaters comparing with vertical seawalls;
- a different recruitment pattern between these two types of environments; and
- a different morphological pattern in mussels from inside and outside the port.

As a first main objective (Chapter 2), was made a description of the patterns of mussel distribution and abundance, and of its dimensional structure inside and outside the port, with the effects of predation and thermal stress, and with recruitment patterns. The second objective was to report a preliminary approach of the morphological differences observed in mussels from inside and outside areas of the Port of Sines (Chapter 3).

CHAPTER 2. Inside a marine harbour: patterns of distribution and abundance of *Mytilus galloprovincialis* in the Port of Sines

2.1 ABSTRACT

Inside the Port of Sines, mussels are abundant in artificial vertical seawalls and buoys, forming thick and dense communities, while in adjacent breakwaters are absent. Outside the port, mussels form patchy monocultures in natural rocky shores. In several areas, the patterns of distribution and abundance of mussels were studied using quadrats in the mid-intertidal zone, as well as its dimensional structure in three different environments. Taking into account its absence in breakwaters, three main factors were studied: predation, thermal stress, and recruitment. Remarkably consistent differences were apparent between orientations inside the port (vertical vs. horizontal surfaces) and among areas inside vs. outside. Adult mussels of outside areas showed significantly shorter length of shell than mussels living inside the port, and predation pressure was significant in all areas studied. Thermal stress was significant among treatments, with apparently greater survival of mussels in seawalls comparing with a breakwater. In terms of recruitment, all areas presented mussel recruits, with significant differences among areas only in one month. Overall, these patterns can reflect differences in growth and post-settlement mortality during earlier colonisation, along with an intensive top-down pressure by predators. Thermal stress and intrinsic properties of the substratum can be extremely important for mussels' survival, especially in first stages of its life history.

2.2 INTRODUCTION

Artificial surfaces are continuously being added to waterways all over the world due to the rapid urbanization of coastal regions. This progressive increase has raised concern about their effects on natural assemblages of organisms (Connell & Glasby, 1999; Glasby, 1999b; Bulleri et al., 2000; Glasby, 2000; Davis et al., 2002; Bacchiocchi & Airoidi, 2003; Chapman & Bulleri, 2003; Moschella et al., 2005; Blockley, 2007). Recent work indicates that assemblages on vertical surfaces of pilings, pontoons and retaining walls are quite different from those on nearby natural rocky reefs (McGuinness, 1989; Connell & Glasby, 1999; Glasby, 1999a,b; Holloway & Connell, 2002; Chapman, 2003; Bulleri, 2005a). Not only do urban structures provide surfaces of different compositions,

they may also provide surfaces of various orientations (Glasby, 2000; Glasby & Connell, 2001). As a consequence, very different types of epibiotic assemblages have been shown to occur on surfaces with distinct orientations: upper vs. lower surfaces, and vertical vs. horizontal surfaces (Chapman, 2003; Chapman & Bulleri, 2003). The reasons for such differences in assemblages are not clear, but could be due to a variety of factors and combinations of influences that determine its distribution and abundance (Glasby, 1999c).

Surprisingly, the epibiota of man-made coastal defence structures have received little attention until the last decade or so (Ardizzone et al., 1996; Connell & Glasby, 1999; Bulleri et al., 2000; Bacchiocchi & Airoidi, 2003; Chapman, 2003). Few studies have specifically attempted to compare artificial and natural surfaces in order to understand their relative effects on species diversity and abundance (but see McGuinness, 1989; Glasby, 1999a,b, 2000; Holloway & Connell, 2002). This limits the possibility to develop models of predicted impacts, and to identify options for the design and management of defence structures (Bacchiocchi & Airoidi, 2003). Although, as referenced in Bulleri & Chapman (2004), it is important to evaluate the ecological value of these artificial surfaces as habitats for epibenthic assemblages, as a way to fully understand the ecology of complex coastal developments that introduce different types of substrata into a relatively small area. This study is unusual in that it took place in a marine harbour, the Port of Sines, being its main purpose the assessment of spatial differences between mussels from two different types of artificial habitats (seawalls and breakwaters).

Mussel populations show a high degree of spatial aggregation or patchiness in space (Sousa, 1984; Littorin & Gilek, 1999; Erlandsson & McQuaid, 2004). Its degree of heterogeneity is scale dependent and the importance of different regulating factors varies through space and time (Connolly & Roughgarden, 1998; Lawrie & McQuaid, 2001; Erlandsson & McQuaid, 2004; Airoidi et al., 2005). Traditionally, the principal factors regulating temperate intertidal communities have been thought to be physical variables, such as wave exposure and shoreline configuration, and biological processes like competition and predation (Paine, 1966; Levin & Paine, 1974; Sousa, 1984; Denny, 1987; Griffiths & Hockey, 1987; Alvarado & Castilla, 1996; Bustamante & Branch, 1996; Hunt & Scheibling, 1996; McQuaid et al., 2000; Underwood, 2000; Carrington, 2002a; Steffani & Branch, 2003). Predation pressure is undoubtedly the single most important source of natural mortality in *Mytilus*, being especially high during

the 3 weeks when it is a planktonic larva (Newell, 1989; Seed & Suchanek, 1992). This is the so-called “top-down” control exerted primarily by seastars (Paine, 1966; Suchanek, 1978; Menge et al., 1994; Saier, 2001; Menge et al., 2004), shore crabs (Ceccherelli & Rossi, 1984; ap Rheinallt, 1986; Ameyaw-Akumfi & Hughes, 1987; Enderlein & Wahl, 2004) and dog-whelks (e.g. Suchanek, 1978). Other gastropods, birds, mammals (including seals, walrus, sea otters, and even turtles), fishes, octopuses, lobsters, sea urchins and polychaetes are also known to feed on mussels and may account for some mortality (Seed & Suchanek, 1992 and references therein). Environmental conditions may alter the behaviour and/or relative abundance of predators, resulting in variation in feeding rate, susceptibility of prey to capture, or size selectivity of predators (Ameyaw-Akumfi & Hughes, 1987; Carroll & Highsmith, 1996). Thus, predator’s handling time and preference may vary with prey size, being mortality rates also size dependent (Dare et al., 1983; ap Rheinallt, 1986; Ameyaw-Akumfi & Hughes, 1987; Griffiths & Hockey, 1987; Seed & Suchanek, 1992; Petraitis, 1995; Nagarajan et al., 2006).

Thermal stress is a matter that is becoming an increasingly important area of scientific interest and concern with the climate changes throughout the world (Helmuth & Hofmann, 2001; Fitzhenry et al., 2004). Temperature varies seasonally and latitudinally in a moderately uniform manner, performing a causal relationship with reproduction and geographical distribution (Seed & Suchanek, 1992; Helmuth & Hofmann, 2001; Somero, 2002; Wetthey, 2002; Harley, 2003). Therefore, physiological intolerance to temperature extremes and desiccation represent one of the most important factors for the determination of upper limits of mussels and their predators in rocky intertidal sites (Suchanek, 1978; Seed & Suchanek, 1992; Hofmann & Somero, 1995; Dahlhoff & Menge, 1996; Hunt & Scheibling, 1997; Buckley et al., 2001; Helmuth & Hofmann, 2001; Sanford, 2002; Blanchette et al., 2007). Although mussels are well-adapted to life in constantly changing environmental conditions (Seed & Suchanek, 1992; LeBlanc et al., 2005), survival depends greatly on acclimation, humidity, food availability, reproductive condition, and especially with its previous thermal history (Buckley et al., 2001; Somero, 2002); but all species live in a characteristic limited range of habitats, and within their range they tend to be most abundant at their particular environmental optimum (Bustamante et al., 1997).

More recently, a big emphasis has been placed on understanding the effect of variation in rates of larval supply, settlement and recruitment to intertidal populations (see

Connell, 1985; Gaines & Roughgarden, 1985; Petraitis, 1991; Caley et al., 1996; Hunt & Scheibling, 1996, 1997; Harris et al., 1998; Helson & Gardner, 2004), and how this variability can sometimes be explained by features of the substratum (Nielsen & Franz, 1995; Connolly et al., 2001; Lawrie & McQuaid, 2001; Erlandsson & McQuaid, 2004), or near-shore processes operating at various spatial (tens to hundreds of kilometers) and temporal scales (annual, decadal, or unusual events) (e.g. Underwood & Denley, 1984; Denny, 1987; Roughgarden et al., 1988; Underwood & Fairweather, 1989; Pineda, 1991; Wing et al., 1995; Connolly & Roughgarden, 1998; Archambault & Bourget, 1999; McQuaid & Phillips, 2000; McCulloch & Shanks, 2003; Shanks et al., 2003; Airoldi et al., 2005; Porri et al., 2006; McQuaid & Lindsay, 2007). Consequently all factors that influence these basic processes will also influence the assemblage present at a given location, such as salinity (Seed & Suchanek, 1992), shading and proximity to the seafloor (Kennelly, 1989; Glasby, 1999b,c), food availability (Sprung, 1984; Gilek et al., 2001), and overcrowding (Skibinski & Roderick, 1991; Seed & Suchanek, 1992). These factors can vary seasonally and their combined and/or synergistic effects occasionally result in spectacular mass mortalities (Seed & Suchanek, 1992).

Inside the Port of Sines, mussels are abundant in the artificial vertical seawalls (intertidal and subtidal) and beneath buoys, forming thick and dense communities. Adjacent to these areas, they are absent in the intertidal and subtidal environments of horizontal breakwaters with different orientations. Outside the port, in natural areas of rocky shores, mussels can be abundant in areas exposed. This suggests that differential hydrodynamics, predation, thermal stress, and recruitment (and interactions among these factors) may play important roles in explaining the above pattern. Experiments were set up to test the following hypothesis inside the Port of Sines: (1) that in breakwaters predation have a greater effect than in adjacent seawalls; (2) that thermal stress in mussels is heavier in breakwaters than in seawalls; and (3) that there is higher recruitment in vertical seawalls than in breakwaters. At the same time, its patterns of distribution and abundance in areas inside and outside the port were studied, as well as the dimensional structure of mussel populations in intertidal and subtidal areas inside the port, and one intertidal area outside.

2.3 MATERIAL AND METHODS

2.3.1 Study area

The Port of Sines is an open deep-water sea port located in the southwest coast of continental Portugal (Latitude: 37°57'N, Longitude: 08°53'W), being the main port in the Ibero-Atlantic front. With relevant national and international importance by its strategic location and natural characteristics, it offers unique facilities to receive any type of vessel. Between the many industrial activities, stands out the charge and discharge of crude oil, liquefied petroleum gas and refined products on the Liquid Bulks Terminal (LBT), and ethylene and propylene on the Petrochemical Terminal (PET; see **Figure 2**). Adjacent to this port, there are moderately to extreme exposed marine areas with regional and national importance for tourism, fisheries and conservation, namely a natural park, “Parque Natural do Sudoeste Alentejano e Costa Vicentina”.



Figure 2. The Port of Sines and shores at north and south of Sines. CSN: Cabo de Sines Norte; VIE: Vieirinha; OLI: Oliveirinha; SAM: Samouqueira; QUE: Queimado; LBT: Liquid Bulk Terminal; PET: Petrochemical Terminal; FH: Fishing Harbour; LH: Leisure Harbour; SH: Service Harbour (*in Google Earth*).

For the purpose of this study, we have chosen some areas inside and outside the Port of Sines for our samplings and experiments (**Figures 3-4**): ten inside the port – two artificial vertical seawalls and three breakwaters located between the Liquid Bulk and Petrochemical Terminals (hereafter SW1, SW2, B1, B2 and B3), the natural and artificial (breakwaters) substrates of the Leisure Harbour (LHn and LHa) and Service Harbour (SHn and SHa), and the Fishing Harbour (FH); and five beaches outside – one north of Sines (Cabo de Sines Norte-CSN), and four south (Vieirinha-VIE, Oliveirinha-OLI, Samouqueira-SAM, and Queimado-QUE). Breakwaters are heterogeneous in shape and age, and were made of concrete and concrete and sand. The vertical seawalls that support the jetties for the vessel's anchorage were built with reinforced concrete. The main structure of the port dates from early 1980s, having been amplified and restructured until 2003.

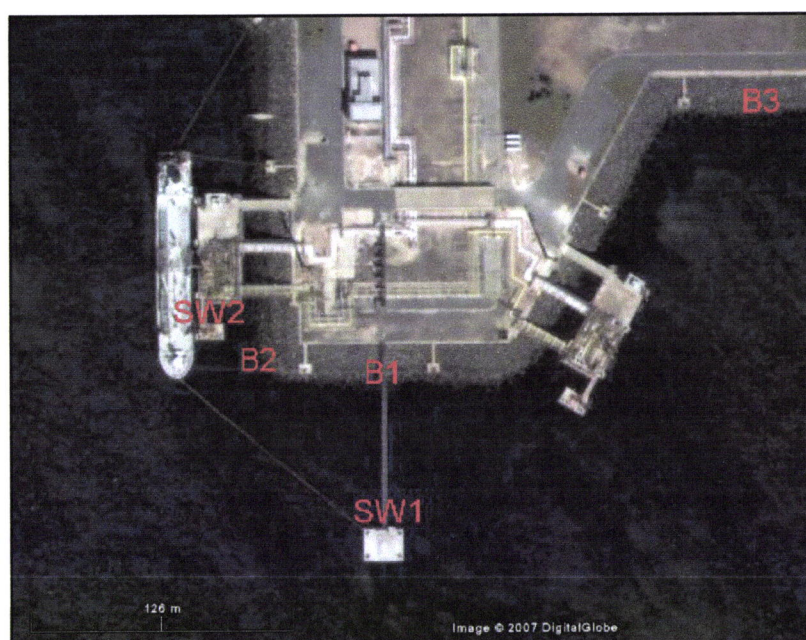


Figure 3. Breakwaters and vertical seawalls located inside the Port of Sines, between the Liquid Bulk and Petrochemical Terminals (*in Google Earth*).

Preliminary observations inside the port suggested that intertidal assemblages on seawalls are composed of fewer species than on breakwaters right beside, although patterns seem to vary among areas. In vertical seawalls much of the space becomes dominated by beds of *Mytilus galloprovincialis* intermixed with the algae *Enteromorpha* spp., *Codium adhaerens* and *Caulacanthus ustulatus*. Sessile animals are relatively common, like barnacles (chiefly *Balanus perforatus*), polychaetes, sponges, ascidians and bryozoans, as well as mobile animals, namely crabs (*Pachygrapsus marmoratus*). The boulders of breakwaters are overgrown by a biofilm, composed mostly by

Cyanophyceae (*Calothrix* spp. and *Oscillatoria* spp.), and some *Enteromorpha* spp. and *Caulacanthus ustulatus*. *M. galloprovincialis* is absent, but other animals are commonly found, such as barnacles (*Chthamalus* spp.), polychaetes, platyhelminths, limpets (mainly *Patella ulyssiponensis* and *Siphonaria pectinata*), the whelk *Melaraphe neritoides*, starfishes (*Marthasterias glacialis*), and crabs.



Figure 4. The artificial and natural substrates of the Leisure (LHa, LHn) and Service (SHa, SHn) Harbours, inside the Port of Sines (*in* Google Earth).

2.3.2 Patterns of distribution and abundance

The distribution and abundance of mussels were investigated during November and December 2005, with spatial variation being studied in eight areas inside the Port of Sines (SW1, SW2, B1, B2, LHn, LHa, SHn and SHa) and four outside (CSN, OLI, SAM and QUE). Sampling was done at spring tides, placing 6 randomly chosen 50×50 cm quadrats (2500 cm²) in the mid-intertidal mussel zone of two sites in each area (see Figure 5). In seawalls, the sea- and landward faces were sampled to maximise differences between exposure to sunlight and wave action. Two random sites (~15-20 m of distance) were selected in each area to unconfound variation among areas. Each replicate consists on a photograph of the quadrat area, avoiding rough and sediment surfaces, crevices and tidepools.

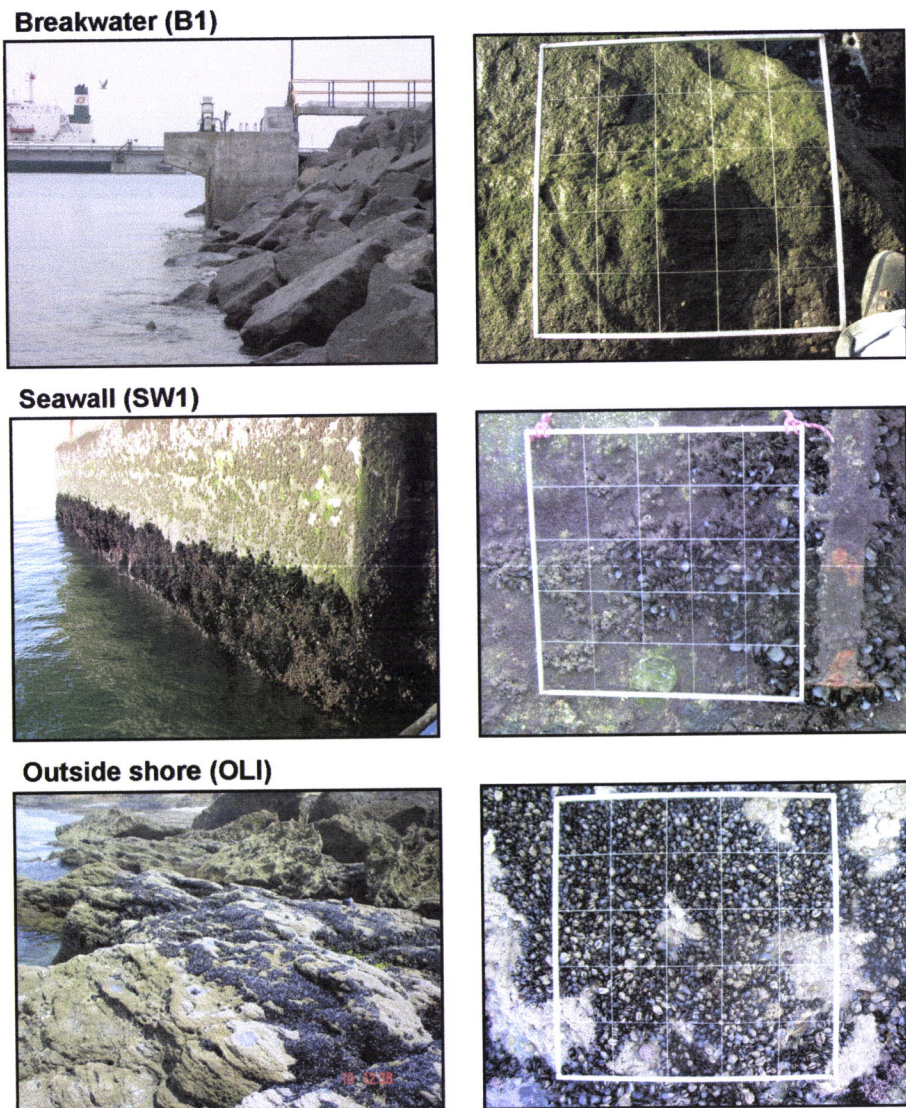


Figure 5. Examples of photo quadrats (50×50 cm) taken in a breakwater and a seawall inside the Port of Sines, and in an outside shore.

The question addressed in this study is: do initial observations represent the distribution and abundance of mussel populations living in this area? What we expect is that mussels are significantly abundant in areas outside the port and in seawalls inside; and in breakwaters and natural substrates inside the port they should be few or rare. Thus, a range of spatial scales were covered: meters (distance among replicate quadrats, and ~20 meters among sites), hundreds of meters (distance among replicate areas) and tens of kilometres (distance among inside and outside areas).

Despite the great amount of methods that can be used for the estimation of surface cover (e.g. Dethier et al., 1993; Benedetti-Cecchi et al., 1996; Pech et al., 2004), photographing a quadrat area, and then determining surface cover in the laboratory using a grid (photo quadrat), can present several advantages (reviewed in Pech et al., 2004). At an easily and relatively low cost way high quality digital cameras allow us to build up an extensive image data base for qualitative and quantitative studies, lowering the costs of monitoring programs.

To obtain reliable photographs, the camera was held perpendicularly to the quadrat frame covering the total screen of the digital camera, thus minimizing possible parallax errors. Photographs (3264×2448 pixel size) were transferred directly from the camera to the computer using JPG format, and photo analysis was performed following standard procedures of image analysis. Projecting the photographs on the computer screen using SigmaScan Pro 5 software (SPSS science 1999), cover was interactively determined by delimiting the edge boundaries of mussels (for an example see **Figure 6**). Abundance of mussels was quantified as cover per quadrat area (%). The spatial patterns of distribution and abundance were analysed in a two-way nested analysis of variance (ANOVA), with the random factor site (two levels) being nested under the main fixed factor area (twelve levels). GMAV5® for Windows (Underwood & Chapman, 1998) was used to carry out all ANOVAs. The assumption of homogeneity of variance was checked by Cochran's test, and the data were arc-sin transformed to stabilise variances. Student-Newman-Keuls (SNK) procedure was used to make post hoc comparisons among levels of significant terms.

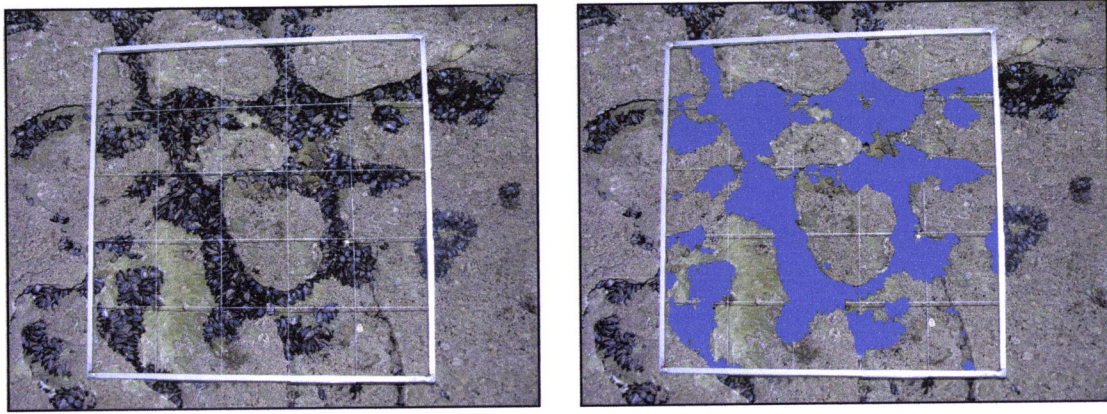


Figure 6. Example of an analysis of mussels coverage in a photo quadrat (50×50 cm) of an outside area (SigmaScan Pro 5 software).

2.3.3 Dimensional structure

The dimensional structure of mussels was studied in November 2005, taking into account that sampling was done in a way that reduces variability from exogenous factors to a minimum. Hence, all mussels were collected from a single clump (mussels attached to each other by byssal threads from an area of about 10×10 cm randomly selected) with a mussel collector, which consists of a small triangular iron frame in the tip of a long wooden stick. This study consisted in a way of knowing the length variability in areas inside and outside the Port of Sines, characterizing its structure among three different environments: intertidal and subtidal inside the port, and intertidal outside the port. Therefore, the hypothesis being tested is that mussels are different in areas inside and outside the Port of Sines, and that they are different among the two types of environments (intertidal vs. subtidal). Regarding this, two inside areas were chosen, an artificial intertidal seawall SW1 and a subtidal buoy anchored in FH; and four areas outside (CSN, VIE, SAM and QUE). Mussels were placed in a labelled plastic bag, returned to the laboratory, and frozen until they were processed.

After unfreezing, as suggest in Westerborn et al. (2002), mussels were distributed evenly on a water-filled tray sectioned in eight parts. Four sectors were randomly chosen, and mussels within each sector counted. In a quantitative way, adult mussels (>10 mm; n~200 ind.) randomly chosen were separated and their length (maximum anterior-posterior axis) measured with a Mitutoyo® digital pachymeter (accurate to 0.01 mm). Small mussels (<10 mm, n~100 ind., designed hereafter as “recruits”) were counted and measured separately under a dissecting microscope fitted with an ocular

micrometer (40× magnification and accuracy of 0.02 mm). In accordance with suggestions in Sokal & Rohlf (1995), abundance of adult mussels was categorised into eighteen 5 mm shell-length classes, and for recruits into five 2 mm classes, except the first one of 1 mm. Usually, adults are considered >30 mm, sub-adults 10-30 mm, recruits 1-10 mm, and settlers <1 mm (Lasiak & Barnard, 1995; Harris et al., 1998; McQuaid & Lindsay, 2000). Abundance was quantified as relative frequencies (%), obtaining length-frequency histograms for each area.

Non-metric multidimensional scaling (MDS) was used to produce two-dimensional ordinations of the rank orders of similarities among samples in the different areas. Thus, a matrix of similarities was calculated using the Bray-Curtis similarity coefficient on square-root transformed data. The contribution of each length class to average areas dissimilarity was determined with similarity percentage analyses (SIMPER), considering important differentiators the ones that contribute at least 10% to dissimilarity. This was done dividing the areas in two groups, one for the areas inside the Port of Sines, and the other outside areas. All non-parametric techniques for communities' analyses were done using PRIMER™ 5.2 software package (Plymouth Marine Laboratory, U.K.) according to Clarke & Warwick (1994). Additionally, for the recruits' dimensional structure, a one-way ANOVA was performed in order to identify the scale at which greatest significant variation occurred between the areas (fixed factor, 6 levels). For this analysis, only the first 72 individuals were considered as replicates, because in CSN the clump sampled didn't achieve the number predetermined. Cochran's test was used to test for homogeneity of variance, being significant without transformation, and differences between appropriate means were compared using an SNK test.

2.3.4 Manipulative experiment of predation

To test for the effect of predation on the distribution and abundance of mussels inside the Port of Sines, a manipulative experiment of transplanted was carried out between August and November 2005. This experiment was done in the intertidal level of mussel beds (+1.4 m above mean lower low water) with three treatments (four replicates each; see **Figure 7**): full cage (FC; -predation, +full cage), excluding all macro-predators; partial cage (PC; +predation, +partial cage), as a control for potential hydrodynamic influences of the cage; and no cage (NC; +predação, -cage), as a control to the manipulation. These cages were made of a 10 mm-mesh galvanized wire

with 8.75×7.5×5 cm (L×W×H). Partial cages excluded only the predators that can't enter through the sides opened, and, despite having the same dimensions, presented rectangular openings on two non-adjacent sides (6.25×2.5 and 5×2.5 cm) and on their top (6.25×5 cm). This wire is available in a variety of industrial suppliers, allowing the construction of robust and long-lasting structures that can withstand harsh field conditions with minimal maintenance (see Como et al., 2006; Miller & Gaylord, 2007).

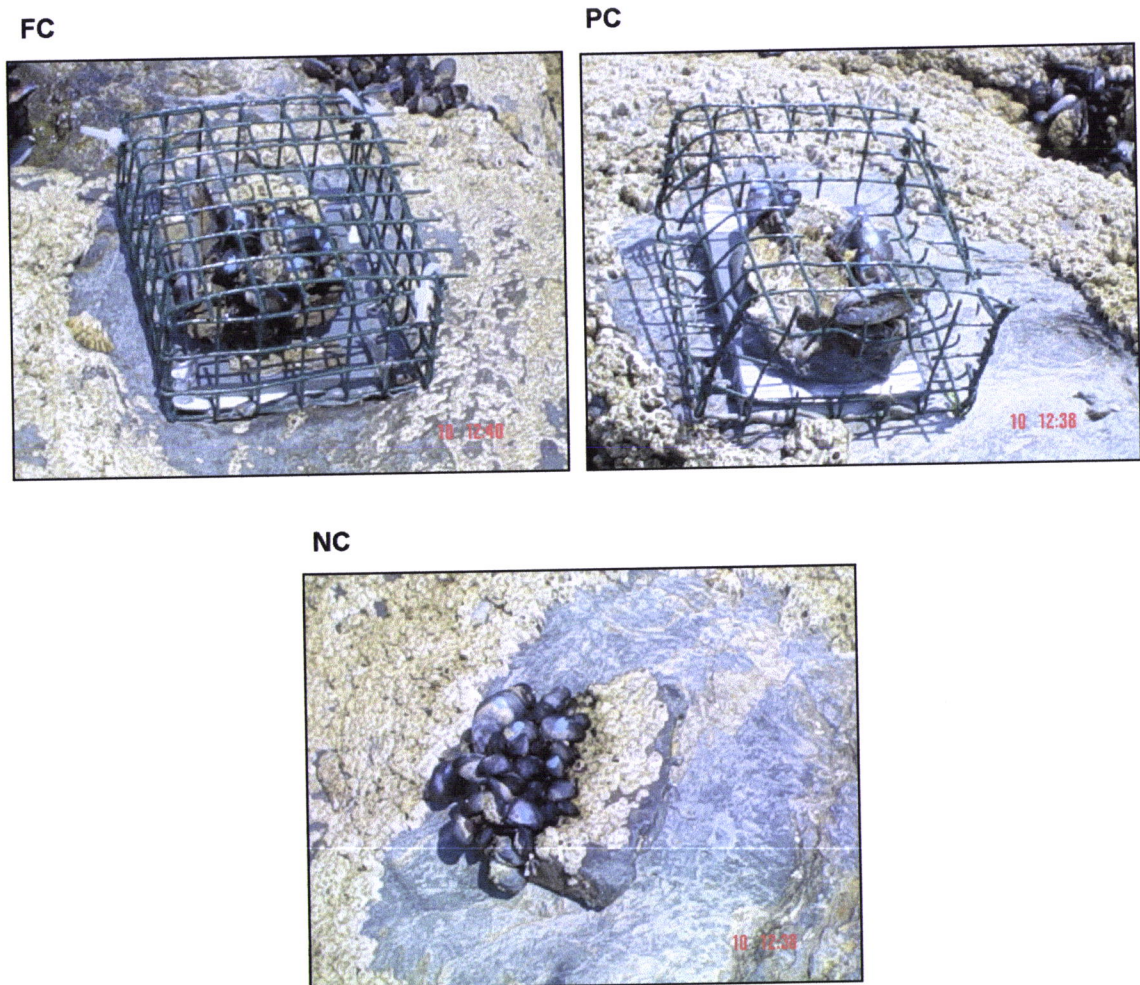


Figure 7. Example of one replicate of each treatment of the manipulative experiment of predation. FC: Full cage; PC: Partial cage; NC: No cage.

For the transplants, rock pieces (<math><100\text{ cm}^2</math>), covered with juvenile mussels (20-30 mm total length) firmly attached, were collected from an area outside the port (QUE), and returned to the laboratory. After dry with paper, they were glued to PVC plates (8×5.5 cm) with a non-toxic submarine epoxy (Underwater Wet Surface Repair Putty®, Devcon) and firmly attached to the bottom of cages with an underwater tape (Pattex® Power Tape). Each transplant contained 10 mussels marked with nail polish for

subsequent identification, along with additional ~10 unmarked mussels. After being held overnight in an aquarium supplied with running seawater, cages and plates were fixed with epoxy over bare scrapped rock in the low intertidal zone of horizontal breakwaters (B1 and B2) and vertical seawalls (SW1 and SW2) inside the port, as well as to the same place where they were collected (QUE, control area). Transplants were placed at random within naturally existing mussel beds in the seawalls and QUE, and at the same level on breakwaters.

Mussel survival was monitored three times a week in the first month, and between 20 to 30 days thereafter (until ca. 90 days of manipulative experiment). Survivorship was defined as the number of identified mussels that persisted in each sampling data until November. With this experiment we seek to determine the hypothesis of having a greater effect of predation in intertidal breakwaters comparing with vertical seawalls inside the Port of Sines, and similar pressure in seawalls and outside areas.

At the same time, TidBit™ loggers (Optic StowAway, Onset Computer Corporation; **Figure 8**) were placed in open rock, at similar tidal height as the experiments, to record water and air temperatures (during high and low tides, respectively). Each logger was programmed to continuously record temperatures throughout the experiment at a frequency of 1 reading/5 min, and had an accuracy and resolution of ~0.3°C. One was deployed on the horizontal surface of B1, and other at the center of a small mussel bed in the landward vertical face SW1. In each site, data-loggers were placed with epoxy away from algal canopies, rocky crevices or sources of shade, and stayed continuously from 8 August to 16 November 2005. From these records, mean low tide air temperatures were calculated at each site, defined as the mean of all readings during a period of two hours before and after each low tide. Standard deviations of mean temperatures at low tides were recorded as a measure of variability. The time of the tides was estimated using Sines' tide charts from Hydrographic Institute of Portugal. Sudden temperature drops (of at least 3°C) are diagnostic of the first instance of wave splash during the return of the tide (Fitzhenry et al., 2004; Blanchette et al., 2007), and were avoided every time they coincided with the period defined for the readings.



Figure 8. TidBit™ logger used during the manipulative experiment of predation.

The effect of predation on mussel survival was evaluated using a two-way ANOVA with two main fixed factors, area (five levels) and predation (three levels/treatments). The data were $\ln(x+1)$ -transformed and a Cochran's test was performed, as necessary to meet assumptions of normality and homogeneity of variance. Finally, SNK tests were used for posteriori comparisons of means.

2.3.5 Manipulative experiment of thermal stress

Since we noticed high divergence in the temperature registered by TidBit™ loggers during the manipulative experiment of predation, and regarding results in mussels' survival in this study, we performed an experimental manipulation of thermal stress in mussels from August to October 2006. The effect of temperature achieved by mussels, designed here as "thermal stress", was assessed inside the Port of Sines using always full cages (12.5×12.5×6.25 cm, L×W×H) and transplants similar to the manipulative experiment of predation. Four treatments were considered: three treatments in the breakwater B1 (see **Figure 9**) – shade (SH), procedural control (PC), which had all the features of the shaded treatment, except the shade itself, and undisturbed control (C) – ; and one treatment in a vertical seawall that consisted of an undisturbed control (the landward face of SW1 for the first 20 days, and until the end of the experiment in the seaward face of SW2 because of maintenance works). The main predictions were that unshaded mussels (control treatment, C) have greater survival in vertical seawalls than in horizontal breakwaters, and that shaded (SH) mussels in breakwaters have similar survival comparing to the ones in unshaded seawalls. In the procedural control treatment of the breakwater we expect to have a similar survival as the one of their unshaded mussels.

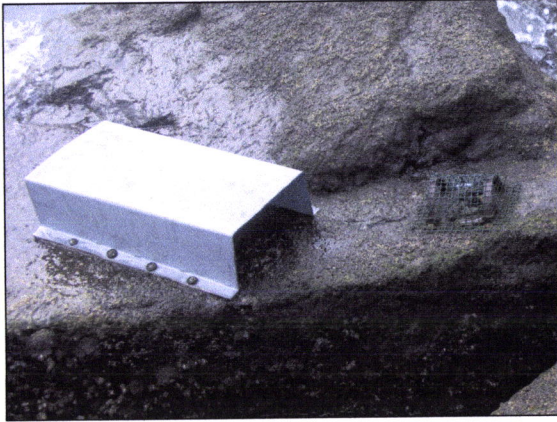
SH and C**PC**

Figure 9. Example of one replicate of each treatment of the manipulative experiment of thermal stress. SH: Shade; C: Control; PC: Procedural control.

Shading structures with 35×17.5×10 cm (L×W×H) were made of galvanized iron plates, ordered specifically to this study. The shade treatment was performed with fully structures of iron, but in the procedural control the top was opened and covered with 4 mm-thick transparent acrylic (32×13.5 cm) fixed with plastic cable ties through holes (30×11.5 cm) drilled into the acrylic and iron plate), in order to test for artifacts associated with the shading structure. The position of each replicate was chosen randomly, with at least 30 cm apart in order to keep them independent.

Like in the predation experiment, transplants were collected from QUE and all fixed to full cages as described above. However, in this experiment groups of 20 mussels were marked with nail polish for subsequent identification. In B1, cages and shading structures were fixed to the boulders with stainless steel screws and washers (after clearing the rock surface and drilling holes) to stabilize the structures; and in SW2 cages were glued with epoxy. To guarantee a better fastening to the substratum, shading structures had a reinforcement of 5 mm-thick black rubber washers in each hole. As after some time some biofilm settled in the acrylic, they were scrubbed twice with a brush in a way to maintain its transparency. Survivorship was defined as the number of identified individuals that persisted until the following monitoring date, being sampled in almost every week until the end of the experiment (ca. 60 days).

In an attempt to confirm the shade effect of the shading structures, thermal time series that approximated to surface temperatures at the three treatments were obtained with high resolution Thermochron® iButtons (temperature loggers from Dallas

Semiconductor; **Figure 10**). These loggers were attached inside one cage of each treatment with plastic cable ties, and programmed to record a single temperature measurement (with a resolution of 0.0625 °C) every 6 min from 12 August to 7 October 2006. With this programme planning, these ThermoChron® iButtons can stay in the field ~11 days, which means that at each sampling date temperature loggers were replaced. Similarly to the records of temperature during the predation experiment, mean low tide air temperatures at each site were calculated.

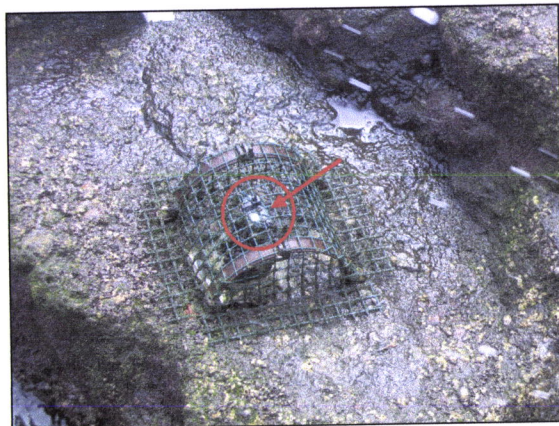


Figure 10. ThermoChron® iButton fixed in a control cage of the manipulative experiment of thermal stress.

The effect of thermal stress on mussel survival was examined with two one-way ANOVA. In the first one we analysed the first 20 days of sampling between B1 treatment and SW1 treatments, and after 20 days between B1 and SW2 (as replicates in the vertical SW1 were moved due to maintenance works). The factor analysed was thermal stress (fixed, four levels), considering the control cages of the seawalls as a fourth treatment. Homogeneity of variance was checked by Cochran's tests with non-transformed data, and SNK procedure was used to identify possible differences among treatments.

2.3.6 Recruitment

To test for potential differences between settlement inside and outside the Port of Sines, recruitment of mussels (*Mytilus* sp.) was measured with plastic mesh dish scrubbers (SOS Tuffy pads, The Clorox Company, Oakland, California, USA) fixed to the rock at each location. These devices mimic the physical structure of filamentous substrata, such as finely branched algae and mussel byssal threads, surfaces

apparently preferred for settlement by mussel larvae (Suchanek, 1978; McGrath et al., 1988; King et al., 1989; Lutz & Kennish, 1992; Seed & Suchanek, 1992; Cáceres-Martínez et al., 1993, 1994; Menge et al., 1994; Hunt & Scheibling, 1996; Pulfrich, 1996; Connolly et al., 2001; Menge et al., 2004; Broitman et al., 2005).

Four replicate tuffies were fastened to the mussels' natural mid-zone of QUE, CSN and SHn, and, at the same tidal level, to artificial boulders of B3 with stainless steel screws and washers inserted in holes drilled into the rock. Other four tuffies were fixed with plastic cable ties into a ladder of the seaward face of SW2. Replicates were spaced ~20-30 cm apart, avoiding rough and sediment surfaces, crevices and tidepools. The purpose of this study was to address the question of recruitment limitation as an explanation for the absence of mussels in breakwaters inside the Port of Sines. We therefore test the hypothesis of having greater recruitment in seawalls comparing with breakwaters inside the port, and that there are no differences in recruitment between seawalls and areas outside the port.

Additionally, in order to study the effects of predation on newly settled recruits as suggest by Smith & Witman (1999), two tuffies in B1 and B2 were caged, along with additional two non caged tuffies, using the same full cages of the manipulative experiment of predation. Pictures representative of replicates of each treatment are represented in **Figure 11**. Each tuffie was left in place for one month, and then returned to the laboratory in plastic bags and frozen until they were processed. The period of sampling was approximately two months, from 27 June through 24 August 2006, but cages were left in the field for 11 months. This two months were chosen for this experiment because a peak of settlement for *M. galloprovincialis* is usually recorded in summer months (north of Spain: Cáceres-Martínez et al., 1993, 1994, Molares & Fuentes, 1995 and Cáceres-Martínez & Figueras, 1998; Mediterranean: de Vooy, 1999). However, cages were left in the field for 11 months in order to test the

Recruits, as well as larvae and sediment, were rinsed from tuffies for several minutes with a moderate flow of fresh water into two jointed sieves (63 and 500 μm). Repeated testing in McCulloch & Shanks (2003) indicated that rinsing removed essentially all organisms within the tuffies, being the use of bleach to dissolve the byssal attachments (see Menge et al., 2004; Rilov & Schiel, 2006) useless. The contents of each fraction were transferred to a labelled jar and maintained in a freezer. These sieves were chosen due to the distinction between primary ($\leq 500 \mu\text{m}$) and secondary settlers (501-

2000 μm ; Gilg & Hilbish, 2000, 2003a,b). The largest fraction ($>500 \mu\text{m}$) of organisms was identified to species when possible under a dissecting microscope and counted; however, as *Mytilus* species are visually indistinguishable at this stage, they were all considered as *M. galloprovincialis*. For the fraction retained in the 63 μm sieve, despite having some shapes already different, all mytilids counted were pooled together as it is almost impossible to separate recently recruited mussels. A variety of recruits were recovered from the tuffies, including other mytilid species (*Mytilaster minimus* and *Musculus costulatus*) and bivalves (*Hiatella arctica*, *Lasaea rubra*, *Ruditapes* spp. and *Tapes* spp.). Densities of recruits are expressed as the monthly averages of the number of individuals collected per tuffy area when opened and spread flat (730 cm^2). Since one tuffy was lost in one place at August, the mean from the remaining three was used for the missing value.

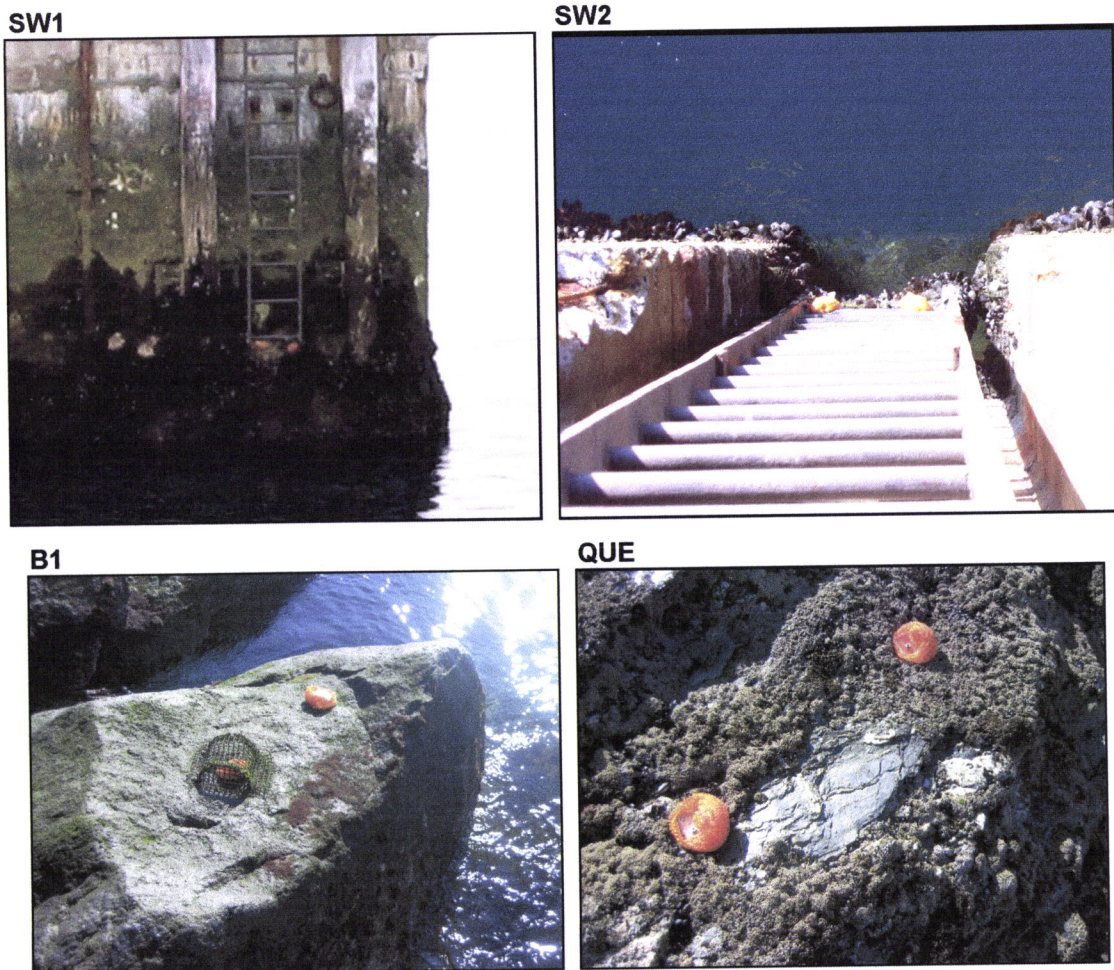


Figure 11. Example of replicate tuffies fixed in the seawalls with plastic cable ties, and to the boulders of breakwaters and rocks with stainless steel screws. SW1, SW2, B1: vertical seawalls and a breakwater located inside the Port of Sines; QUE: Queimado.

Despite caging usually increases settlement rate and/or early post-settlement survival (Petraitis, 1991; Hunt & Scheibling, 1997), cages had no effect in the number of recruits (in Appendix). Therefore, we considered all four replicates in each site as replicates of each area for the analysis of variance. Mussel recruitment (separated in two fractions and two months) was analyzed using one-way ANOVA to test for differences in numbers of settled mussels among areas. In both fractions, data from July were found to be heterogeneous from a Cochran's test, so data were transformed ($\ln(x)$ and square-root) and performed an SNK test; for August no transformation was needed.

2.4 RESULTS

2.4.1 Patterns of distribution and abundance

Cover varied between 0 and 59%, with total absence of *Mytilus galloprovincialis* in the breakwaters inside the Port of Sines (see Figure 12). Artificial vertical seawalls achieved percentages between 34 and 52%, and outside areas between 24-59%. The natural substrates inside the port presented 5 to 22% of mussel cover. In general, there was a large spatial heterogeneity in abundance of *M. galloprovincialis* among areas, what was confirmed by the statistical significance of the main factor area in the nested ANOVA ($p < 0.0001$; Table 1). Factor site was not significant, and the SNK test confirmed that all breakwaters are similar and also that the natural substrate of the Service Harbour (SHn) is different from the other areas.

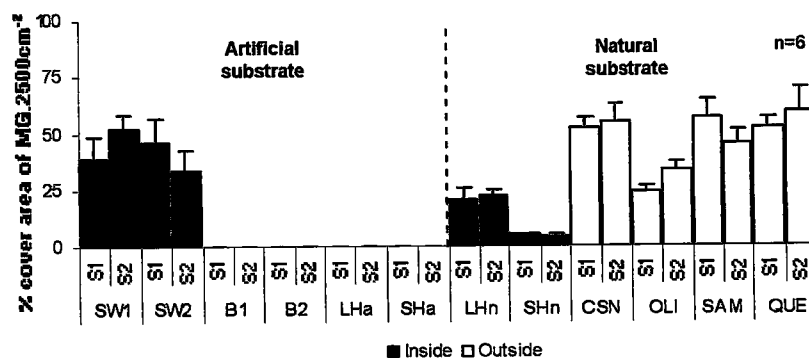


Figure 12. Cover area (%) of *Mytilus galloprovincialis* (MG) in two sites (S1 and S2) of twelve sampling areas inside and outside the Port of Sines. SW1, SW2, B1, B2: vertical seawalls and breakwaters located inside the port; LHa/n, SHa/n: artificial and natural substrates of the Leisure and Service Harbours; CSN: Cabo de Sines Norte; OLI: Oliveirinha; SAM: Samouqueira; QUE: Queimado.

Table 1. ANOVA and SNK test of the cover area of *Mytilus galloprovincialis* on quadrats of 2500 cm² in two sites of twelve sampling areas. *N*=6.

ANOVA					
Source of variation		d.f.	MS	F	P
Area	=ar	11	5071.08	78.35	0.0000
Site (ar)	=si(ar)	12	64.72	0.89	0.5611
Residual		120	72.88		
Total		143			
Cochran test	C=0.1456 (n.s.)				
Transformation	ArcSin (%)				
SNK					
	ar				
	n.p.d.>SHn>B1=B2=LHa=SHa				

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s. or =) - no significant differences, $p > 0.05$; n.p.d. - no pattern defined of variation; (>) - significant differences, $p < 0.05$.

2.4.2 Dimensional structure

The dimensional structure of adult and recruit mussels sampled in six areas inside and outside the Port of Sines is represented in **Figure 13**. Adult mussels of outside areas showed significantly shorter length of shell (individuals <55 mm) than mussels living inside the port, where SW1 has 9% of mussels >55 mm, and FH 7%. The area which presented the biggest individuals measured was the Fishing Harbour (FH), the only subtidal area sampled. This implies that in the MDS ordination (**Figure 14**) we have clearly two groups, one from areas inside the port and other from the outside areas. This representative illustration is like a variability axis, with the largest variability in SW1 and the less in QUE. The SIMPER test for areas resulted in 83% of similarity for the group of inside areas, and 76% for the outside. The main contributors for the percentage dissimilarity among both (22%) were the individuals between 10-15 mm and 21-25 mm (for areas outside 35% and 18%, and for areas inside 36% and 10%, respectively). The average contribution of 10-15 mm class was 12%, and for individuals from 21-25 mm 21%.

In terms of recruits, the length-frequency distribution doesn't have a well defined pattern, but we can see that in FH, median classes had similar abundances, and that in QUE the most abundant classes are the two first. In the areas SW1, CSN, VIE and SAM, the great majority of recruits belong to the second class; but in CSN the first class has fewer individuals, and the last class more, than the other areas. This pattern is very well represented in the MDS graph, where only the recruits of SW1, VIE and SAM appear grouped. In terms of analysis of variance represented in **Table 2**, the FH recruits are equal to the ones from CSN, and significantly bigger from a group formed

by the other four areas. The SIMPER analysis for areas showed that the inside areas have 76% of similarity, and the outside 74%, being the main contributors for their dissimilarity (24%) the individuals from the two first shell-length classes, 0.1-2.0 mm and 2.1-4.0 mm (for areas outside 22% and 40%, and for areas inside 13% and 33%, respectively). The average contribution of 0.1-2.0 mm class was 29%, and for individuals from 21-25 mm 22%.

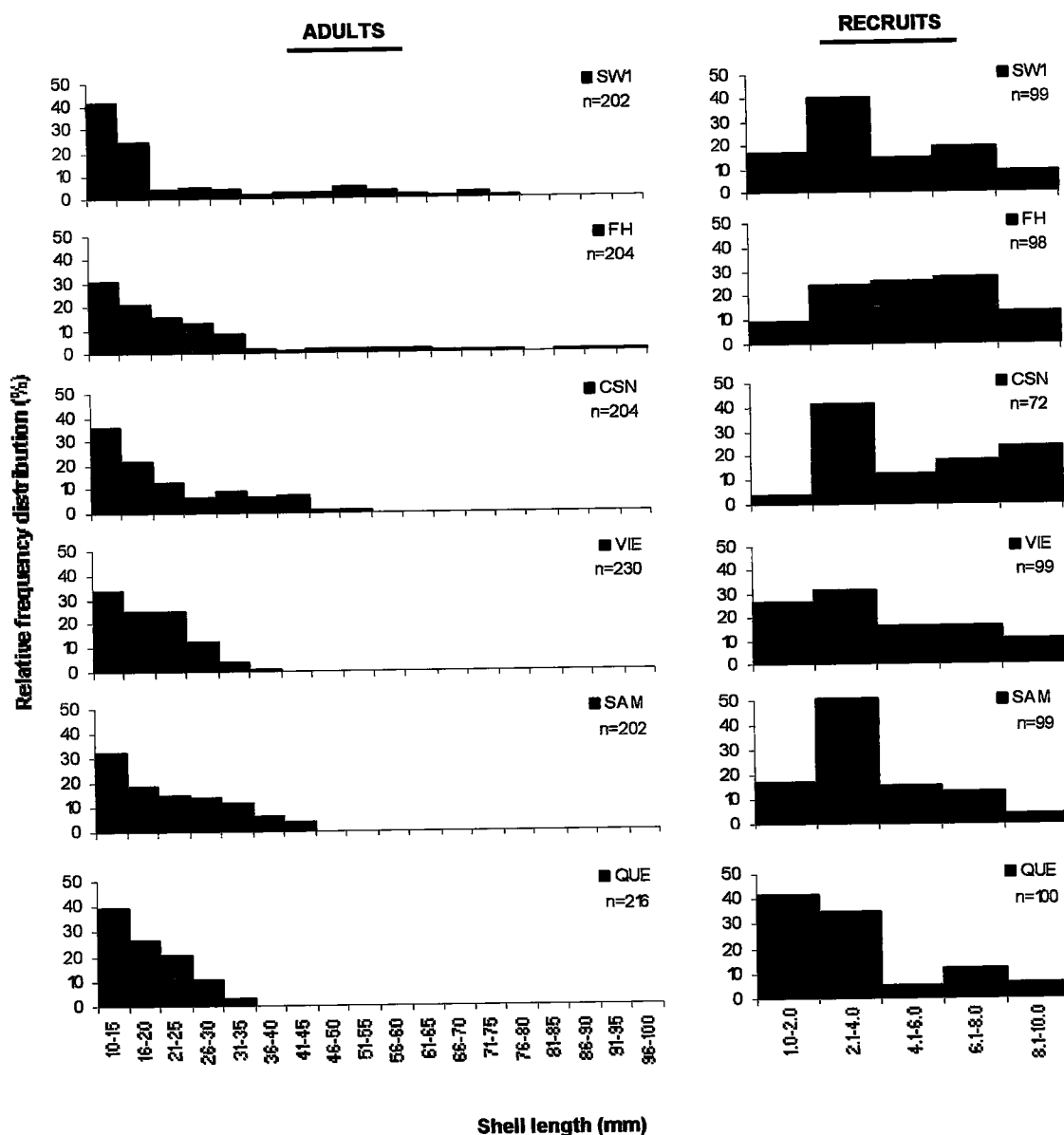


Figure 13. Length-frequency distributions (in %) of *Mytilus galloprovincialis* adults and recruits at low intertidal level of five areas, and subtidally in one (FH), inside and outside the Port of Sines. The values of *n* are the total number of individuals sampled in each area. SW1: vertical seawalls located inside the Port of Sines; FH: Fishing Harbour; CSN: Cabo de Sines Norte; VIE: Vieirinha; SAM: Samouqueira; QUE: Queimado.

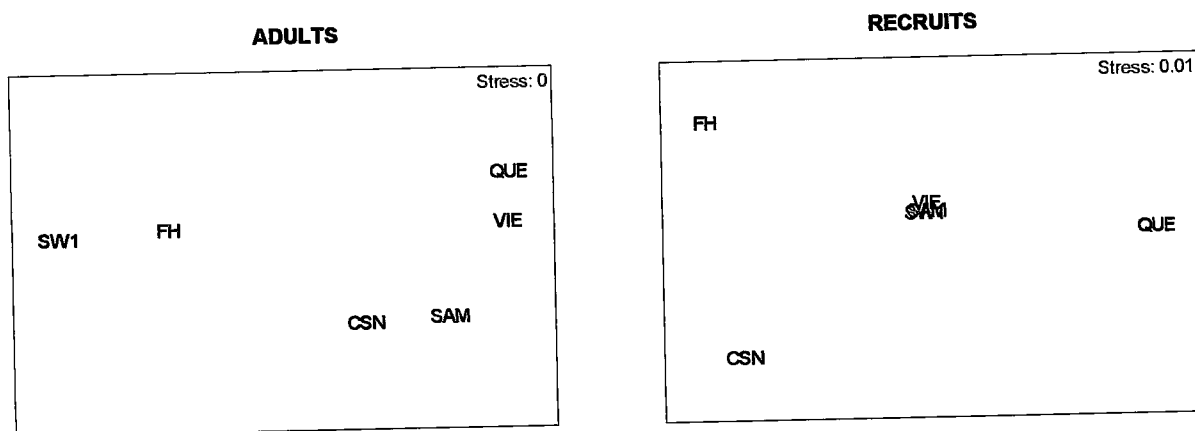


Figure 14. Dimensional structure of *Mytilus galloprovincialis* adults and recruits: ordination of sampling areas by MDS of the shell-length classes (Bray-Curtis coefficient and square-root transformed data).

Table 2. ANOVA and SNK test of the length-frequency distributions of *Mytilus galloprovincialis* recruits in six sampling areas. $N=72$.

ANOVA					
Source of variation		d.f.	MS	F	P
Area	=ar	5	43.69	7.42	0.0000
Residual		426	5.89		
Total		431			
Cochran test	C=0.1917 (n.s.)				
Transformation	No transformation				
SNK					
	ar				
	FH=CSN>SW1=VIE=SAM=QUE				

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance ratio; P - probability; (n.s. or =) - no significant differences, $p>0.05$; (>) - significant differences, $p<0.05$.

2.4.3 Manipulative experiment of predation

In the manipulative experiment of predation of mussels inside and outside the Port of Sines, we registered along the time a decrease in the number of mussels alive in all areas (Figure 15). Until 25 days, transplants in the outside area (QUE) have greater survival than the ones of inside the port, but in the end of the experiment the number of mussels alive is similar to the ones of seawalls (SW1 and SW2). Despite having a quick mortality in seawalls (as PC and NC treatments decrease sharply in the beginning), they seem to persist longer than in breakwaters' replicates (FC treatments in SW1, SW2, B1 and B2). Therefore, predation seems to have a different effect in these two types of areas inside the port.

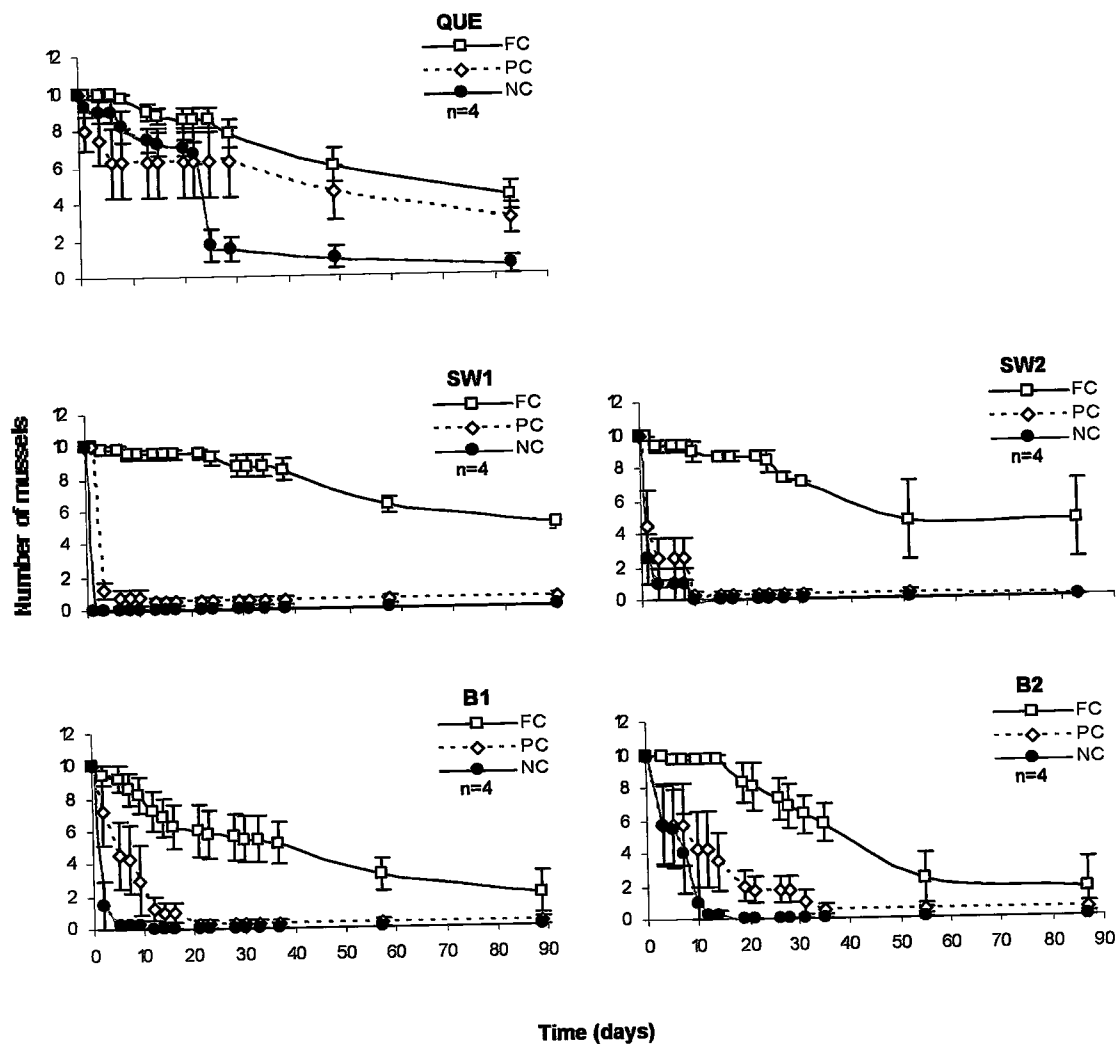


Figure 15. Number of mussels (mean values and standard errors) observed in the three predation treatments (FC – full cage; PC – partial cage; NC – no cage) of the experiment performed to test the survival along the time in different areas. QUE: Queimado; SW1, SW2, B1, B2: vertical seawalls and breakwaters located inside the Port of Sines.

The analysis of variance to factors areas and predation (Table 3) resulted in a non-significant interaction, with significant differences for each factor. This means that the areas are different, and that the effect of predation is important for mussels' survival. SNK tests hadn't revealed a defined pattern of variation for the factor area, but survival was greater in the outside area (QUE) and lower in breakwaters inside the Port of Sines. For predation SNK tests showed the following pattern: FC>PC>NC.

Table 3. ANOVA and SNK tests to the survival of mussels after ca. 90 days of manipulative experiment of three predation treatments at five sampling areas. $N=4$.

ANOVA					
Source of variation		d.f.	MS	F	P
Area	=ar	4	1.20	4.29	0.0050
Predation	=pr	2	7.63	27.22	0.0000
ar x pr		8	0.52	1.87	0.0893
Residual		45	0.28		
Total		59			
Cochran test		C=0.2571 (n.s.)			
Transformation		Ln (x+1)			
<hr/>					
SNK	ar	pr			
	n.p.d.	FC>PC>NC			

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s.) - no significant differences, $p>0.05$; n.p.d. - no pattern defined of variation; (>) - significant differences, $p<0.05$.

The mean temperature registered in the diurnal low tide periods along the experiment is represented in Figure 16, being notorious that the values achieved in the breakwater analysed is far superior to the ones from the seawall. In various periods of time, the maximum daily temperature achieved values superior to 38°C (the maximum temperature measured by this type of thermometers) in the breakwater, and only the maximum of 25°C was registered in seawalls.

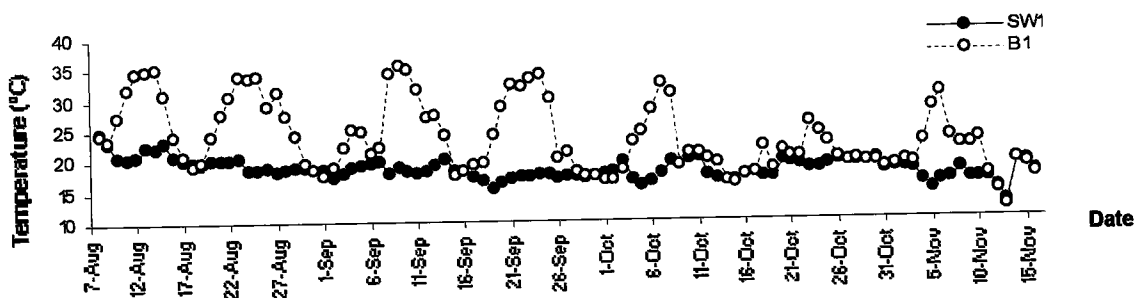


Figure 16. Mean temperature in diurnal low tide periods (temperatures two hours before and after the point of maximum low tide) along the time of predation experiment in two areas inside the Port of Sines, a vertical seawall (SW1) and a breakwater (B1).

2.4.4 Manipulative experiment of thermal stress

For the manipulative experiment of thermal stress in mussels inside the Port of Sines, we also registered their survival along the time, as is represented in Figure 17. All the thermal stress treatments presented losses of mussels, being notorious the difference between the survival in the control (C) treatment of the breakwater B1 and the seawalls (SW1 and SW2). In the breakwater, it seems that the number of mussels decreases

similarly in the three treatments along the experiment. The analysis of variance to the four treatments resulted in significant differences (Table 4), but the SNK test hadn't defined a pattern of variation among them. However, survival seems greater in seawalls than in all treatments of the breakwater studied, being the control treatment the one which presents greater mortality of mussels in this horizontal surface.

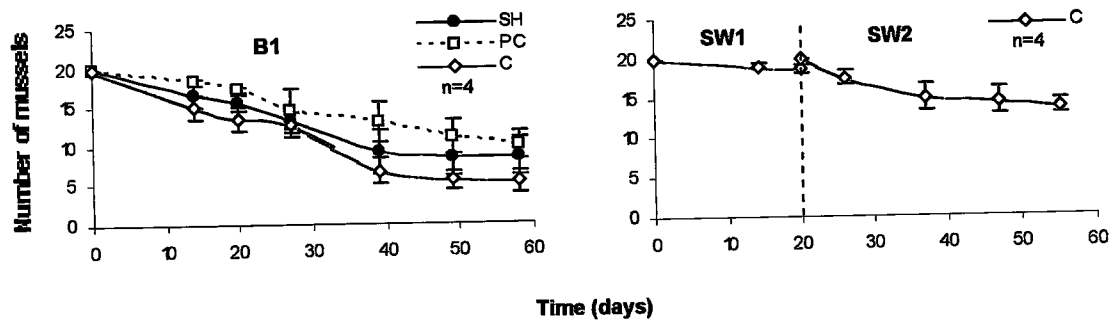


Figure 17. Number of mussels (mean values and standard errors) observed in the three thermal stress treatments (SH – shade, PC – procedural control and C – control) of the experiment performed to test the survival along the time in two areas inside the Port of Sines, a breakwater (B1) and two vertical seawalls (SW1 and SW2).

Table 4. ANOVA and SNK test to the survival of mussels after ca. 60 days of manipulative experiment of four thermal stress treatments inside the Port of Sines. $N=4$.

ANOVA					
Source of variation		d.f.	MS	F	P
Thermal stress	=ts	3	49.75	3.97	0.0354
Residual		12	12.54		
Total		15			
Cochran test	C=0.4850 (n.s.)				
Transformation	No transformation				
<hr/>					
SNK	ts n.p.d.				

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s.) - no significant differences, $p>0.05$; n.p.d. - no pattern defined of variation.

Although only full cages were used in this experiment, caging seem to had no effect on mussels growth inside the port, since in the end of the experiment all individuals left inside full cages in seawalls had grown considerably, almost fulfilling its total volume.

In terms of temperature, we can see in Figure 18 that the SH treatment resulted in less values of temperature registered by ThermoChron® ibuttons in the diurnal low tide

periods along the experiment (maximum of 23°C). Unlike the temperatures registered in the predation experiment, in various periods of time the mean temperature achieved in SW2 are not so different from the ones recorded for the two control treatments in B1 (C and PC). The maximum temperature registered for SW2 was 38°C, while in C was 36°C and PC 34°C.

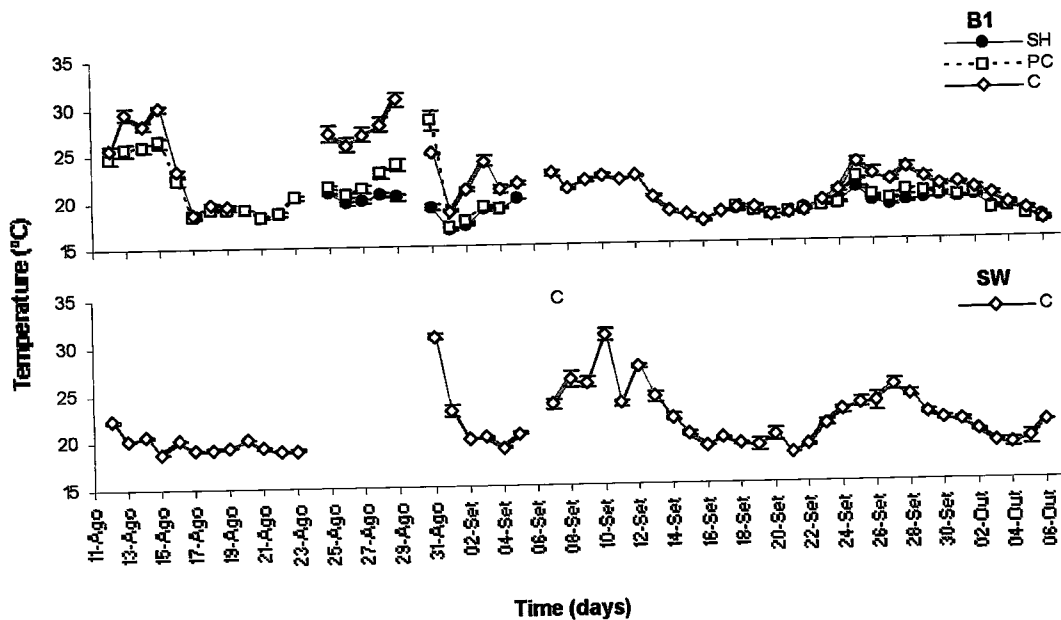


Figure 18. Mean temperature in diurnal low tide periods (temperatures two hours before and after the point of maximum low tide) along the time of thermal stress experiment in two areas inside the Port of Sines, a breakwater (B1) and a vertical seawall (SW1 in the first 20 days, and SW2 until the end of the experiment).

2.4.5 Recruitment

The recruitment in July and August 2006 of *Mytilus galloprovincialis* and other mytilids in seven areas inside and outside the Port of Sines is represented in Figure 19. There was less recruitment of *M. galloprovincialis* in the artificial substrates of SW2, B1 and B2, except for B3 in the month of July where the recruits achieved values similar to QUE. The natural substrate of the SH had similar medium values of recruitment for both months; and the outside areas achieved the biggest values of recruitment, except for CSN in the month of July. The mytilids recruitment was notably bigger in August than July, with the artificial and natural substrates inside the port having the higher numbers of recruits. Opposite to *M. galloprovincialis*, for mytilids the areas outside the port have few recruits in the two months.

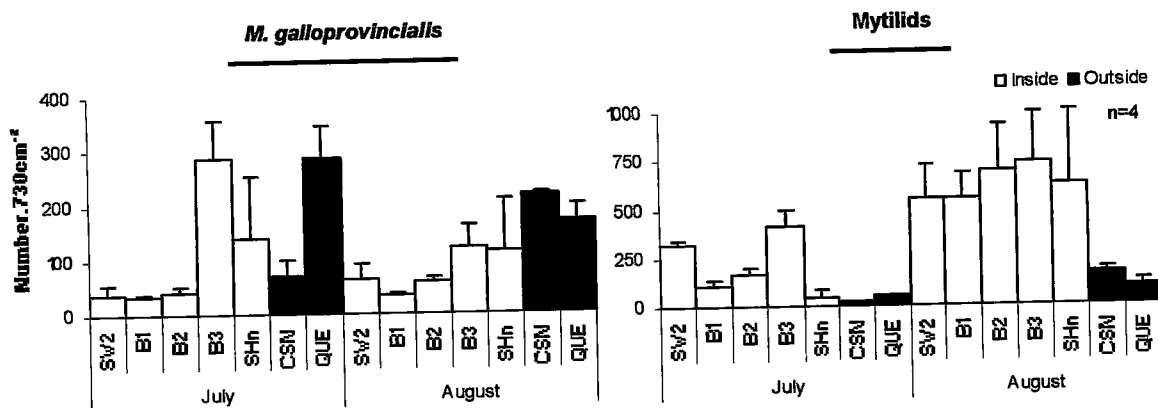


Figure 19. Number of *Mytilus galloprovincialis* and mytilids recruits (mean values and standard errors), retained in different sieves (500 and 63 μm -mesh, respectively), by an area of 730 cm^2 of SOS Tuffy pad in seven sampling areas inside and outside the Port of Sines. SW2, B1, B2, B3: vertical seawall and breakwaters located inside the Port of Sines; SHn: natural substrates of the Service Harbour; CSN: Cabo de Sines Norte; QUE: Queimado.

In terms of ANOVAs (Table 5), only July had significant differences in the main factor area for both *M. galloprovincialis* and mytilids. The SNK tests defined the following patterns: for *M. galloprovincialis* all the areas are equal, and for mytilids recruits, $B3=SW2 > \text{n.p.d.}$. However, SW2 apparently has less number of recruits than QUE in July.

Table 5. ANOVAs and SNK tests to the recruitment of *Mytilus galloprovincialis* and mytilids in seven sampling areas inside and outside the Port of Sines. $N=4$.

ANOVA: <i>M. galloprovincialis</i>			July		August			
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar	6	3.71	3.54	0.0140	4384.45	0.63	0.7051
Residual	21	1.05			6965.73			
Total	27							
Cochran test		C=0.3551 (n.s.)				C=0.7149 (n.s.)		
Transformation		Ln (x)				No transformation		
SNK	ar	SW2=B1=B2=B3=SHn =CSN=QUE						
ANOVA: Mytilids			July		August			
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar	6	151.74	19.57	0.0000	255641.29	1.39	0.2644
Residual	21	7.75			183853.10			
Total	27							
Cochran test		C=0.3610 (n.s.)				C=0.4501 (n.s.)		
Transformation		Sqrt (x+1)				No transformation		
SNK	ar	B3=SW2 > n.p.d.						

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s. or =) - no significant differences, $p > 0.05$; (>) - significant differences, $p < 0.05$; n.p.d. - no pattern defined of variation.

After 11 months, the cages used to test the effect of predation on newly settled recruits were almost full colonized by mussels in both breakwaters studied (see **Figure 20**).



Figure 20. Full cage used to see the effect of predation on newly settled recruits after 11 months left in a breakwater inside the Port of Sines. In this picture the cage was removed and placed in the right side in order to reveal the area covered.

2.5 DISCUSSION

2.5.1 Patterns of distribution and abundance

Cover varied among areas inside and outside the Port of Sines, achieving similar percentages in vertical seawalls inside the port comparing with outside areas. As it was expected, in breakwaters mussels were absent, and in natural substrates inside the port mussels were less abundant than in natural shores in outside areas. The coverage of defence structures by epifaunal invertebrates have been extensively documented, depending on both density and individual body size (e.g. Seed & Suchanek, 1992; Petraitis, 1995). These intertidal communities are generally composed by few species, being epibiota dominated by large dispersal range taxa (Bacchiocchi & Airoidi, 2003; Chapman, 2003). Not surprisingly, *Mytilus* and *Enteromorpha* spp. were the dominant species on vertical seawalls inside the Port of Sines, as both are characterised by larvae and propagules that disperse over long distances (Ceccherelli & Rossi, 1984). However, the null existence of mussels in breakwaters is more difficult to ascertain, being much of its space occupied by barnacles and biofilm in this port.

Intrinsic properties of the surfaces have been reported as causes that generate different assemblages in artificial surfaces like breakwaters (e.g. Chapman, 2003;

Blockley, 2007). Hence, they do not function as complete analogues of natural rocky substrata (see Bulleri & Chapman, 2004 for a review), which suggests that in some systems surfaces do not provide suitable habitats for a number of taxa. One explanation could be the existence of competition for space with macroalgae, as suggested in Witman & Dayton (2001) and references therein. However, this is not the case of the Port of Sines as its artificial substrates aren't dominated by them.

Otherwise, complexity and heterogeneity of habitat structure in hard-man made surfaces can play an important role structuring epibenthic assemblages by affecting a variety of ecological processes (Hawkins & Hartnoll, 1983; McGuinness, 1989; Archambault & Bourget, 1996; Thompson et al., 1996; Glasby, 2000). This variation is mostly attributable to different structural components of the surface, particularly microhabitats (pits, depressions, cracks, crevices, overhangs, rock-pools, etc.) that retain water during low tide and provide refuge and shelter for sessile invertebrates (Underwood & Denley, 1984; Littorin & Gilek, 1999; Bertness et al., 2002; Chapman, 2003; Chapman & Bulleri, 2003; Bulleri & Chapman, 2004; Moschella et al., 2005). Although in the Port of Sines artificial substrates cemented with concrete may have some shallow cracks, microhabitats such as rock-pools are absent, except in very few places where the surfaces are degraded and have slumped. This could be determinant for mussel colonization in areas inside this port, as in such crevices and depressions (irregularities on substrate topography) of its natural substrates greater abundances of mussels were evident (personal observations).

Along with these, a variety of other factors determine the large-scale spatial variability and composition of intertidal communities, as for example the slope and orientation of the substratum. Steep slopes, as the ones in vertical seawalls, contrast with the near horizontal or more gently sloping breakwaters, a major determinant of its structure (Leichter & Witman, 1997; Benedetti-Cecchi et al., 2000; Glasby, 2000; Connell, 2001; Witman & Dayton, 2001; Chapman & Bulleri, 2003); and as a consequence, intertidal area available extends 10s of metres in rocky shores, and just a fraction of ca. 2 m in seawalls (Buschbaum & Saier, 2001; Chapman, 2003; Chapman & Bulleri, 2003). This may limit the number of species living on it. Other surface characteristics, like the material, texture, size, age, and interfacial alkalinity (namely in concrete surfaces), have been found to influence the species and relative abundance of fouling organisms on artificial surfaces (McGuinness, 1989; Connell & Glasby, 1999; Glasby, 1999a, 2000; Witman & Dayton, 2001; Chapman & Bulleri, 2003) and should be considered.

Differences between assemblages on seawalls and breakwaters may also result from interactive effects of different habitat structure, depositional processes, and local water motion. Water flow and turbulence are likely to vary among rocky shores and artificial structures, influencing mussels in a range of ways, primarily in its recruitment and food supply, and hence in its growth rate and condition (Raubenheimer & Cook, 1990; Hunt & Scheibling, 1996; McQuaid & Lindsay, 2000; Branch & Steffani, 2004; Bulleri & Chapman, 2004). As reported by Bulleri (2005b) for other harbour, seawalls may experience more intense hydrodynamic forces from breaking waves throughout the tide, being higher biomasses of mussels frequently recorded at more exposed environments due to higher water turnovers and consequent increase in the supply of food (Bustamante & Branch, 1996; Bacchiocchi & Airoidi, 2003). In breakwaters, particularly at mid to high tidal levels due to their limited vertical extension, waves pass over the tops of vertical ledges possibly affecting turbulence and flow of the water in the vicinity of its substratum (Denny et al., 2003), enhancing the lift and drag forces (Witman & Suchanek, 1984; Denny, 1987; Bell & Gosline, 1997; Gilek et al., 2001). Although important, wave exposure was not measured in this study, but it seems that hydrodynamics is not the major determinant for the different patterns of distribution and abundance of mussels observed inside the Port of Sines.

2.5.2 Dimensional structure

In the study of dimensional structure of mussels, intertidal samples with high biomass outside the Port of Sines consisted of small and young mussels (mono-layers); while high biomass subtidal and intertidal samples within a buoy and a seawall inside the port housed considerable amounts of large and old mussels (usually bi- or multi-layered matrices). This results support our initial hypothesis of having differences in dimensional structure of mussels inside and outside the port. As suggested by some authors for another areas (e.g. Witman & Suchanek, 1984; Gardner & Skibinski, 1991; Hunt & Scheibling, 2001; Carrington, 2002a,b; Steffani & Branch, 2003), this is likely a reflection of moderate recruitment and slow growth in areas with stronger hydrodynamic forces, which generally increase demands for byssus production and shell thickness, and reduces the energy available for growth. Also, heavy predation can influence the size structure of mussel populations, being noticeable that mussels achieve lower maximum sizes where predation is high (Suchanek, 1978). Both explanations can be considered for the areas studied outside the Port of Sines, as these are areas exposed to great wave action and that have a high predation pressure.

Mussels inside harbours are generally characterized by a rapid growth at seawalls, probably facilitated by the relative absence of large predators and a potential increase in feeding time and protection by the densely packed communities (Buschbaum & Saier, 2001; Blanchette et al., 2007). Therefore, this can decrease the metabolic cost of byssus production, allowing more energy to be invested in growth and reproduction (Zardi et al., 2006a). However, other studies recorded a faster growth and better physiological conditions in exposed compared to sheltered situations (Bustamante & Branch, 1996; Dahlhoff & Menge, 1996; Leichter & Witman, 1997; McQuaid & Lindsay, 2000; McQuaid et al., 2000; Steffani & Branch, 2003; Branch & Steffani, 2004; Westerbom & Jattu, 2006; McQuaid & Lindsay, 2007). Hence, this comparison between growth in inside and outside areas of the Port of Sines is difficult due to variations in time and space, and also to the degree of interaction that can occur between recruitment, size, growth, density and biomass (as suggested in McQuaid & Lindsay, 2007).

Other important factor to consider is shoreline configuration, which as been described as one of the main modifiers of the hydrodynamic regimes over large spatial scales, which can in turn influence the distribution of phytoplankton and zooplankton (food supply) to shallow and subtidal filter feeders (Archambault & Bourget, 1996, 1999). For example, shoreline irregularities (such as embayments) are extremely important as they could entrap inert particles and nutrients (presumably because of longer retention times) and increase the local abundance of suspended particles in the water column (Archambault & Bourget, 1996, 1999). This enhances the growth, larval retention and settlement of filter feeders (Petraitis, 1991; Alvarado & Castilla, 1996; Gilek et al., 2001; Shanks et al., 2003; Branch & Steffani, 2004), and could be the main factor that determines the greater dimensional structure of mussels inside the Port of Sines. Food availability is thus considered by some authors as the single most important environmental variable regulating growth rates, increasingly substantially with time of submergence (or duration of air exposure in subtidal vs. intertidal environments; Seed & Suchanek, 1992; Buschbaum & Saier, 2001; Blanchette et al., 2007). Probably this is the reason for the larger dimensions achieved by subtidal mussels in the Fishing Harbour (FH).

Finally, the pattern of recruits' dimensional structure is characteristically a reflection of the extended periods of recruitment in mussels, and of the variable individual growth rates that usually merge the age classes (Barkati, 1989; Seed & Suchanek, 1992).

Therefore, its interpretation is by no mean easy to explain without further analysis in a more extensive spatial and temporal design.

2.5.3 Manipulative experiment of predation

In terms of predation, our experimental manipulation of top-down predators showed that along the time there is a decrease in the number of mussels alive in all areas. Until 25 days of manipulation, the area outside the port (QUE) had greater survival than the ones inside the port, but towards the end it presents similar number of mussels alive as the seawalls studied. Mussels in seawalls have a quick mortality after transplantation but persist longer than in breakwaters, which suggest that predation can have a heavier effect in breakwaters comparing to seawalls.

Although our experiment may not have lasted enough time to detect a significative effect of predation in breakwaters, which would support our initial hypothesis, the evidence is that its pressure weights against the patterns of distribution of mussels inside the Port of Sines. Many studies have demonstrated that predators are capable of controlling prey communities on temperate and tropical rocky coasts and in many other marine habitats (Menge, 2000; Menge et al., 2004), and may also allow competitors of the preferred prey to persist (Petraitis, 1990; Helmuth et al., 2005). Therefore, models of foraging assume that predators depress the density of preys of a particular type or in a particular location, and consequently slowly move to neighbouring less favourable categories or patches of prey as depletion proceeds (Van de Koppel et al., 2005). As a reaction to predation, mussels developed better morphological and behavioural anti-predator defences such as producing stronger byssus attachment, increasing detachment force, and enhancing shell strength and thickness (Kautsky et al., 1990; Nagarajan et al., 2006 and references therein).

Different predators use specific techniques to break the shell and get the flesh of mussels (Petraitis, 1995). For example octopus and dogwhelks bore a hole in the shell and suck out the flesh (Griffiths & Hockey, 1987); fish and ducks swallow the mussel whole complete with shell (Kautsky et al., 1990); birds drop mussels onto stones, stab at opened valves, or hammer them through the dorsal or ventral valves (Griffiths & Hockey, 1987); starfish force the shell open by exhausting the adductor muscle with their tube-feet (Kautsky et al., 1990; Norberg & Tedengren, 1995); and crabs by shell crushing (ap Rheinallt, 1986; Ameyaw-Akumfi & Hughes, 1987; Bertness et al., 1999).

As in many sampling data cages were observed as forcedly pushed in the sides, perhaps this is a foraging behaviour of one of the main predators inside the Port of Sines. Probably seastars are the support top-predator, as they were commonly visualized near the cages. This was only seen in the breakwaters, evidencing that local predation in seawalls is more improbable, and that on breakwaters can be heavy and cause rapid mortality.

A possible artifact to this experiment is that we did not completely excluded crabs of very small size that can pass through the mesh and find refuge inside these structures (see Petraitis, 1991; Hunt & Scheibling, 1997; Miller & Gaylord, 2007), being only effective the removal of seastars. In fact, small crabs were observed in large numbers either on seawalls as in breakwaters inside the port. Thus, some mortality in the Port of Sines has to be unambiguously attributed to crab predation, and because of the characteristic shell breaking patterns sometimes seen on the recovered shells.

Other factors, like a stronger flow that could possible dislodge mussels (Denny, 1987; Alvarado & Castilla, 1996; Bell & Gosline, 1997; McQuaid & Lindsay, 2000; Hunt & Scheibling, 2001; Carrington, 2002a,b; Menge et al., 2004; Schneider et al., 2005), or the presence of avian predators or other top predators (e.g. Griffiths & Hockey, 1987; Carroll & Highsmith, 1996), can be potentially excluded because we have made our study during summer, when wave action is minimal, and we never observed other types of predators in our study sites. Loss from waves would be more likely to remove the entire clump (Menge et al., 2004), while loss from predation usually remove individual mussels at a more steady rate, which was the pattern observed in this experiment. Transplantation of mussels from one place to another could be other cause of disturbance (Honkoop et al., 2003), but some studies have proved that mussels are quite adaptable to manipulation (e.g. Petraitis, 1990; Widdows & Donkin, 1992; Menge et al., 1994; Robles et al., 1995).

Overall, assuming that predation can control abundance of mussels, along with other detrimental features that unable its persistence, in inside areas of the Port of Sines this can be a possible factor that explains its absence in breakwaters and the existence of dense beds in seawalls. The more probable scenario is that crabs eat particularly small sized mussels in both types of artificial surfaces, and that starfish fulfill a structuring top-down linkage between subtidal and intertidal habitats mostly in breakwaters. Without these main predators, mussels in artificial surfaces inside this port could

probably invest in its defences and persist. Also, as documented by some authors (e.g. Seed & Suchanek, 1992; Menge et al., 1994; Branch & Steffani, 2004), at exposed locations there are usually few shore crabs and predation is probably less intense, and at more sheltered areas predators are more active and can limit mussel abundance to crevices and other microhabitats. This is another evidence for the pattern of predators observed in areas outside and inside the Port of Sines, respectively.

2.5.4 Manipulative experiment of thermal stress

As the mean temperature registered during the predation experiment was notoriously superior in breakwaters comparing to seawalls, and taking into account that predation pressure in breakwaters seemed to be heavier, an experimental of thermal stress was considered as the next step to explain mussels' absence in breakwaters inside the Port of Sines. In this experiment, there were significant differences in the survival of mussels among treatments, with apparently heavier mortality also in the breakwater studied comparing to a seawall. However, results were insufficient to support the hypothesis of having greater survival in unshaded seawalls comparing with unshaded breakwaters (control treatment, C); or that shaded breakwaters (SH) were similar do unshaded seawalls. Also, the procedural control (PC) was not equal to the unshaded treatment in the breakwater. Potentially, thermal stress in mussels is not the main cause for its absence in breakwaters inside the Port of Sines.

In terms of temperature measurements, the range of values registered in unshaded seawalls were a consequence of orientation within the harbour (SW2 faced south, towards the sun; and SW1 faced north, exposed indirectly to the sun), what was sufficient to register temperatures of up to 10°C and less than 5°C in SW2 and SW1, respectively. Horizontal breakwaters, located only a few cm away, often experience temperature maxima over 10°C hotter than vertical substrata (California: Helmuth & Hofmann, 2001), but this was not what we registered in the present study for its unshaded treatment. The only approach to these values is the ones achieved during the manipulative experiment of predation in summer 2005. This could be to the fact that the temperature loggers used (ibuttons) had several problems along the experiment, with many of these malfunctioned and causing gaps of measures along the time. Probably this result from the fact that they are not adapted to full submergence. In a future study, we could substitute them with TidBit™ loggers, as they seem to be more

appropriate to register temperatures in this environment; or use ibuttons inserted in mussel shells filled with silicone (e.g. Fitzhenry et al., 2004).

High temperatures characteristic of summers may possibly reduce growth in mussels and even cause mortality from heat stress in some areas (Seed & Suchanek, 1992; Hofmann & Somero, 1995; Buschbaum & Saier, 2001; Somero, 2002; Wetthey, 2002; Rius & McQuaid, 2006), as temperature extremes during low tide can far exceed those experienced during submersion (often by 20°C or more in California: Helmuth & Hofmann, 2001; Sanford, 2002). However, this was not what we observed in the Port of Sines. Despite temperatures registered (mostly in summer 2005) being closer to the lethal thermal tolerance limits described by Blanchette et al. (2007), probably mussels can withstand higher temperatures for short periods of time in this port.

As ectothermic organisms, body temperatures in mussels are determined by external climatic conditions, as water or air temperatures, wind speed, cloud cover, solar radiation and relative humidity (Helmuth, 2002). Therefore, obtaining measurements realistic of the microclimatic data within the rocky intertidal over long periods of time is difficult (Helmuth & Hofmann, 2001; Fitzhenry et al., 2004). As a consequence other temperature loggers (thermal “mimics”) have been developed as a way to face the large extent of lack data from intertidal environments (Helmuth & Hofmann, 2001; Helmuth, 2002; Menge et al., 2002; Somero, 2002; Harley & Helmuth, 2003; Fitzhenry et al., 2004; Blanchette et al., 2007).

2.5.5 Recruitment

Contrasting to the hypothesis previously considered, the recruitment pattern observed in areas inside and outside the Port of Sines was representative of a system that has a heavier larval supply of mussels in all areas. However, *M. galloprovincialis* had differences between areas only in July, with less recruitment in the artificial surfaces of SW2, B1 and B2 (with the exception of B3). Natural substrates inside the port had similar values of recruitment for both months, but outside areas achieved the biggest values of recruitment, except for CSN in the month of July. Mytilids recruitment was notably heavier in August than July, with the artificial and natural substrates inside the port having the higher numbers of recruits. Therefore, the hypothesis initially proposed was rejected as we didn't notice greater recruitment in seawalls comparing with

breakwaters, and also seawalls didn't have similar number of recruits as the outside areas.

Although patterns of distribution of organisms can often be determined by recruitment into specific habitats (Underwood & Fairweather, 1989; Hunt & Scheibling, 1996; Blockley & Chapman, 2006), this was not the general outcome of the manipulative experiment of recruitment done in the Port of Sines. Therefore, post-recruitment processes such as competition, predation, or physical stress (see revisions of Newell, 1989; Hunt & Scheibling, 1997 and Todd, 1998) can be the main determinants of its initial patterns. This differential mortality at the time of settlement, or shortly thereafter (post-settlement mortality), may also affect patterns of distribution and abundance (Connell, 1985; Underwood & Fairweather, 1989; Hunt & Scheibling, 1997), which is often difficult to distinguish from variable initial settlement.

Among different hard-man made structures, much of the variation in assemblages may be due also to larval responses to a suitable substratum, such as surface complexity (texture, size, thermal capacity, surface energy and charge, colour) and a range of microhabitats (McGuinness, 1989; Petraitis, 1990; Seed & Suchanek, 1992; Archambault & Bourget, 1996; Walters & Wethey, 1996; Hunt & Scheibling, 1997; Wahl & Hoppe, 2002), or the material from which it is composed (McGuinness, 1989). Nevertheless, mussels are known to settle in very large numbers in harbours, particularly on intertidal seawalls (Glasby & Connell, 2001; Chapman, 2003; Chapman & Bulleri, 2003), being differences in early patterns of colonisation of space probably the result of a distinct establishment of mature assemblages on these structures (Bulleri, 2005b).

The present study had also higher recruitment in areas inside the port probably as a result of larval retention in this embayment environment, as suggested in Branch & Steffani (2004). Therefore, differences in abundances are unlikely to be due to differences in larval supply, but responses of the recruits to the different habitats (recruitment processes) or post-settlement mortality occurring within a short time. This have been shown to vary spatially and temporally, especially for intertidal plants and animals (Connell, 1985; Petraitis, 1991; Menge et al., 1994; Hunt & Scheibling, 1996; Broitman et al., 2005; McQuaid & Lindsay, 2005), being this the most probable scenario as mortality of mussel larvae approaches or exceeds 99% (Lutz & Kennish, 1992).

CHAPTER 3. Morphological study of mussels inside and outside the Port of Sines

3.1 ABSTRACT

In areas inside the Port of Sines, mussels from seawalls and buoys achieve greater lengths than the ones outside, and have more sinusoidal shapes and different patterns of shell colour. In order to make a preliminary morphological study, qualitative and morphometric characters were analysed in three different environments. Despite the significant differences among areas, both approaches separated mostly mussels from inside and outside the port. The main contributors for differences in the qualitative analysis were the inner and outer surface colours of the shell. These characteristics are the most plastic ones and usually generate unclear identifications. In morphometric analysis, despite the great amount of characters assessed, dissimilarities among areas were only 7-10%. Altogether, data were insufficient to truly report that the similarities or differences between mussels in these areas are meaningful of a single species or not. The fact is that they are different and we are yet to understand the implications of genotype-specific differences and environmentally-induced changes in the ecology of mussels in these coastal areas.

3.2 INTRODUCTION

For many years, the taxonomy of individuals belonging to the genus *Mytilus* (Mollusca: Bivalvia) has been subject to controversy, because the accurate establishment of the taxonomic status of their species, namely *M. edulis* and *M. galloprovincialis*, has proved to be difficult (McDonald et al., 1991; Gosling, 1992a,b; Suchanek et al., 1997). Initial taxonomic studies on this group were based solely on shell characteristics, but the high phenotypic plasticity and diversity of environments where this group inhabits, have generated unclear identifications (e.g. Skibinski et al., 1983; Gosling, 1984; Johannesson et al., 1990; Gosling, 1992a,b; Bates & Innes, 1995; Inoue et al., 1995; Fish & Fish, 1996; Inoue et al., 1997; Cárcamo et al., 2005; Beaumont et al., 2006). Where hybridization is taking place, the large number of intermediate forms makes accurate identification even more difficult or almost an impossible task, especially at exposed locations (Gosling, 1984). As a consequence, populations frequently differ in growth rate and size, as well as in morphology of shell and soft parts (Blot et al., 1988;

Kautsky et al., 1990). A large part of this variation may result from environmental factors such as temperature, salinity, and wave exposition, with genetic differentiation in growth rate and in morphological traits observed (Gosling & Wilkins, 1981; Skibinski et al., 1983; Gardner & Skibinski, 1991; Bates & Innes, 1995; Gardner & Thompson, 2001).

After the first studies, the technique of gel electrophoresis (in conjunction with morphological analyses) was used in an attempt to quantify the genetic differences between the two forms (e.g. Skibinski et al., 1978; Gosling & Wilkins, 1981; Grant & Cherry, 1985; McDonald et al., 1991; Bates & Innes, 1995; Inoue et al., 1995, 1997; Suchanek et al., 1997; Toro, 1999). Several allozyme loci were considered as absolute diagnostic for the certain separation of mussels (e.g. Lobel et al., 1990; Gosling, 1992a,b), although no single locus seems to be diagnostic between the three species of the *Mytilus* complex (McDonald et al., 1991; Gosling, 1992a). Only since 1995 that truly diagnostic DNA-based molecular methods have become available (e.g. Inoue et al., 1995; Quesada et al., 1995; Rawson & Hilbish, 1995; Rawson et al., 1996; Inoue et al., 1997; Quesada et al., 1998; Daguin et al., 2001; Bierne et al., 2002, 2003; Wood et al., 2003a,b; Cárcamo et al., 2005). This is the most promising approach to this problem, in conjunction with the use of the polymerase chain reaction (PCR), which allows also the analysis of extremely low DNA amounts typical of young bivalves (e.g. Rawson et al., 1996; Daguin & Borsa, 1999, 2000; Skurikhina et al., 2001; Toro et al., 2002, 2005; Coghlan & Gosling, 2007).

As stated above, there have been several attempts to find a single morphological character that would consistently discriminate between *M. edulis* and *M. galloprovincialis*. The characters which have previously been considered as the most useful for distinguishing these two species, considering the extensive worldwide study of McDonald et al. (1991), were the length of the anterior adductor muscle scar and length of the hinge plate. However, revisions by Gosling (1984, 1992a) documented that the separation of these two forms of mussels can be based primarily on external shell contours, internal features of the shell valves and the colour of the mantle edge. As stated recently by Skurikhina et al. (2001), although morphological criteria are available, identification of mytilid species is difficult even for experts in morphology, in particular, due to the overlapping of morphological characters of closely related species in the same habitat (McDonald et al., 1991; Innes & Bates, 1999). It would appear, therefore, that overall shell shape in *Mytilus* is so variable that it has little if any value in

taxonomic studies (Gosling, 1984; McDonald et al., 1991). In general, *M. edulis* and *M. galloprovincialis* are considered the most closely related species and *M. trossulus* the most divergent (Rawson & Hilbish, 1995; Quesada et al., 1998; Hilbish et al., 2000). Between the first two, *M. galloprovincialis* seems to be the more variable form (Gosling, 1984). Since the three species of the *M. edulis* complex are at least independent populations and have different phenotypic properties, it is important to identify them whatever the purpose of the study (Inoue et al., 1997).

In areas inside the Port of Sines, mussels from seawalls and buoys achieve greater lengths than the ones from outside shores (see Chapter 2), with a shell shape more sinusoidal than the outside specimens. The colour of the outer surface of the shell is black, with some individuals sometimes brown and a radial pattern. Mussels from moderately to exposed shores at north and south of Sines are more elongated and round, with shells, in general, externally intensely blue. Taking into account these patterns, we addressed the hypothesis of having two different species inside and outside the Port of Sines. Thus, a preliminary morphological and morphometric description of mussels was made, as suggest by some authors (Gosling, 1984; McDonald et al., 1991; Gosling, 1992a).

3.3 MATERIAL AND METHODS

The study of morphology of mussels in areas outside and inside the Port of Sines attempted to see if there were differences between mussels adapted to different environments and was divided in two parts.

First, was made a qualitative analysis of some shell characteristics described in the literature as diagnostic of the two species that can be found in Portugal and form hybrids, *Mytilus edulis* and *M. galloprovincialis* (McDonald et al., 1991; Sanjuan et al., 1994; Quesada et al., 1995; Daguin et al., 2001; Bierne et al., 2002, 2003; Śmietanka et al., 2004). Shape variation is strongly associated with length (Lobel et al., 1990; Innes & Bates, 1999), so we attempted to remove the effect of size by randomly sampling 25 mussels within a small range of length-size (25-40 mm of maximum shell length) from samples of three areas of the dimensional structure study (see Chapter 2): SW1, FH and QUE. After registering the length of each mussel, they were cleaned with a brush and jets of seawater, and then analysed as presence or absence of each characteristic (attributing 1 and 0, respectively).

Six morphological qualitative characters were used to differentiate between the two species (described in Saldanha, 1974; Gosling, 1984; Van Erkom Schurink & Griffiths, 1990; Gosling, 1992a; Fish & Fish, 1996), but altogether 14 different traits were characterized:

- (1) **mdc**: form of the dorsal margin of the shell, rounded and slightly concave (ventral flattening) for *M. galloprovincialis* (hereafter MG), or less rounded and slightly sinusoidal for *M. edulis* (hereafter ME);
- (2) **pac**: form of the anterior end of the shell, distinctly beaked and incurved pointing downwards in MG, or rounded with a snub-nosed appearance in ME;
- (3) **umb**: form of the hinge plate (umbo), in MG is smaller and forms a tighter arc with its rear end more clearly delimited from the adjacent ventral edge of the valve, or bigger and a gently curving structure in ME;
- (4) **sec**: colour of the outer surface of the shell, typically black shading to brown ventrally (rarely light brown throughout) in MG, or dark blue, sometimes brownish with a radial pattern, in ME;
- (5) **sic**: presence of pearly iridescence and dark blue edge in the inner surface of ME, or absence in MG, with a whitish inner surface;
- (6) **bvl**: presence of longitudinal purple-violet bands in the inner surface of the shell of ME, or absence in MG.

As the colour of the outer and inner surfaces (**sec** and **sic** characteristics) is very plastic, each was divided into three types: for **sec**, the typically black of MG (**sec-MG**), the dark blue for ME (**sec-ME**), and brown for both (**sec-ME/MG**); and for **sic**, the pearly iridescence in ME (**sic-ME1**), the blue edge of ME (**sic-ME2**), and the white inner surface more common in MG (**sic-MG**). In **Figure 21** there are some representative pictures of the outer and inner surface of a shell of mussel. This study did not include some characters which have previously been considered useful for distinguishing these two species, such as the mantle edge colour (e.g. Gosling, 1984; McDonald et al., 1991; Gosling, 1992a; Fish & Fish, 1996) because these characters were difficult to measure in the majority of the mussels sampled. Probably this was a consequence of the frozen procedure.

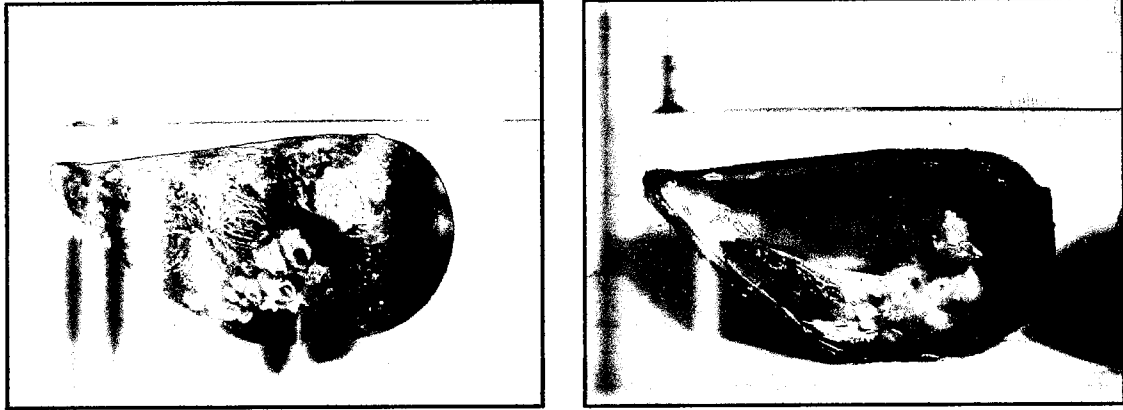


Figure 21. Example of an outer and inner surface of the shell of a mussel.

The second part corresponds to a morphometric analysis of 17 characteristics which were previously shown by some authors (Gosling, 1984; McDonald et al., 1991; Gosling, 1992a; Innes & Bates, 1999; Gardner & Thompson, 2001) to be most useful for discriminating species of mussels (the majority represented in **Figure 22**):

- (1) **aam**: length of anterior adductor muscle scar;
- (2) **dpr**: distance between the anterior end of posterior retractor muscle scar and dorsal shell margin;
- (3) **hp**: length of hinge plate;
- (4) **ht**: shell height;
- (5) **len**: shell length;
- (6) **lig**: distance between umbo and posterior end of the ligament;
- (7) **ligto**: ligament length;
- (8) **lpr**: length of posterior retractor muscle scar;
- (9) **pad**: length of posterior adductor muscle scar;
- (10) **padp**: distance between anterior edge of posterior adductor muscle scar and posterior shell margin;
- (11) **padv**: distance between ventral edge of posterior adductor muscle scar and ventral shell margin;
- (12) **pal**: distance between pallial line and ventral shell margin midway along shell;
- (13) **pmlc**: the position of the point of maximum shell width, measured from the umbo;
- (14) **ppad**: distance between posterior edge of posterior adductor muscle scar and posterior shell margin;
- (15) **vpr**: distance between ventral edge of posterior retractor muscle scar and dorsal shell margin;

- (16) **wid**: shell width;
 (17) **wpr**: width of posterior retractor muscle scar.

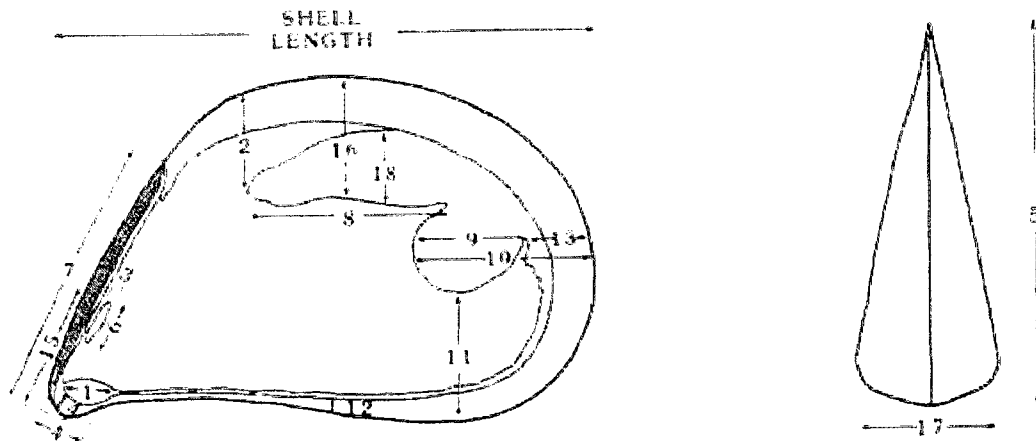


Figure 22. The 18 morphometric characters used by McDonald et al. (1991) to distinguish among different forms of *Mytilus*. 1, **aam**: length of anterior adductor muscle scar; 2, **dpr**: distance between the anterior end of posterior retractor muscle scar and dorsal shell margin; 3, **war**: width of anterior retractor muscle scar; 4, **hp**: length of hinge plate; 5, **ht**: shell height; 6, **lar**: length of anterior retractor muscle scar; 7, **lig**: distance between umbo and posterior end of the ligament; 8, **lpr**: length of posterior retractor muscle scar; 9, **pad**: length of posterior adductor muscle scar; 10, **padp**: distance between anterior edge of posterior adductor muscle scar and posterior shell margin; 11, **padv**: distance between ventral edge of posterior adductor muscle scar and ventral shell margin; 12, **pal**: distance between pallial line and ventral shell margin midway along shell; 13, **ppad**: distance between posterior edge of posterior adductor muscle scar and posterior shell margin; 14, **teeth**: number of major teeth on hinge plate, excluding any small crenulations which may appear, especially on the posterior ventral face of hinge plate; 15, **ular**: distance between umbo and posterior end of anterior retractor scar; 16, **vpr**: distance between ventral edge of posterior retractor muscle scar and dorsal shell margin; 17, **wid**: shell width; 18, **wpr**: width of posterior retractor muscle scar. (After McDonald et al., 1991).

All measurements were made under a dissecting microscope fitted with an ocular micrometer (40× magnification and accuracy of 0.02 mm), except **ht**, **len**, **pmlc** and **wid**, which were measured with a Mitutoyo® digital pachymeter accurate to 0.01 mm. Again, this study did not include some characters which have previously been considered useful for distinguishing *M. edulis* from *M. galloprovincialis*, such as the

length of the anterior retractor muscle scar (**lar**), the distance between umbo and posterior end of anterior retractor scar (**ular**), and the width of anterior retractor muscle scar (**war**) (see McDonald et al., 1991), because they were almost impossible to measure in the mussels sampled. However, as suggest by McDonald et al. (1991), we included the position of the point of maximum shell width (**pmic**), as a new tool that might yield a better discrimination among these species.

These morphometric data are commonly analyzed by canonical variates analysis (e.g. McDonald et al., 1991; Innes & Bates, 1999; Gardner & Thompson, 2001); however, we used multivariate analyses of MDS, PCA, SIMPER and ANOSIM, according to Clarke & Warwick (1994) to test the patterns among areas. In the qualitative analyses, multivariate comparisons between areas were examined by means of MDS, based on the Bray-Curtis measures of similarity calculated from untransformed data; and in the morphometric analyses, by principal component analysis (PCA). In both, ANOSIM and SIMPER analyses were done in order to test for differences between areas, and determine which morphological indices are responsible for significant differences among areas.

3.4 RESULTS

In the qualitative analysis of the morphology of mussels, the MDS representation of areas (**Figure 23**) separates the mussels from QUE from the ones of inside the Port of Sines, with some individuals of FH overlapping SW1 mussels. Despite the significant differences among areas detected in the ANOSIM analysis (**Table 6**), the group of QUE is more significantly different from the other two areas (bigger R). Dissimilarities were bigger between comparisons of QUE and the areas inside the port (SW1-FH 21%, SW1-QUE, 39% and FH-QUE 31%). Dispersion was bigger in SW1 and QUE (similarity of 77% and 78%, respectively, in each area) than in FH (similarity of 87%). Among each comparison, the main contributors for the percentage dissimilarity were **sec** and **sic**. In general, mussels from SW1 and FH have morphological characteristics that are diagnostic of both species, being its description very mixed. However, in this approach mussels in QUE seem to present more tendencies to be *M. edulis*, as the average abundance of the characteristics **sec-ME** and **sic-ME1** is 100% in comparisons made among individuals of this area and the ones from inside the Port of Sines.

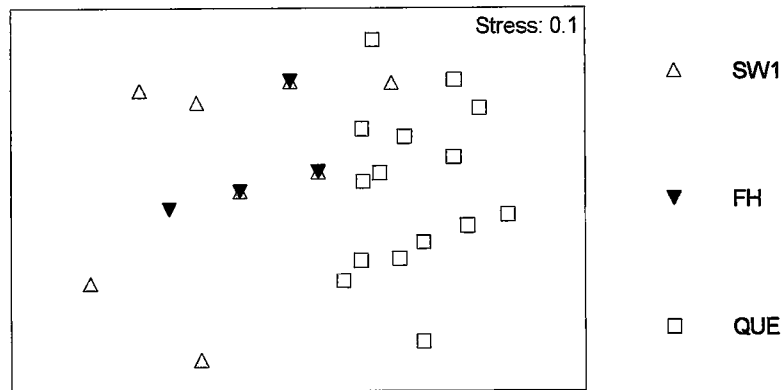


Figure 23. Qualitative analysis: classification by MDS of the individuals sampled (total N=84) and visualization by sampling areas.

Table 6. Qualitative analysis of mussels. ANOSIM and SIMPER results of three areas inside and outside the Port of Sines.

ANOSIM						
Global R	0.392					
P value	< 0.01					
Pairwise tests	SW1-FH	SW1-QUE		FH-QUE		
- R statistic	0.134	0.568		0.507		
- Significance level (%)	0.1	0.1		0.1		
SIMPER						
Similarity (%)	SW1	FH		QUE		
	77.49	87.04		77.88		
Dissimilarity (%)	SW1-FH	SW1-QUE		FH-QUE		
	21.06	39.32		30.63		
Contribution to dissimilarity (>10%)	sic-ME1	23.04	sec-ME	19.38	sec-ME	24.87
	sic-MG	23.04	sic-ME1	11.92	sic-ME2	13.57
	sec-ME/MG	16.52	sic-MG	11.92	sec-ME/MG	11.73
	sec-MG	16.52	sic-ME2	10.57		
Average abundance (%)	SW1	FH	SW1	QUE	FH	QUE
- sec-ME			0	100	0	100
- sec-ME/MG	73	68			68	60
- sec-MG	27	32				
- sic-ME1	38	86	38	100		
- sic-ME2			0	57	0	57
- sic-MG	62	14	62	0		

The results of the morphometric analysis of mussels were represented in a PCA (Figure 24), where along PC2 axis QUE individuals appear again separated from the mussels inside the port. However, it is apparent that FH individuals are more different from the QUE mussels than the ones from SW1. PC1 separates the majority of outside mussels from a great part of the inside ones. The morphometric characteristics that contributed (all negatively) for the formation of the PC1 axis are, in a decreasing order of importance, *len*, *ht*, *padp* and *pmlc*. For PC2, the biggest positive contribution is

markedly from *wid*, followed by *lpr* and *lig*, and negatively by *vpr*. In general, mussels from QUE tended to have less *len*, *ht*, *padp*, *pmic* and *vpr*, and more *wid*, *lpr* and *lig* than the mussels from SW1 and FH. The ANOSIM analysis represented in Table 7 shows that all mussels from the three areas are different, but these differences are greater between QUE and the areas inside the port (bigger R). The similarities between individuals in each area are all >90%, and the dissimilarities between each comparison of two areas are very low: SW1-FH 7%, SW1-QUE, 10% and FH-QUE 10%.

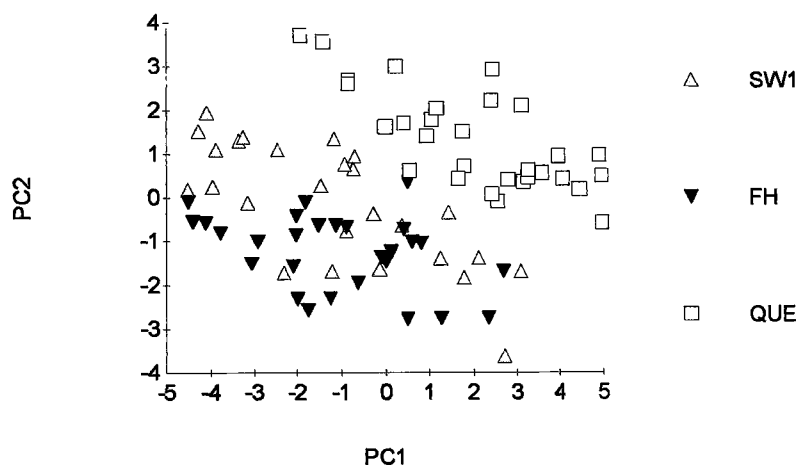


Figure 24. Morphometric analysis: classification by principal components analysis of the individuals sampled and visualization by sampling areas. The two first axes (PC1 and PC2) explain 51.8% of the total variation of data.

Table 7. Morphometric analysis of mussels. ANOSIM and SIMPER results of three areas inside and outside the Port of Sines.

ANOSIM						
Global R	0.367					
P value	< 0.01					
Pairwise tests	SW1-FH	SW1-QUE		FH-QUE		
- R statistic	0.108	0.405		0.570		
- Significance level (%)	0.4	0.1		0.1		
SIMPER						
Similarity (%)	SW1		FH		QUE	
	92.48		93.96		92.68	
Dissimilarity (%)	SW1-FH		SW1-QUE		FH-QUE	
	7.00		9.52		9.70	
Contribution to dissimilarity (>10%)	<i>len</i>	13.15	<i>ht</i>	13.29	<i>ht</i>	17.41
	<i>pmic</i>	10.89	<i>pmic</i>	11.42	<i>pmic</i>	13.65
			<i>len</i>	10.48		
Average abundance (%)	SW1	FH	SW1	QUE	FH	QUE
- <i>ht</i>			17.25	13.34	18.58	13.34
- <i>len</i>	30.76	30.80	17.52	14.67		
- <i>pmic</i>	17.52	18.54	30.76	29.35	18.54	14.67

differences could be applied to mussels in areas of overlap and hybridization, as intermediate forms makes accurate identification of *M. edulis* and *M. galloprovincialis* an almost impossible task (Gosling & Wilkins, 1981; Gosling, 1992a).

Of the various ecological factors known to influence shell shape, population density seems to play one of the most important roles (Alunno-Bruscia et al., 2001). High density has generally been reported to lead to elongated and narrower (i.e. filater) shells in mussels (Seed & Suchanek, 1992; Gardner et al., 1993), although extreme deformation may be observed (Zardi et al., 2006a). Crowding can result from food regulation, physical interference or their interaction (Alunno-Bruscia et al., 2001; Steffani & Branch, 2003). In the Port of Sines, the high density of bigger mussels in both subtidal FH and intertidal SW1 can be the cause that explains the more elongated (+len, +ht) and narrower (-wid) shapes reported in its morphometrics. In QUE, as populations consisted of small and young mussels (mono-layers), this reflects its less length (-len) and height (-ht) of the shell, being alternatively more width (+wid).

Other differences have been allocated to differentiate *M. edulis* from *M. galloprovincialis*, like the growth potential (Gosling, 1992a; Seed & Suchanek, 1992): *M. galloprovincialis* have a slightly higher growth rate and a greater survivorship than *M. edulis* (Skibinski et al., 1983; Gardner et al., 1993; Wilhelm & Hilbish, 1998; Comesaña et al., 1999; Hilbish et al., 2003), and its growth is proportionally less diminished by exposure to air (Branch & Steffani, 2004) and parasitism (Secor et al., 2001). In this perspective, the classification of mussel species in areas inside and outside the Port of Sines becomes even more confused, as inside the port they can be *M. galloprovincialis* because they have higher growth rate, and in outside areas can be also this species as it faces more exposed exposition.

Along with this, it is commonly found that in hybrid populations' larger mussels tend to be *M. galloprovincialis*-like than smaller mussels, and that *M. edulis*-like individuals assume a slightly more eccentric shape (Skibinski & Roderick, 1991; Gardner et al., 1993; Fish & fish, 1996; Wilhelm & Hilbish, 1998; Innes & Bates, 1999; Hilbish et al., 2003). This complicates even more when we consider exposure to wave action, where maximum size ranges from about 1 cm on an inhospitable windswept wave-beaten shoreline to perhaps 10 cm and more width in a sheltered bay or estuary (Lobel et al., 1990; Steffani & Branch, 2003). Taking into account the above assumptions, in the present study we could consider the large mussels of areas inside the port as being *M. galloprovincialis*-like, and the wider (eccentric) mussels in outside areas as *M. edulis*.

However, the dominance of mussels on shores with high wave exposure have been reported as belonging to *galloprovincialis* genotypes, which despite having greater shell area can compensate the big hydrodynamic forces experienced with stronger attachment strength than *edulis* (Gosling & Wilkins, 1981; Skibinski et al., 1983; Gardner & Skibinski, 1991; Skibinski & Roderick, 1991; Gardner et al., 1993; Schneider et al., 2005). Opposite to this, *M. edulis* predominates in estuarine and more sheltered environments (Skibinski et al., 1983; Gardner et al., 1993; Bierne et al., 2002, 2003; Hilbish et al., 2003), and possibly subtidal sites (Riginos & Cunningham, 2005). Perhaps this trend is not related to wave action, but to the reduction of area over which hydrodynamic lift acts, and is a response to the increased risk of dislodgement (Denny, 1987; Bell & Gosline, 1997).

This shift in the mussels characteristics can be also a result of strong natural selection and genetic drift, that usually occurs in hybrid zones mostly against *M. edulis*-like genotypes, where differential mortality apparently leads to its elimination (Skibinski et al., 1983; Skibinski & Roderick, 1991; Gardner et al., 1993; Wilhelm & Hilbish, 1998; Daguin et al., 2001; Secor et al., 2001; Bierne et al., 2002; Hilbish et al., 2002, 2003; Schneider et al., 2005). Therefore, hybrid genotypes usually have an intermediate fitness level compared to *M. edulis*-like and *M. galloprovincialis*-like genotypes (Gardner et al., 1993; Innes & Bates, 1999; Hilbish et al., 2003), but viability selection usually favours hybrid mussels with genetic compositions similar to *M. galloprovincialis* rather than those more similar to *M. edulis* (Wilhelm & Hilbish, 1998). However, the strength of selection is habitat-dependent and can induce reproductive isolation (Lutz & Kennish, 1992; Seed & Suchanek, 1992; Chícharo & Chícharo, 2000; Hilbish et al., 2003), being exogenous selection the main contributor for the coexistence of different morphotypes in hybrid zones and other systems (Gardner & Skibinski, 1990; Gardner et al., 1993; Wilhelm & Hilbish, 1998; Gardner & Thompson, 2001; Toro et al., 2004; Schneider et al., 2005).

The maintenance of *M. edulis* in some areas usually is explained by its immigration of numerous spat (Gardner & Skibinski, 1990; Skibinski & Roderick, 1991; Gardner et al., 1993; Wilhelm & Hilbish, 1998; Hilbish et al., 2002; Gilg & Hilbish, 2003b), and by spawning asynchrony (McGrath et al., 1988; Gardner & Skibinski, 1990; Seed & Suchanek, 1992; Cáceres-Martínez et al., 1993; Secor et al., 2001; Bierne et al., 2003), as *M. edulis* populations' spawns earlier than *M. galloprovincialis* (Seed & Suchanek,

1992; Secor et al., 2001; Gilg & Hilbish, 2003a). This could tend to overlap both of the monospecific populations in areas where they could coexist.

As this is a main port in the Ibero-Atlantic front, sporadic episodes of introduction by means of ships can be relatively abundant and create strong interactions between native and invasive species (as also reported in other systems by Grant & Cherry, 1985; Carlton, 1992; Inoue et al., 1997; Suchanek et al., 1997; Branch & Steffani, 2004; Rius & McQuaid, 2006). However, *M. galloprovincialis* presents several dominant characteristics as a competitor (rapid growth rate, high fecundity and recruitment rate, and higher resistance to desiccation and parasitism), which should make this introductions of potential *M. edulis*-like mussels less effective (Sanjuan et al., 1994; Harris et al., 1998; Śmietanka et al., 2004; Zardi et al., 2006a).

CHAPTER 4. General considerations and future perspectives

Deploying a number of human-made constructions in coastal areas has inevitably an impact on the abundance and distribution of species on a regional scale. Only through an understanding of the mechanisms which cause assemblages on artificial structures to differ from those occurring on natural habitats, can the design of artificial structures be improved (Benedetti-Cecchi et al., 2000; Bulleri, 2005b).

Mussels (*Mytilus* spp.) belong to a group of key species in coastal marine ecosystems, being commonly the dominant invertebrates on hard surfaces in the mid- to lower intertidal range in all temperate seas (Seed & Suchanek, 1992). Therefore, characterisation of their populations is extremely useful for monitoring programmes and for predicting diversity changes at intra-specific and inter-specific levels, as well as in the communities of organisms associated with them (Blanchette et al., 2007). With implications in understanding the biodiversity changes in coastal ecosystems in all Europe, the identification of how selective forces act to maintain isolation between taxa, or differentiated geographic species, is a key strategy in addressing these issues.

With respect to the hypotheses posed at the beginning of this thesis, our results indicate that: (1) mussels are abundant on intertidal seawalls inside the Port of Sines, as well as in outside areas, being absent in all breakwaters; (2) the dimensional structure of mussels inside the port is different from the outside areas, as individuals achieve larger dimensions and different morphologies inside the port; (3) predation pressure as an effect in all areas studied, being one of the main negative determinants for mussels' survival in breakwaters inside the port; (4) thermal stress can have an important effect in mussels living inside the port, but by its own doesn't seem to be the main cause for their absence in horizontal breakwaters; (5) the supply of mussels recruits is not the factor responsible for mussels distribution inside the port, but it can remarkably have differences between areas inside and outside; and, finally, (6) morphological and morphometric analyses of mussels distinguished mussels from areas inside and outside the port, and among different environments (subtidal vs. intertidal), but were insufficient to discriminate similarities or differences meaningful of one or two species.

The true extent of the mosaic of population structure across this study area is completely unknown, being warranted the need of further investigations. Consequently,

there is an increasing awareness that a multidisciplinary approach is the only way to effectively solve the problem. Promising areas of further research should include:

- Examination of how byssal thread material properties and their dependence on environmental and physiological conditions influence mussel attachment strength;
- More predation experiments, as this factor provides a good predictor of mussels' distribution and abundance in this area. However, more careful must be taken with the possible artifacts of the structures employed; for example, diminishing mesh size could be an important improvement, or even trying to study predation at the level of recruits or settlers;
- Study of predators inside the Port of Sines, as studying them can be also important to understanding its influence in mussels;
- Other factors affecting post-settlement mortality (e.g. different types of substratum, like its composition or material; damp conditions; and the presence or absence of biofilms and chemical cues);
- Epibiosis, as it can have a great impact on the mortality of mussels and could contribute significantly to the dynamics and structure of local benthic communities;
- Understanding the relative importance of temperature during submersion versus aerial exposure is crucial for decipher the effect of this factor in adults and, mostly, in early settlers;
- Biochemical indicators of stress, particularly heat shock proteins, in mussels from different areas (mainly in breakwaters and seawalls);
- Genetic studies of mussels inside and outside the Port of Sines, regarding also its differences related to exposure. More identification of *Mytilus* morphotypes and genotypes is needed before we can truly say that the similarities or differences we report are real and meaningful characteristics attributed to a single species;
- Determination of genetic composition of primary and secondary settlement mussels, as well as trying to distinguish early post-metamorphic and juvenile stages. This early mussel life history stages may have an important influence on later performance, and species discrimination may be important also because possible control methods may differ according to species;
- Detailed study of the possibly non-native species that can be entering in this system by ship translocations;
- Lastly, understanding the link between mussel dislodgement and mortality is a necessary component in verifying the role that wave forces may play in driving selection patterns between horizontal and vertical surfaces in the Port of Sines.

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APPENDIX

Table 1. ANOVAs to the recruitment of *Mytilus galloprovincialis* in caged and non-caged Tuffy pads deployed in each area inside the Port of Sines. *N*=2.

ANOVA – SW2		July				August		
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar 1	1482.25	1.52	0.3425	3025.00	0.93	0.4369	
Residual	2	973.25			3258.00			
Total	3							
Cochran test		C=0.8641 (n.s.)				C=0.9972 (n.s.)		
Transformation		No transformation				No transformation		

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s) - no significant differences.

ANOVA – B1		July				August		
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar 1	110.25	2.38	0.2626	49.00	0.66	0.5025	
Residual	2	46.25			74.50			
Total	3							
Cochran test		C=0.6541 (n.s.)				C=0.9698 (n.s.)		
Transformation		No transformation				No transformation		

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s) - no significant differences.

ANOVA – B2		July				August		
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar 1	930.25	3.40	0.2063	625.00	4.08	0.1807	
Residual	2	273.25			153.00			
Total	3							
Cochran test		C=0.9963 (n.s.)				C=0.9412 (n.s.)		
Transformation		No transformation				No transformation		

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s) - no significant differences.

Table 2. ANOVAs to the recruitment of mytilids in caged and non-caged Tuffy pads deployed in each area inside the Port of Sines. $N=2$.

ANOVA – SW2		July			August			
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar	1	3540.25	6.52	0.1253	132860.25	1.04	0.4147
Residual		2	543.25			127478.25		
Total		3						
Cochran test		C=0.8509 (n.s.)			C=0.9830 (n.s.)			
Transformation		No transformation			No transformation			

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s) - no significant differences.

ANOVA – B1		July			August			
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar	1	4830.25	6.14	0.1314	5476.00	0.05	0.8459
Residual		2	786.25			112540.50		
Total		3						
Cochran test		C=0.8598 (n.s.)			C=0.9942 (n.s.)			
Transformation		No transformation			No transformation			

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s) - no significant differences.

ANOVA – B2		July			August			
Source of variation	d.f.	MS	F	P	MS	F	P	
Area	=ar	1	7140.25	1.89	0.3032	447561.00	3.54	0.2008
Residual		2	3783.25			126565.00		
Total		3						
Cochran test		C=0.9046 (n.s.)			C=0.8973 (n.s.)			
Transformation		No transformation			No transformation			

Legend: d.f. - degrees of freedom; MS - mean squares; F - variance rate; P - probability; (n.s) - no significant differences.