UNIVERSIDADE DO ALGARVE

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THE STATUS OF THE INVASIVE SEA SQUIRTS AND BARNACLES FOUND IN THE MARINAS AND PORTS OF ALGARVE, SOUTHERN PORTUGAL

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“Declaração de autoria de trabalho”

“Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.”

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RESUMO

As espécies invasoras são consideradas por vários autores como uma das maiores ameaças que podem ocorrer nas faunas nativas, a par com as alterações climáticas, a destruição dos habitats naturais e a sobre-exploração pesqueira. O principal problema das espécies invasoras é a sua grande competência e capacidade de crescimento em relação às nativas, provocando alterações na estrutura da comunidade, podendo chegar a ter impacto na saúde humana e nas economias locais. Estas ameaças ocorrem quando um organismo ou grupo de organismos são introduzidos (intencional ou acidentalmente) e se estabelecem fora da sua área natural de distribuição, apresentando capacidade de sobreviver e se reproduzir. As espécies invasoras sésseis apresentam como principais mecanismos de introdução as águas de balastro dos navios, assim como a fixação nos cascos das embarcações (*biofouling*), e a aquacultura. Da atual transferência global e europeia de espécies invasoras surge a necessidade de criar listas de identificação que englobem estas espécies. Portugal possui alguns trabalhos de compilação de espécies invasoras. No entanto, não existe atualmente nenhuma base de dados, sendo essa a principal razão para a execução deste trabalho. Para isso, este trabalho centrou-se na deteção e validação taxonómica de ascídias e cracas invasoras encontradas nos principais portos e marinas do Algarve, na costa sul de Portugal. Foram realizadas comparações para verificar as diferenças na abundância daquelas espécies invasoras em termos sazonais (inverno, primavera e verão), tipos de portos (recreativos e de pesca), localização geográfica dos portos (este, centro e oeste) e a localização do porto (abrigados ou desprotegidos da corrente marítima). Finalmente, foi realizado um caso de estudo para determinar qual a melhor superfície para a fixação destas ascídias e cracas invasoras, assim como para comprovar as alterações produzidas na comunidade durante as tarefas de eliminação de espécies invasoras. A metodologia aplicada foi diferente para cada um dos grupos de interesse. As cracas foram estudadas usando 10 quadrículas aleatórias em cada porto, com fotografias e com contagens para determinar as suas abundâncias, através do programa ImageJ. No estudo das ascídias, devido à impossibilidade de realizar mergulhos, foram realizadas análises qualitativas de proporções, identificando 30 ascídias em 10 pontos aleatórios de cada porto. Devido à dificuldade de identificação externa deste grupo, foi criada uma chave de identificação com caracteres morfológicos relevantes para a sua identificação *in-situ*. Uma amostra

aleatória de indivíduos foi selecionada, levada para o laboratório, para confirmação final de identificação (e validação da chave) através de disseção, para obter um grau máximo de precisão. Para o caso de estudo, foram instalados 10 dispositivos no porto de Olhão, dos quais 5 serviram como controlo e os outros 5 foram manipulados para eliminar consecutivamente as espécies invasores encontradas. Os dispositivos foram formados por 3 objetos diferentes: uma “carteira”/bolsa cheia de pequenas pedras, uma peça de PVC e uma placa de cimento. Através desta experiência foi possível obter recrutamento para ambos os grupos de interesse. Todas as análises dos dados obtidos foram realizadas nos seguintes softwares: Excel (para abundâncias e frequências de ocorrência), PRIMER-6 (para diversidade e comparações de comunidades) e R (para ANOVAs). Foram encontradas 19 espécies de ascídias ecracas, das quais 11 pertenciam ao Grupo Cordata e 8 ao Grupo Artropoda, respetivamente. Pode destacar-se que 58.3% das espécies de ascídias eram invasoras, e 60% no caso das cracas. Adicionalmente, foram encontradas outras espécies invasoras durante as amostragens, como é o caso do gastrópode *Pollia assimilis*, encontrado pela primeira vez em costas europeias. Nas quadrículas ocorreram 5 espécies, das quais 2 eram invasoras (*Amphibalanus amphitrite* e*Austrominius modestus)*. Em termos de frequências de ocorrência, ambas as espécies apresentaram valores elevados. No entanto, em abundância, apenas *A. amphitrite* apresentou elevadas percentagens. Para as quadrículas em geral, foram observadas diferenças significativas em todas as comparações realizadas (ANOSIM e ANOVA, $p<0.05$), excetopara a sazonalidade, na qual não foram encontradas diferenças significativas (ANOSIM e ANOVA, $p>0.05$). Nas contagens de ascídias, tendo sido apenas consideradas ascídias solitárias, encontraram-se 9 espécies, sendo que 4 delas eram invasoras (*Microcosmus squamiger, Styela plicata, Styela canopus* e *Corella* cf. *eumyota*). Destaca-se o facto de terem sido as espécies *M. squamiger* e *S. plicata* que para além de apresentarem maior percentagem de frequência de ocorrência e abundância, também contribuiram em maior parte para a existencia de dissimilaridades entre as amostras. Para as ascídias, com exceção do tipo de porto, todas as outras comparações apresentaram diferenças significativas (ANOSIM e ANOVA, $p<0.05$). Neste estudo tornou-se claro que as espécies observadas/estudadas encontraram condições ótimas para o seu estabelecimento nestes portos do Algarve. Foram apresentadas diferenças entre a abundância de espécies invasoras nos diferentes portos e no tipo de embarcações que neles circulam, tendo-se observado um maior numero de espécies nas marinas devido eventualmente a uma maior entrada destas espécies e uma elevada circulação de embarcações internacionais. No caso de estudo, várias espécies invasoras foram encontradas nos dispositivos (*A. amphitrite*, *Amphibalanus* cf. *eburneus, S. plicata* e *Botryllus schlosseri*), sendo de assinalar que *B. schlosseri* apenas ocorreu de forma pontual, razão pela qual não foi incluída nos cálculos de recrutamento. Para*A.* cf. *eburneus* e *S. plicata*, não se observaram diferenças significativas no seu recrutamento em relação a: tipo de superfície de fixação, eliminação ou não de indivíduos de espécies de invasoras, e na combinação destes dois fatores (ANOSIM, $p>0.05$). A espécie *A. amphitrite* apresentou preferência pelo cimento para o seu recrutamento e fixação. É possível deduzir que, para o caso destas três espécies invasoras, a utilização de placas de cimento garante uma alta taxa de fixação. Pode referir-se também que, comparando as comunidades em relação ao tipo de superfície de fixação, foram observadas as maiores diferenças entre as “carteiras”/bolsas vs as outras duas superfícies (pvc e cimento). Quanto à eliminação de indivíduos de espécies invasoras, foram observadas maiores diferenças entre a primeira amostragem e todas as seguintes (incluindo o controlo), devido ao facto de que na primeira amostragem apenas foi realizada uma observação (sem eliminação). Foram também encontradas diferenças entre a segunda e terceira amostragem quando comparada com a última, na qual foram recolhidos os dispositivos (ANOSIM, $p<0.05$). Isto poderia ser explicado pela ocorrência de uma elevada abundância destas espécies invasoras, pelo que a eliminação destas apenas tem efeito a curto prazo. Isto deberia ser estudado mais a fondo por as necesidades explicadas.

Abstract

Exotic species have been considered by several authors as the worst threat that can occur to native biodiversity. Due to the lack of information on sea squirts and barnacle invaders in southern Portugal, the first step of this study aims to detect and identify their presence in the ports of the region. A comparison reporting differences between these ports was performed: i) fishing and recreational; ii) port location; iii) sheltered versus unsheltered facilities. The localities of Lagos, Quarteira-Vilamoura, Faro and Olhão were the study areas where seasonal sampling was done. A case study to obtain the total recruitment for the invasive species was also carried out, comparing surfaces and removal of invaders. Higher percentages of invasive species were found (60% for barnacles and 58.3% for sea squirts). The appearance of one invasive gastropod, originating in the West African coast, was reported in European waters the first time. Besides, for barnacle and sea squirt counting, there were significant differences in all comparisons except between seasons for barnacles and type of port for sea squirts. The main differences in the abundance, frequency of occurrence and communities seems to be related with the transit of international vessels, the settlement period and the conditions presented between ports in terms of exposure to the sea. Differences between surfaces during the case study were found, showing wallets as the best surface for sea squirts and cement plates for barnacles in general. Cement can be considered as the best surface for recruitment for the invasive *Amphibalanus amphitrite* in relation with the huge abundance found. The other two invasive *Amphibalanus* cf. *eburneus* and *Styela plicata* found during the experiment, did not present any preference between surfaces. So in general for the three invasive species of this area the use of cement plate guarantees a high rate of recruitment.

Key words: Invasive species, macroinvertebrates, sea squirts, barnacles, Algarve, southern Portugal.

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LISTS OF ABREVIATION

ANOSIM: Analysis of similarities

ANOVA: Analysis of variances

BOE-A: Boletin Oficial del Estado-Andalucía

CBD: Convention on Biological Diversity

CIESM: Mediterranean Science Commission

d: Margalef diversity index

DAISIE: Delivering Alien Invasive Species Inventories for Europe

EG: the Exotic Guide

Foc %: percentage of Frequency of occurrence

GLERL: Great Lakes Environmental Research Laboratory

H': Shannon-Wiener diversity index

INSPECT: The project "*Espécies exóticas marinhas introduzidas em estuários e zonas costeiras Portugueses: padrões de distribuição e abundância, vectores e potencial de invasão"*

IO-FCUL: Instituto de Oceanografia, Faculdade de Ciências da Universidade de Lisboa

I.P. (IPTM): Instituto Portuário e dos Transportes Marítimos

ISSG: Invasive Species Specialist Group

IUCN: International Union for Conservation of Nature

J: Pielou evenness index

LPN: Liga para a Protecção da Naturaleza

MarLIN: The Marine Life Information Network

MDS: Multidimensional organization method

MPA: Marine Protected Area

MSFD: Marine Strategy Framework Directive

NHM: Natural history museum of UK

NOAA: National Oceanic and Atmospheric Administration

NOBANIS : European Network on Invasive Alien Species

NSS: National Strategy for the Sea

S: Species richness

SIMPER: percentages of similarity method

UAC: Universidade dos Açores

UE : Universidade de Évora

WoRMS: World Register of Marine Species

WRIMS: World Register of Marine Introduced Species.

1. INTRODUCTION

In the last decades global biodiversity has experienced several important threats. Biological invasions together with global climate change, habitat destruction and overfishing are now accepted as the 4 largest human impacts on nature. A major concern deals with non-native species that compete and overgrow the native existing fauna. This biological threat occurs when an organism or group of organisms are introduced (accidentally or intentionally) and establish out of their distribution area (Williamson 1996); it happens when a species is settled in a place where it has never been before (Shigesada & Kawasaki, 1997 *in* Silva *et al.*, 2008)

Exotic species have been considered by several authors as the worst threat that can occur to native biodiversity (Vitousek *et al*., 1997 in Carlton, 2001). An example, pointed out by several authors is the Japanese oyster drill, *Ocenebra inornata* (Récluz, 1851) (Goulletquer *et al.* 2002, Buhle & Ruesink, 2009 and Afonso, 2011). This invasive marine gastropod that originates from the NW Pacific region is not only considered as a threat to oyster farming in North America and Europe, for predation of juveniles (seeds) of oysters, but also to local biodiversity by consuming mussels, native bivalves and barnacles.

Another example is the North Pacific sea star, *Asterias amurensis* Lutken, 1871, which has increased by over 100 million individuals showing higher biomass than all species caught in the San Francisco bay. This species covers about 1500 km^2 of bay and predates on many native species of benthic invertebrates (Bax *et al.*, 2003).

Others surveys report significant impacts on local economies and human health (Schaffelke *et al*., 2006). The European green crab, *Carcinus maenas* (Linnaeus, 1758), native to Europe and North Africa has been found almost all over the world. This important invasive species, listed among the 100 "world's worst alien invasive species is reported to severely affect the bivalve fisheries in Northern America (Lowe *et al.*, 2004).

The Asian clam *Potamocorbula amurensis* (Schrenck, 1861) is also considered as one of the 100 world´s worst alien species (Lowe *et al.*, 2004). According to Bax et al. (2003) it’s responsible for the collapse of the local fisheries of San Francisco Bay by covering the seafloor.

Exotic species have also been linked to changes in the structure of a given ecosystem, modifying the food web and changing the faunal community (Thresher, 2000 *in* Raffo *et al.*, 2014). An example is the New Zealand screw shell, *Maoricolpus roseus* (Quoy & Gaimard, 1834) that has invaded Australian waters. This gastropod invades soft sediments and maybe found in very high abundances. Their shells are an attachment point for other marine fauna (Bax *et al*., 2003).

To distinguish differences between a native and a non-native species the International Union for Conservation of Nature (IUCN) defines as native "a species, subspecies or lower *taxon* which occurs within its natural range of dispersal potential" and as exotic "a species, subspecies or inferior *taxon* introduced outside of its natural distribution range that might survive and reproduce" (Silva *et al.*, 2008). An exotic, introduced, non-indigenous, non-native or alien species make mention to the same thing, so any of these terms could be use to describe these species (National Oceanic and Atmospheric Administration (NOAA, 2015)).

Exotic species that occasionally reproduce outside of a "farming" area or captivity but cannot maintain a population in stables values without human intervention and need to repeat the introduction to persist in the new ecosystem are normally labelled as casual or non-established species (Pysek*et al.*, 2009). On the other hand a naturalized or establishedexotic species are the ones that build up stables populations maintained for themselves and preserved in a wild region without help of humans (Occhipinti-Ambrogi & Galil, 2004 and Pysek *et al*., 2009).

Non-indigenous species are generally defined as invasive only if they are able to spread by expanding their range beyond their points of initial arrival or introduction (Martel *et al*., 2004; Richardson *et al.*, 2000 and Rehage & Sih, 2004 *in* Silva *et al.*, 2008). Thus, dispersal ability is generally expected to be a key factor determining invasion success (Ehrlich, 1986; Sakai *et al.*, 2001 *in* Silva *et al.*, 2008).

According to the IUCN (2015) exotic invasive species can be defined as "an exotic species which established in a natural or semi-natural ecosystem and is an agent for change and a threat to native biodiversity".

Another definition of alien invasive species from Nature and Biodiversity Heritage is a species which is introduced or established in an ecosystem or in a natural or semi-natural habitat and acts as an agent of change causing threatens to the native biological diversity, either by its invasive behaviour or by the risk of genetic contamination (LEY 42/2007, de 13 de Diciembre, Del Patrimonio Natural y de la Biodiversidad, BOE-A-2007-21490).

Under the Convention on Biological Diversity (CBD), invasive alien species (IAS) are those that are introduced, establish or naturalize, and that spread outside of their home range, and whose impacts involve significant harm.

Of the three interpretations to characterize what is an alien invasive species, the one by UICN seems to be the most appropriate definition for this study and will be the chosen one.

According to Parker (1999) the biological invasions that currently exist around the globe have been produced intentionally, in the past to improve the lack of food, to provide medicines or even for aesthetic reasons. However the majority of species have been introduced accidentally through crop contaminants or any other way of transport from their native to the invaded zone (Rejmánek *et al.*, 1991 *in* Silva. *et al.*, 2008). It has been stated that some species by its own means would need 5000 years to reach a new region, while today in one day it could reach a new biogeography area (Vilá *et al.*, 2008).

Today human development is the major cause for rapid dispersal of invasive species, due to the intensification of trade and altered ecosystems through multiple human movements (Vilá *et al.*, 2008; Williansom, 1996 and Silva *et al*., 2008).

In the marine environment dispersal vectors for invasive species have been increasing over the years. The main mechanisms are currently ballast water and fouling on ship hulls, followed by fisheries which include aquaculture involving the cultivation of marine organisms for food or other proposes. Other important vectors are public aquariums, although private aquariums with pets are also contributing for this problem (Carlton, 2001; Wittenberg & Cock, 2001).

It is interesting to know that in Europeover 300 species that originated from the Red Sea have invaded the Mediterranean Sea via Suez Canal in less than a century (Vila *et al.*, 2008). It can be said that this is the main door for the introduction of marine invasive species in European waters.

Other sets of input mechanisms that help invasions to occur are the use of live bait and subsequent released, oil rigs, seaplanes, navigational buoys, floating marine debris, diving equipment, dry docks, certain research activities, and restorations carried out in natural ecosystems (Carlton, 2001); however these being more important in some areas than in others.

According to Parker (1999) impacts can manifest at five levels: a genetic level, individuals, populations, communities and ecosystems. But it cannot be forget the yield losses of anthropogenic activities such as agriculture, fisheries and aquaculture, and damage to infrastructure and stored products, plus the cost to control these invasions (Silva *et al*., 2008).

In order to know what invasive species may cause larger damage to the ecosystem and to the native fauna at a global or national level there is a growing need to create specialized species lists for this propose.

Several studies were conducted to create different lists of invasive species globally; also European and national lists were created. Today a list of the worst global invasive species can be obtained from the Invasive Species Specialist Group (IUCN/ISSG) at: http://www.issg.org. Some of these species compose several groups of marine invertebrates.

According to the IUCN/ISSG (2015) worldwide list the most important marine invasive marcoinvertebrateof our group of interest are bryozoans (e.g. *Schizoporella unicornis, Watersipora subtorquata, Zoobotryon verticillatum*), tunicates (e.g. *Ciona intestinalis, Styela clava, Styela plicata,*), crustaceans (e.g. *Carcinus maenas, Chthamalus proteus, Elminius modestus)* and molluscs (e.g. *Crassostrea gigas, Crepidula fornicata, Mytilopsis galloprovincialis, Ostrea edulis, Perna perna,*).

There are many other lists of invasive species for Europe, another one of them is developed by Delivering Alien Invasive Species Inventories for Europe (DAISIE, 2015) with the 100 most dangerous invasive species. In this list many species belong to macroinvertebrate category, (e.g. molluscs: *Musculista senhousia, Brachidontes pharaonis* and *Crassostrea gigas*; custaceans: *Marsupenaeus japonicus, Balanus improvisus* and *Paralithodes camtschaticus*; tunicates: *Styela clava*).

Certain species of macroinvertebrates may be found in both lists. Some examples are the tunicate *Styela clava* and molluscs such as *Musculista senhousia, Crassostrea gigas, Crepidula fornicata* and *Rapana venosa*. This clearly reflects that certain species are highly damaging to the ecosystem they invade.

According to Katsanevakis *et al*. (2013), Portugal is included within the 13 countries with the lowest number of reported marine alien species. Katsanevakis *et al*. (2013) also mentions the lack of online databases in these countries, namely Portugal.

At the Portuguese level, between the years of 2004 and 2006, the "Instituto de Oceanografia, Faculdade de Ciências da Universidade de Lisboa (IO-FCUL), the *Universidade dos Açores* (UAC), the *Universidade de Évora* (UE), the *Instituto de Conservação da Natureza e da Biodiversidade* (ICNB), the *Instituto Portuário e dos Transportes Marítimos* (IPTM) and the *Liga para a Protecção da Naturaleza* (LPN)" carried out the project *"Espécies exóticas marinhas introduzidas em estuários e zonas costeiras Portugueses: padrões de distribuição e abundância, vectores e potencial de invasão - INSPECT"* in order to evaluate the current status of the invasive species occurring in Portugal.

Through INSPECT some macroinvertebrate invaders were discovered in Portugal. The phylum Cnidaria, Annelida, Mollusca, Arthropoda, Chordata and Bryozoa were highlighted in this project. Several major invasive species were dated before and during this project such as the Black sea jellyfish (*Blackfordia virginica* (Mayer, 1910)), the tubeworm *Ficopomatus enigmaticus* (Fauvel, 1923), the Striped barnacle (*Amphibalanus amphitrite* (Darwin, 1854)), the bryozoan *Zoobotryon verticillatum* (Della Chiaje, 1828) and the Golden star tunicate (*Botryllus schlosseri* (Pallas, 1766)).

Through the INSPECT project, other important invaders such as the Asian clubbed tunicate (*Styela clava* (Herdman, 1882)) reported for the island of São Miguel (Azores) and the Japanese oyster (*Crassostrea gigas* (Thunberg, 1793)) for the entire coast of Algarve (southern Portugal) were also reported.

However, it must be said that there are still very few studies when it refers to invasive marine macro invertebrate species present in Portugal; most works like Ribeiro *et al*. (2009) have been conducted on invasive marine algae like the Wireweed *Sargassum muticum* ((Yendo) Fensholt, 1995)) and for freshwater fishes (e.g. the

Goldfish *Carassius auratus* (Linnaeus, 1758) or the Tinfoil barb *Barbonymus schwanenfeldii* (Bleeker, 1854)).

The lack of information in Portugal led Chainho *et al*. (2015) to conduct a compilation and updated work on invasive marine species occurring in this geographical region. The update accounted species reported from continental Portugal (which includes estuaries, coastal areas and lagoons) and also the ones found in the Macaronesian Islands of the Azores and Madeira. The output of this study resulted in alist of 133 species clustered in distinct 12 phyla know until the year 2015. Chainho *et al.* (2015) may now be considered as the most comprehensive compilation of marine invasive species occurring in Portugal and serve as a useful step stone mark for future related studies.

From a regional point of view, some invasive species have already been reported from the Algarve coast located in southern Portugal. Some examples are the Brine shrimp *Artemia franciscana* (Kellog, 1906) studied via water birds and recognized in Castro Marim by Green*et al.* (2005). Chícharo (2009) conducted a study on the potential impacts of the hydromedusa *Blackfordia virginica* Mayer, 1910 and the Oriental shrimp *Palaemon macrodactylus* Rathbun, 1902in the Guadiana estuary.The Japanese oyster drill *Ocenebra inornata* (Récluz, 1851) was also reported for a long time series in Sagres by Afonso (2011). However, there is still much to be done making this one of the main reasons for choice of this study.

Such being said this study primarily focused on the detection and taxonomic validation of invasive marine tunicates and barnacle species, occurring in some of the most important ports and marinas in the Algarve, southern Portugal. Species composition was studied seasonally and comparisons made between western, centre and eastern ports, between marinas with national and international vessel traffic *versus* local fishery ports with regional boat traffic and ports exposed to open ocean conditions (unsheltered) *versus* ports situated in a closed lagoon system, Ria Formosa (sheltered). Finally, a case study to determine the best surface (closed plastic wallet filled with stones, PVC surfaces and cement plates) for barnacle and tunicate attachment was carried out. Community changes were verified by the removal of invaders from theses surfaces during this case study.

2. MATERIALS AND METHODS

2.1. CHARACTERIZATION OF STUDY AREA

The Algarve is located in the south of Portugal, highlighting Faro as capital of this region. Eastward includes the coordinates 37º 1’ N and 8º 59’O (Sagres) to37º 11’ N and 7º 24’ O (GuadianaRiver) and northwards from Sagres to 37º 26’ N and 8º 48’ O (Odeceixe). This area is visited during the summer by millions of foreign tourists for its beaches and natural areas.

The Algarve coastline is characterized by a high lithological and morphological diversity (Dias, 1988). It is the most southern region of Portugal and has about 458,000 inhabitants (Aguilera & Botequilha-Leitão, 2012). It has a width of 50 km from Sagres to Odeceixe and about 160 km from Sagres to the GuadianaRiver (Dias, 1988). The marine platform has a small size among 7-28 km, extends to small depths (110-150 m) and is characterized by simplicity in its forms (Magalhães, 2001).

In regard to climate differences may also be found. The northern part of Cabo de São Vicente, an open region where the Atlantic maritime agitation is dominant, is characterized by strong to moderate winds and wave action (Dias, 1988). The cliffs of carbonate rock are the most prevalent in this coastal area. In contrast from Cabo de São Vicente eastward warmer conditions prevail and the wave’s action ismoderate (Dias, 1988). An extensive coastline with sandy ridges is what characterizes this part of the coast.The temperature of the seawater in the Algarve coast lies between 21ºC in August and 17ºC in January, leading to stratification during the summer months. Below the 50 m depth mark temperatures of 15° C are normally recorded year round (Sanchez & Relvas, 2003).

According to Aguilera & Botequilha-Leitão (2012) the littoral concentrates 90% of the population and where coastal activities are predominant. This area is characterized by a high development and population dynamics, which is driven by the increase in urban population in the municipalities with major tourist character.

Regardless of high dynamism several protected areasmay be found in the Algarve region. There are about 8.6% of protection for terrestrial habitats and nearly 60% for the coastal zone areas, which increases its coastline interest (Aguilera& Botequilha-Leitão, 2012).

2.2. SAMPLING AREAS

The study area corresponded to the different recreational harbours/marinas and fishing ports located in the Algarve region in southern Portugal. The most important oneswere selected for this survey which are characterized by a large transit of foreign vessels entering the Algarve coast, mainly during the summer months, so there is a risk increase of embedded species in the hulls of ships or/and the transfer of fouling species between different countries, for that reason seasonality was studied (winter, spring and summer).

Another aspect considered in the choice of these ports and marinas were their geographic location (western *versus* eastern *versus* centre ports) and the port location (sheltered *versus* unsheltered ports and harbours).

The sampling was carried out in areas where both local fishing boats and recreational boats (sailboats and private boats) arrive to the port. The sampling areas chosen for this study are pointed out in Figure 2.1 and are represented by the localities ofLagos, Vilamoura-Quarteira Faro and Olhão. The coordinates of the sampling areas are detailed in Table 2.1.

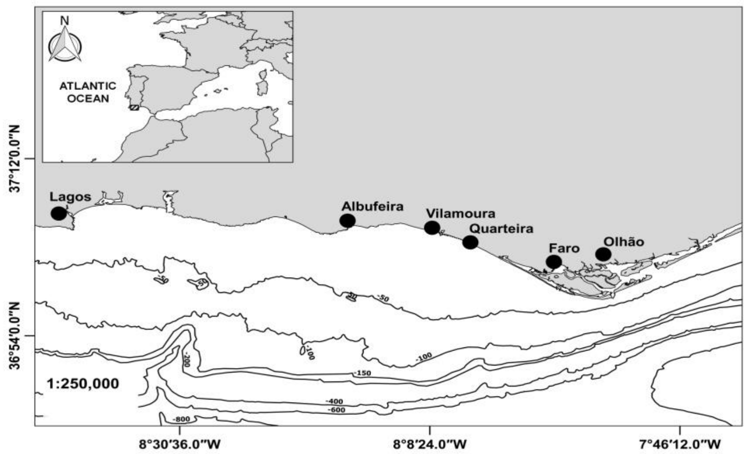

Figure 2.1 The different sampling areas in the Algarve region, southern Portugal representing by the localities of Lagos, Vilamoura-Quarteira, Faro and Olhão (image by Pedro Monteiro).

The Lagos areais characterized by a fishing port and a recreational marina located just a few hundred meters apart and both in the estuary of a small river, Ribeira de Bensafrim. In this study area sampling was conducted at two different points, one corresponding to the fishing port and another in the marina.

In the Vilamoura locality, where the largest recreational marina of Portugal is located, sampling took place in the more confined areas and the main dock where all foreign vessels have to check in when entering the marina. Adjacent to the Vilamoura marina we may find the fishing port of Quarteira. At this location points located on opposite sides of the entrance, where the fishing boats enter the port, was studied.

Also Olhão was sampled and there it can be foundone of the largest fishing ports of Algarve.In this area fishing port and a recreational marina are not so close to each other, they are positioned just a few km apart. So the sampling took place at the two different zones (marina and fishing port).

The last sampling zone was conducted at Faro. Similar to that of other sampling locationsthis area is characterized by fishing and recreational port and both of them were sampled.

Faro and Olhão are located in the Ria Formosa which, according to Ribeiro *et al*. (2006 and 2008) is characterised by having calm waters besides a good conditions to the species to live there. The Ria Formosa has been classified as natural park since 1987 and also accepted as a Natura 2000 and Ramsar site.

Table 2.1 Coordinates of the different sampling areas located in the Algarve region, southern Portugal.

Location	**Latitude**	**Longitude**
Lagos	37° 6' 19.45'' N	8°40' 17.25'' W
Vilamoura	37° 4' 19.69'' N	8° 7' 26.59'' W
Quarteira	37° 4' 03.26'' N	8° 6' 35.85'' W
Faro	37º 0' 33.92'' N	7º 56' 23.55'' W
Olhão	37° 1' 28.68'' N	7°50' 5.98'' W

2.3. MAIN STUDY GROUPS OF INTEREST

The groups of invertebrates that this study was mainly focused on are tunicates (also known as sea squirts) and barnacles. A brief description of each group is listed below:

1 - Sea squirts are marine invertebrates which are sessile as adults. They belong to the group of tunicates, characterized by having a tunic around the body. They

are both efficient filtering plankton and particulate organic matter in the water column. They are hermaphrodites. They can be both solitary (sexual reproduction) as colonial that can be both asexual and sexual (Sanchez, 2013).

2 - Barnacles are crustaceans that as adults are usually sessile and embedded to other objects or hard substrate (fixed or floating). Its carapace covers their entire body helping with the excretion of calcareous shell. The majority of adult barnacles are easily recognized as crustacean when they are dissected however, their body has experienced an evolutionary reduction and some of its crustacean features have been lost(Newman *et al.,* 1980).

2.4. SAMPLING TECHNIQUES

Fishing and recreational ports/harbours and a marina were inspected in order to detect and identify potential invasive species. Invasive, native, established and unknown status were the categories in which the species found were classified.

At each targeted location sampling was carried out seasonally (between winter and summer 2015) and according to the tidal cycles. Low tide is the best time to identify and sample specimens of interest, which are normally exposed. The scraping of walls, poles and rocks, where mainly barnacles attach to, was sampled using a quadrate. Observation of ropes, buoys and deployed devices where mussel rafts are formed was inspected for fouling species such as sea squirts.

2.4.1. BARNACLE IDENTIFICATION AND COUNTING

Before the field work began barnacles species were studied with some level of detail using species identification sheets listed in ANNEX I. This would be useful to latter be able to identify *in situ* many of the native and non-native species during the sampling research. At each of the targeted localities the best fixation substrates for barnacles were identified before performing 10 randomly selected points at mid tide level. This group of organisms was particularly common on artificial rock walls, boulders and pillars. In each one of the random samples a quadrate (10cm x 10cm) was used (Jekins *et al*. 2000) to determine barnacle densities or abundances (Figure 2.2).

In order to analyze and count the number of species present in each quadrate at the laboratory facilities digital images were taken *in situ*. Additionally, a few specimens of each of the observed species were collected using the scraping method. These specimens would be used for taxonomical purposes in order to differentiate the different native and non-native species to serve as back up identification material.

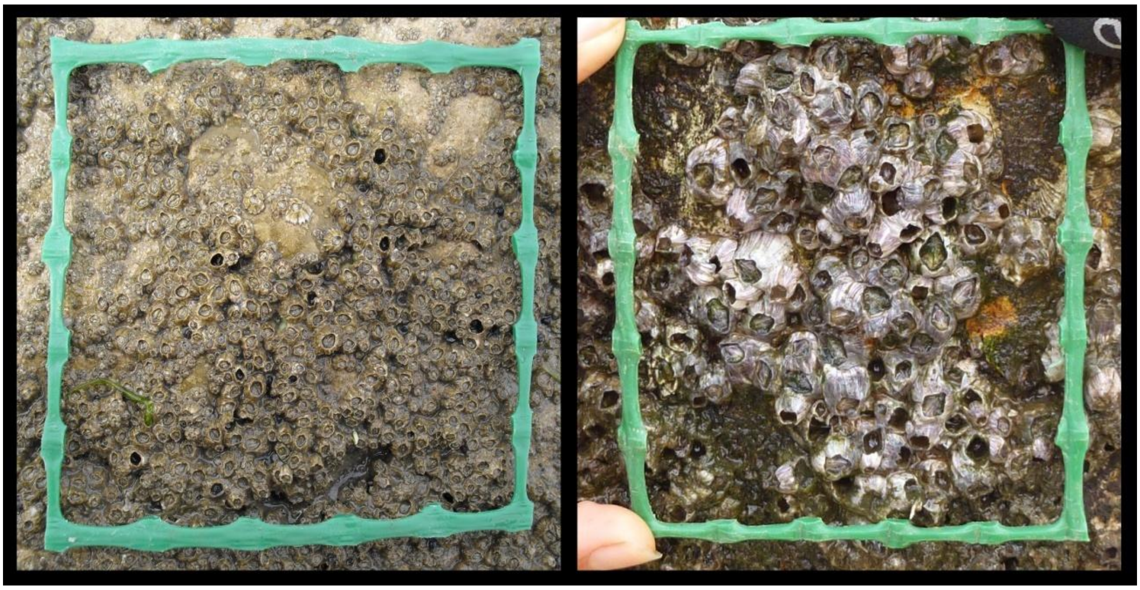

Figure 2.2 Examples of grids (10cm x 10cm) used in the recreational port of Lagos and fishing port of Quarteira for barnacle counting (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015) .

2.4.2. TUNICATE IDENTIFICATION AND COUNTING

Similar to what was done with the barnacles, 10 random points where sampled in each study ports/harbours and marina for the tunicates. The best way to count these organisms is by using underwater grids but it was not possible to carry out diving activities due to the water quality in ports (sewage discharges and high turbidity which involving low visibility), in addition to bureaucratic problems (to obtain license for diving activities in ports) and finally for security purpose (especially in fishing port where the traffic boat is quite busy).

Here, instead of using the quadrate method, a qualitative analysis ofthe proportion of sea squirts in the different port was performed. For that, 30 sea squirts were randomly counted and individually identified *in situ* for each randomly chosen point (Figure 2.3). This would allow determining the relative proportions of each species at each study port area and to compare between them. Before field work began this group of organisms was also intensively studied using species identification sheets listed in ANNEX I. All the species found during winter were well studied and identified by dissection before the counting process was carried out.

Sea squirts are not identified easily by their external morphology and in most cases need to be dissected for proper taxonomical identification. To solve the problem of *in situ* identification, external characteristics patterns were established as key factors to differentiate tunicate species in the field. Three significant external characteristics for each species were selected to create this following identification key:

Styela plicata

- Rough and stiff tunic showing bumps.
- Four-lobed siphons showing red or purple stripes on the inside of the siphons.
- The colour of the tunic varies from light white to gray.

Microcosmus squamiger

- The smooth tunic is not very stiff, presenting a black-purple colour.
- Elongate and red siphons showing white or yellow stripes.
- Siphons separate approximately one third of the total length.

Molgula occidentalis

- Small and rounded sea squirt.
- Leathery and dark tunic.
- Short and red siphons located closed to each other, making difficult the difference between oral and atrial siphons.

Phallusia mammilata

- Very big sea squirts with a leathery and smooth tunic.
- The colour of the tunic varies from white to light brown.
- Siphons separate approximately one third of the total length and with the oral opened it is possible to see the smooth oral tentacles.

Ciona intestinalis

- Elongate, thick and transparent tunic.
- Slightly pronounced siphons (very wrinkled). The oral used to be bigger than the atrial siphon.
- Very soft yellow tunic.

Styela canopus

- Small and rounded sea squirts with transparent and soft tunic.

- Short red or orange siphons located closed to each other, making difficult the difference between oral and atrial siphons.
- The tunic presents a light orange colour.

Ascidiella aspersa

- Small and transparent tunic.
- Siphons not very long from each other, but more separated than *S. canopus* or *M. occidentalis*
- Mostly all the internal organs could be observed through the tunic.

To minimize systematic errors the same researcher carried out the same species counting procedure along the entire study period. Additionally a portion of the specimens identify *in situ* were randomly selected and brought back to laboratory facilities for taxonomical re-confirmation; 100% of success was obtained using the purposed external identification keys listed above.

An attempt to estimate the total amount of sea squirts occupying each study area was also attempted by direct observation. Here, different values in relation to the amount of sea squirts observed at each study area were established:

1→>1.000 organisms

2→ between 1.000-10.000 organisms

3→between 10.000-100.000 organisms

4→>100.000 organisms

Figure 2.3 The counting procedure at the fishing port of Olhão: a rope with several sea squirts (Image by Sofia Tristancho and Carlos M. L. Afonso, Algarve, Portugal, 2015).

2.4.3. CASE STUDY

A case study was performed with the main objective to obtain surface´s preference of invasive sea squirts and barnacles recruitment and see if there are communities' alterations with the removal of invasive species.

To study the alteration in the communities through the times of visits 5 different groups, 1- first observation, 2- second observation and first removal, 3-third observation and second removal, 4- last observation and final removal and Control (remain all the experience in the water not removal) were established and 3 different surfaces were also taken into account to this purpose: a) closed plastic wallet filled with stones, b) PVC surfaces and c) cement plates. These 3 types of surfaces are proxies of complex materials (artifacts, fishing gears) and the general construction materials found in ports (pillars, piers, passage ways, berths, wharfs ...)

For this purpose 10 ropes with 3 panels of different material surface were deployed at the recreational port of Olhão which has a high abundance of sea squirts. The methodology carried out was adapted from Blum *et al.* (2007).

The first panel of the device was 11cm x 11cm closed plastic grid wallet filled with smooth river stones, the second 11cm x 11cm panel composed of a PVC surfaces and a third of cement plate with an area of 11cm x 11cm (Fig. 2.4). On the upper portion of each rope containing the 3 panels a white plastic identification tag (with the university name and a telephone contact of the researcher) were placed.

The total length of each individual device was 2.2 m and the distance between all the surfaces was aproximately 19 cm between each material surface. All case study devices were deployed at a suspended depth of 1m from a floating dock for four and a half months and at different locations distributed around the port of Olhão. The devices remained in the water one and a half months before any kind of treatment began.

Two different treatments were tested while performing this case study: the removing treatment and control treatment. In both procedures cases 5 replicates used. Fouling panels with invaders removed and not removed were compared to determine how invasive species can affect the community structure.

Removing treatment:

For this treatment once a month the suspended devices were retrieved from the water and all the invasive sessile species were recorded and then removed using a tweezers.After the removal the devices were returned to the water. All the invaders taken for each of the panel were counted, labelled and kept in individual plastics bags, previously filled with sea water, and brought to the laboratory facilities to re-confirmation identity.

Control treatment

For this treatment once a month the suspended devices were retrieved from the water and all the invasive sessile species were recorded: At the end, ropes were removed and panels kept in plastic containers with sea water suitable for transportation to the laboratory facilities. All species attached to panels were counted and identified.

Figure 2.4 The three different material surface components used in the case study performed at the Olhão recreational port. A) Plastic grid wallet filled with stones, B) PVC surfaces, C) Cement plates and D) The final device with the 3 surface components and identification tag on the upper portion.

2.5. LABORATORY METHODS

In the laboratory facilities, researchers analyse the samples in order to differentiate a native from none native species. Once a species was considered none native a taxonomic validation process was initiated with the help specific literature and websites specialized in the identification of invasive species. The most important bibliographic references and specialized websites were used:

Cirripedia (barnacles): Southward (2008).

Catalogue des principales salissures marine. Vol 1. Balanes: Organisation de cooperation et de développement économiques

Mollusca (bivalves andgastropods): Poppe & Gotto (1993), Gofas *et al.* (2011); Macedo *et al.*(1999); Nobre (1938-40).

Bryozoa (false corals): Lopez (1990); Hayward (1985), Hayward & Ryland (1985; 1998 & 1999); Gómez J.C.G. (1995).

Tunicata (ascidians or tunicates): Monniot & Monniot (1972); Ramos (1991);

In addition to the literature some web specialized in invasive species were also used for species identification procedure.

http://www.exoticsguide.org

http://www.ciesm.org

http://www.europe-aliens.org

http://www.marlin.ac.uk

http://www.issg.org

http://www.nobanis.org

http://marinespecies.org

http://www.marinespecies.org/introduced

http://species-identification.org

After taxonomic validation some individuals of the invasive invertebrates were kept in an aquarium. This process was maintained specimens alive making them useful to be photographed or to serve as backup in the case further confirmation or comparisons might be needed.

Another portion of the individuals was preserved in 70% ethanol solution (barnacles) or in formalin 10% (tunicates); all properly labelled with the indication of species name, sampling place and date of capture for future ID confirmation or genetic studies (in the case of the barnacles).

Finally, the help of different specialists in each of the studied groups was the final process for the re-confirmation status of each invasive species found. Images of the species as well as preserved specimens were sent to specialists for this final determination.

Barnacle counting

Images of each randomly selected point using the grid method were properly labelled and organized in an Excel computer database. To count the number of individualsper species in each quadrate *Image J* program was used (Collins, 2007). With this program it is able to associate a number and a colour to each species and to live and dead specimens (Figure 2.5). At the end of each counting process an Excel sheet was obtained showing the final number of individuals per species. Data was then grouped in a database specifying the location, season, port type, position, number of the grid and port features in addition to taxonomic information and author of each species.

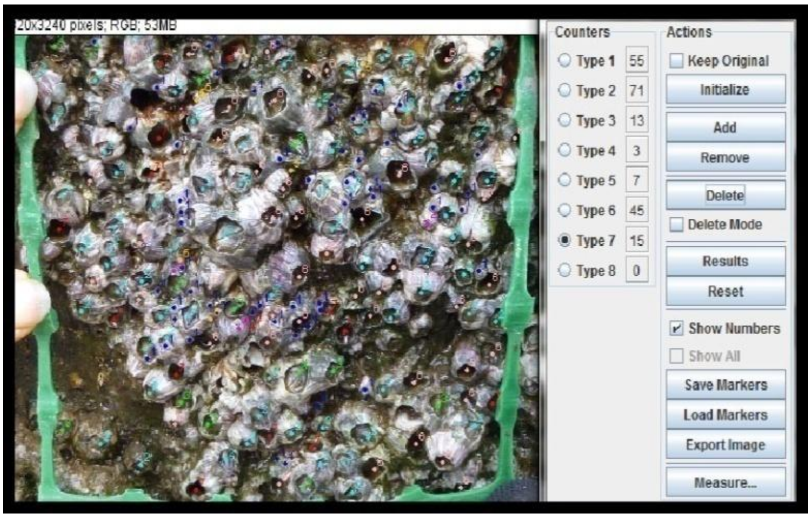

Figure 2.5 An example of a grid (10cm x 10cm) representing the different species using the Image J program. Type 1 (dark blue) - *Chthamalus montagui*, Type 2 (turquoise) - *Amphibalanus amphitrite*, Type 3 (green) – *Amphibalanus* sp., Type 4 (purple) - *Austrominius modestus*, Type 5 (orange) - *Chthamalus montagui* empty shell, Type 6 (pink) - *Amphibalanus amphitrite* empty shell, Type 7 (red) - *Amphibalanus* sp. empty shell, Type 8 (yellow) - *Austrominius modestus* empty shell, Type 9 (grey) - *Perforatus perforatus* and Type 10 (light blue) - *Perforatus perforatus* empty shell.

Sea squirts dissection

To correctly identify sea squirts species a dissection process is normally recommended. Dissection methodology was adapted from Ramos (1991).However, before dissection takes place some fixation and preservation procedures must be performed in order to completely relax tissues. The first step is finding an appropriate anaesthesia for this group of organisms. This is a delicate and critical process; if the anaesthesia's effect lasts for a long period, the internal tissues maybe be damaged, on the other hand, if the process is not completed in due time the contraction of the individual can occur. The relaxation of the individuals is fundamental, for that a mixture of sea water and menthol crystals was prepared. Menthol crystal helps the relaxation process but may take a few hours for its full effect. To know if the anesthesia process is working when the siphons stop responding to touch or when the individuals are removed from the water and there is no contraction. At that moment the relaxation process is completely finished.

After relaxation, a fixation process based on a formalin solution of 1:10 should be used. The solution is prepared with *Formaldehyde 35-40% w/v stabilized with methanol QP* and a sodium borate dust is added to neutralize the formalin until the saturation point was reached. This procedure is done to preserve the calcareous structures and not be dissolved (Machado, 2007), as certain species present calcareous syphonal spines (Barnes, 1987). But in our case, the species studied showed chitinous spines (Turron, 1987).

All dissected individuals were preserved in this fixation dissolution for at least 48h (for the Didemnidae, also known as mate tunicates, the fixation process needs at least one month to be effective). According to Ramos (1991) to preserve dissected specimens, the fixation solution can be used as a liquid but has to be dissolved to a 5% solution of formalin. It is not advisable to use alcohol or any acidic solution as fixation liquid with the risk that the calcareous spicules can be dissolved. The only exception is the interstitial sea squirts that according to Monniot (1972) can be persevered with a 75% alcohol concentration solution. Before specimens were studied the formalin used in the fixation process has to be removed because of its toxicity. For this purpose, persevered individuals were placed under running water for at least an hour and then placed in a container with fresh water to rest.

After the formalin was removed the dissection procedure took place with the help of scissors and ophthalmic tweezers. For solitary sea squirts, as the ones studied, a few steps have to be followed. These steps are described bellow:

1. To remove the tunic: different ways are possible to carry out this task. The one used during this studywas an incision on the oral siphon all around the sea squirt to reach the cloacae siphon.

2. To open the sea squirt: the same procedure was carried out for the mantle, an incision from the oral to the cloacae siphon following the endostyle. It is important to perform this step very carefully not to damage the internal structures; this process should be performed with the use of a binocular lends.

3. Preparation of individual: The opened individual was extended, put on a thin sponge and placed on Petri dish. Needles were used to keep immovable the opened individual above sponge; branchial sacs were removed showing the internal structures (Fig. 2.6). The dish was filled with water to facilitate the identification process.

4. Identification: the final step was the identification process, carried out with the use of specific dichotomy keys (e.g. Ramos, 1991). Different internal organs were analyzed as branchial sacs, oral tentacles, gonads and vibrating tubercle (Figure 2.7).

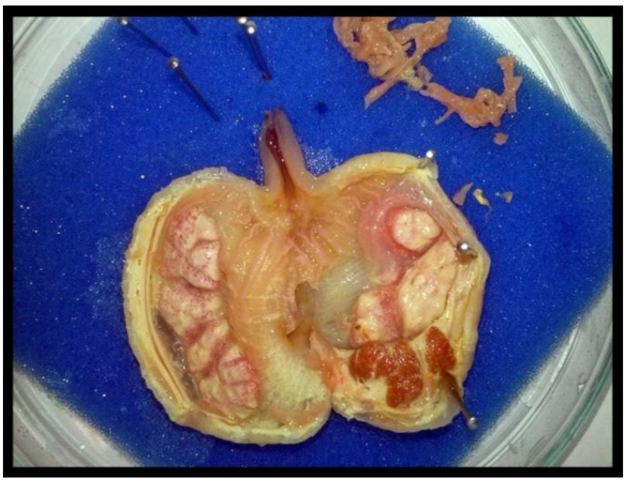

Figure 2.6 Dissected specimen of *Microcosmus squamiger* Michaelsen, 1927 with most of the branchial sacs removed and showing all the internal structures (Image by Sofia Tristancho, Algarve, Portugal, 2015).

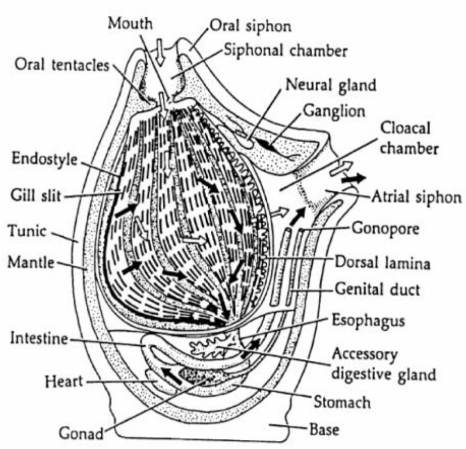

Figure 2.7 Explanatory drawing showing all the internal structures of a sea squirt (adapted from Satoh, 1994); some of these structures are useful to be kwon before using the identification dichotomy keys.

2.6. INTRODUCTION VECTORS

An attempt to explain what introductions vectors are mainly responsible for the dissemination of invasive species in ports and marinas in the Algarve was undertaken.

The invasive species studied are mainly sessile and encrusting animals that usually arrive by fouling on ship hulls. But there are other means by which mobile species reach new communities (e.g. fisheries and aquaculture).

So being said the different input vectors for invasive species to arrive in a new ecosystem are of high importance and need to be taken into account. In this work the identification of the main mechanisms of introduction of marine invasive species and the evaluation of the risk of each dissemination vector were done based on the study of Carlton, (2001) (Table 2.2).

Table 2.2 Main introduction vectors and their risk for the transport of marine invasive species (Adapted from Carlton, 2001).

Vectors	Transport	Risk
Aquariums (public and private)	Organisms transported with target species which accidental or intentional are released.	(medium)
Ballast Water	Organisms floating in loading areas	(high)

Buoys (floating and sailing), Dry docks and Seaplanes	Fouling organisms	(medium)
Divers and diving equipment	Fins, neoprene or other diving equipment.	(low)
Floating marine debris	Species transported by floating elements of anthropogenic origin.	(medium)
Restoration of natural ecosystems	Invading organisms transported accidentally with natives species to restore the native wildlife.	(medium)
Fisheries and Aquaculture	Farmed sea food such oyster, mussels, clams, etc, and those which are released intentionally.	(high)
	The shellfish market exchange and accidental transport of these because of the current growing stocks.	(high)
	The use of live bait and subsequence released.	(high)
	The processing of both fresh and frozen seafood and the discharge of waste that contains living organisms associated or attached .	(high)
Fouling boats	Organisms attaches to hulls, chains and anchors.	(high)
Oil rigs	Organisms attached and free-living and in ballast water.	(medium)

Research activities	Organisms released intentionally o accidentally.	(low)
	Organisms transported in sampling and diving equipment.	(low)

2.7 DATA PROCESSING

The data processing will be performed for all samples during three seasonal periods (winter, spring and summer) along the year 2015. Both barnacles and sea squirts were analyzed with the same process. The case study was performed in different way.

2.7.1 ANALYSIS OF UNIVARIATE STATISTICS

Quantitative index

For each *taxa*a percentage of Frequency of occurrence (Foc %) was estimated. by using the following equation:

$$Foc\ \% = (Ai/At) \times 100$$

Where Ai is the number of samples in which the species/*taxon* (i) is detected and At the total number of samples to be analyzed. A classification of individuals based on their frequency of occurrence was calculated, where R (Rare: Foc < 10%), U (Uncommon: 10% ≤ Foc ≤ 50%), C (Common: 50% ≤ Foc <90%) and VF (Very Common: Foc ≥ 90%).

Diversity index

For biomass data diversity analysis was performed using the Index purposed by Clarke & Warwick (2001):

Species richness (S): This is an index of species diversity that estimates the number of different species represented in a sample/location or area. This could be adapted to none native species by counting the number of invasive species in a given sample, taking into account the sample size.

Diversity index Shannon-Wiener (H'): Is the most widely index used to measure diversity. This index is based on the ratio of species abundance and total species, taking into account their specific richness and evenness.

$$H' = - \sum_i p_i log\ (p_i)$$

The values of *(H')* are typically between 1.5 and 3.5 and rarely reaches 4.5 (Magurran, 1988).

Diversity index Margalef (d): this index incorporates the total number of individuals (N) and the total number of species (S), so its measures the number of species present for a given number of individuals:

$$d= (S-1)/\log N$$

Here the relative importance of species is not taken into account; Margalef Index is based on the number of the total species found. When the diversity of the sampling units is low, this means lower species richness (close to 0) and when the species richness is close to 1, the diversity index shows higher numbers.

Evenness Index of Pielou (J'): This index will give the distribution shape of individuals among the different species:

$$J' = H'/H'_{max}$$

H' is the value of Shannon-Wiener Index and H'_{max} is the maximum possible value for this index. According to Magurran (1988) the index ranges between 0 and 1, being 1 the maximum (the abundance of all species caught is nearly the same) and 0 the minimum (the abundance of one species dominates all others).

An ANOVA and T-test analysis for all the diversity indexes and percentages of abundance were applied to test the existence of significant abundances between all them through all the factor of study.

2.7.2. MULTIVARIATE ANALYSIS

The computer program PRIMER 6 β © (Clarke && Warwick, 2001)was used for the characterization of species based on variability factors as geographical location

(E-W-Centre), the port type (recreational/international andfishing local/regional), port location (sheltered/unsheltered), season (winter, spring and summer)and local (Faro, Lagos, Olhao and Quarteira-Vilamoura).

A numerical matrix was used for each *taxon* at each sampling time (geographical location, type of port, port location, port characteristics, seasonality and port abundance). A square root transformation was applied to the data to increase the importance of the less abundant species. The coefficient of Bray-Curtis similarity was applied to obtain a final similiraty matrix.

Through the multidimensional organization method (MDS) a metric diagram representing the distance between samples as degrees of similarity was obtained (Wickelmaier, 2003). Stress coefficients may be applied to the samples. An analysis of similarity (ANOSIM – Permutation based analysis) was used to detect statistical differences in species composition depending on the required parameters (geographical location, type of port, port location, local and seasonality).

In addition, the contribution of each species was examined whenever differences were shown through the percentages of similarity method (SIMPER) using the index Bray-Curtis dissimilarity (Clarke && Warwick, 2001). This method shows the contribution in percentage of each speciesfor the similarity or dissimilarity between samples (in an increasing order). This allows the identification of the most important species according to the similarity or dissimilarity patterns (Quinn & Keough, 2002).

According with Clarke & Warwick (1994), by using SIMPER, the species which present the ratio Diss/Sd> 1, are those that presented higher consistence between samples. Whilst when this ratio is lower than 1, the variability of the samples can be considered as lower.

Finally for the case study, to determine the alteration produced in the community when the removal of invasive was done and the control, a PRIMER analysis was carried out. But to determine the differences in the recruitment between surfaces and treatments an additional two-ways factorial ANOVA analysis was performed using the free software R-statistical. When significant differences were showed post-hoc comparisons as Tukeys were conducted.

3. RESULTS

In this section data obtained during this study is presented. First, a description of all the Barnacles and Sea squirt species (native, invasive and unknown) observed during the sampling period of the selected ports of the Algarve is given. Average percentage of abundance, frequency of occurrence, diversity index (just for barnacles) and the analysis of dissimilarities through PRIMER-6 are presented. Finally, results of the case study are shown using dissimilarities analysis between samples, analysis of variance (ANOVA) and post-hoc comparison. All scientific names for the studied species have been based on the World Register of Marine Species (WoRMS, 2015 accessed on the 18th of September, 2015).

3.1 Invertebrates sampled and identified in ports/harbour and marinas of Algarve

The method used to detect the species of interest for this study in the selected ports, was direct observation. Table 3.1., displays the category for each of the identified species (invasive, native and of unknown origin) during the study period. Most of the species listed in Table. 3.1 appeared consistently during sampling, while a few others (e.g. *Corella* cf. *eumyota*) were only found sporadically.

Table 3.1 Barnacles and Sea squirts species observed and identified during sampling of ports/harbours and one marina in Algarve, south Portugal.

Species	Native	Invasive	Unknown
Sea squirts			
Ascidiella aspersa (Müller, 1776)	■		
Molgula occidentalis Traustedt, 1883	■		
Ciona intestinalis (Linnaeus,1767)	■		
Phallusia mammilata (Cuvier, 1815)	■		
Microcosmus squamiger Michaelsen, 1927		■	
Styela plicata (Lesueur, 1823)		■	
Styela canopus (Savigny, 1816)		■	
Botryllus leachii (Savigny, 1816)		■	
Corella cf. *eumyota* Traustedt, 1882		■	
Botryllus schlosseri (Pallas, 1766)		■	
Didemnum cf. *vexillum* Kott, 2002		■	
Ascidia cf. *mentula* Muller, 1776	■		
Barnacles			
Amphibalanus sp.			■
Perforatus perforatus (Bruguière, 1789)	■		
Chthamalus montagui Southward,1976	■		
Amphibalanus amphitrite (Darwin, 1854)		■	
Amphibalanus cf. *eburneus* (Gould, 1841)		■	
Austrominius modestus (Darwin, 1854)		■	
Hesperibalanus fallax (Broch, 1927)		■	

Megabalanus tulipiformis (Ellis, 1758)			
Megabalanus tintinnabulum (Linnaeus, 1758)			

A total of 19 species (native, invasive and unknown) were identified during this study: 11spp. of Sea squirts (Phylum Chordata) and 8 spp. of Barnacles (Phylum Arthropoda). Of the total observed species there was a larger percentage of invasive species, 58.3% Sea squirts and 60% of Barnacles.

Additional other invasive species belonging to different taxonomical groups were also detected and identified during this study (table 3.2). A total of 3 spp. of Bryozoa and 3 spp. of Molluscs were confirmed. One of the molluscs, *Pollia assimilis*, was later found to be an invasive species originating for the West African coast. This was the first time that this species was reported in European waters (Afonso *et al*. 2015 under revision).

Table 3.2 Species observed and identified during sampling of ports/harbours and one marina in Algarve, south Portugal.

	Invasive	**Established**	**Unknown**
Bryozoans			
Watersipora subtorquata (d'Orbigny, 1852)			
Bugula neritrina (Linnaeus, 1758)			
Membranipora membranácea (Linnaeus,1767)			
Molluscs			
Chaetopleura angulata (Spengler, 1797)			
Crassostrea gigas (Thunberg, 1793)			
Pollia assimilis (Reeve, 1846)			

None of these taxonomic groups present commercial and/or ornamental interest, so accidental introduction should have been and remain the gateway for most of these invasive invertebrate species.

3.2 Characterization of the sample:

3.2.1 Barnacles identification and counting.

During the sampling period a total of 178 grid squares were used to obtain the data for later analysis. A total of 33,194 individuals were counted among different sampling points. A total of 5 species were identified in the counting grids, 4 of which were correctly classified as species: *Chthamalus montagui, Perforatus perforatus, Amphibalanus amphitrite* and *Austrominius modestus*.The fifth species was listed as doubtful (*Amphibalanus* sp.) and taxonomic identification was only possible at the genus level. Finally, four specimens of one species of gastropod were also found in one grid which was not taken into account for the analysis. In general terms, the species which showed the higher percentage of abundance and frequency of occurrence were *C. montagui*, *A. amphitrite* and *Amphibalanus* sp.

a) Season

When the mean percentages of abundance for the different seasons was studied, the dominance of the native *Chthamalus montagui* was clear, followed by the unknown origin species *Amphibalanus* sp. and the invasive *Amphibalanus amphitrite* (Fig. 3.1). The other two species, invasive *Austrominius modestus* and native *Perforatus perforatus* presented very low percentages of abundance, which are barely noticeable in some cases (Fig 3.1; ANNEX II).

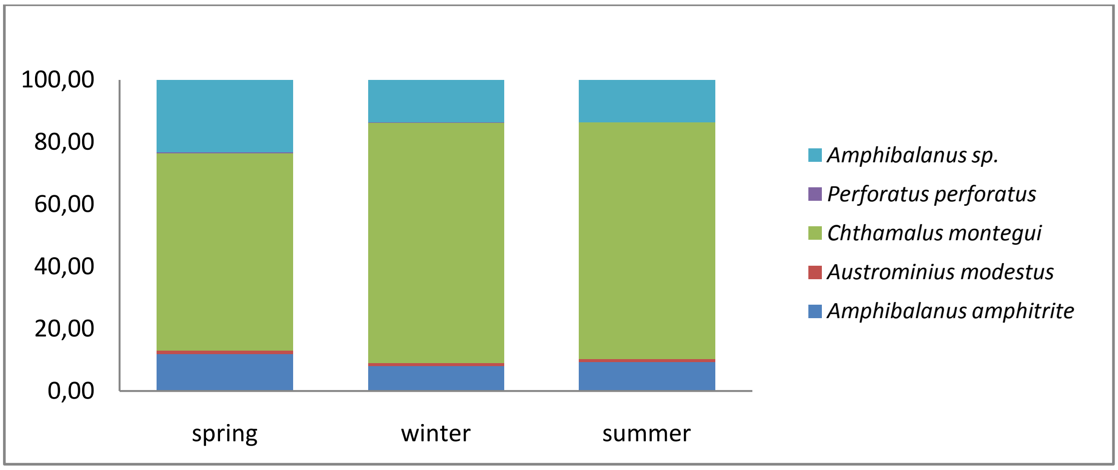

Fig. 3.1 The average percentage of seasonal abundance for barnacle species found in the sampled grids.

For the spring season, mean percentages obtained for *C. montagui* were 64%, for *Amphitrite* sp. 23% and 12% was found for *A. amphitrite*. In winter, the mean values were 77%, 14% and 8%, respectively, and for the summer season, a percentage of 76% for the native *C. montagui*, 14% of unknown origin species *Amphitrite* sp. and 9% of the invasive *A. amphitrite* were obtained (Fig. 3.1). The mean percentages of abundance for the remaining two *P. perforatus* and *A. modestus* were below 1% in all seasons (Fig 3.1; ANNEX II).

Regarding *C. montagui*, the highest percentage of abundance was observed during winter and summer periods while *Amphitrite* sp. showed the maximum percentage in the spring season. The invasive *A. amphitrite* presented some what constant fluctuation during all observed seasons (Fig. 3.1).

For species that showed higher abundances the ANOVA analysis detected no significant differences in the mean percentages of abundance per season for any of them (ANOVA, $p > 0.05$).

In general the frequency of occurrence (Foc%) of the species in the different season was variable (Fig. 3.2) The species *A. amphitrite* and *Amphibalanus* sp. showed similar Foc% along seasons (70-80%). The highest Foc% obtained for *C. montagui* was during the winter (72%) and summer (75%) periods while during spring this native species showed a decrease, reaching 53%. The invasive *A. modestus* appeared with a lower frequency between 40 and 50% while native *P. perforatus* presented the lowest frequency for all the seasons studied (Fig. 3.2; ANEXO III). For all species (*C. montagui*, *A. modestus*, *A. amphitrite* and *Amphibalanus* sp.) the frequency of occurrence can be catalogued as common ($50\% \leq Foc < 90\%$), unless *P. perforatus* was presented as Rare ($Foc < 10\%$).

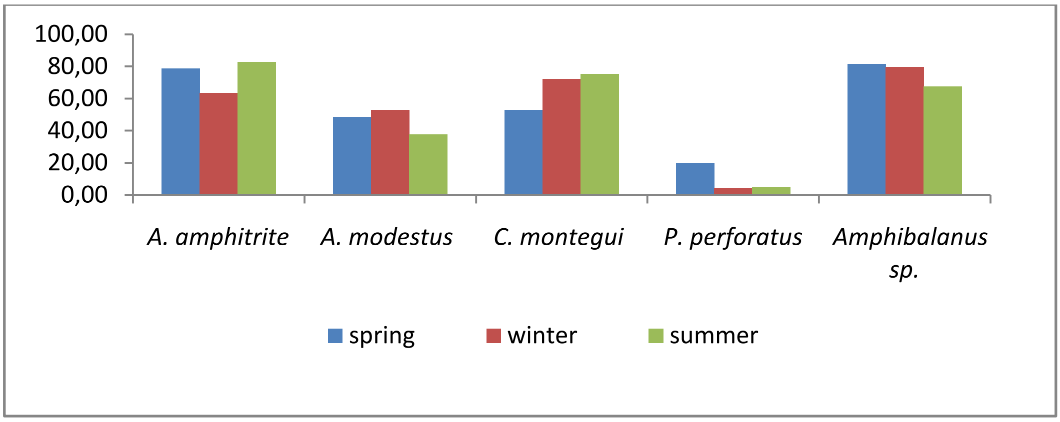

Figure 3.2 Frequency of occurrence (%) of barnacle species during different seasons (spring, winter and summer).

By analyzing the diversity index from all the seasons (Fig. 3.3), the higher values of diversity were observed during summer (d: 0.41; H': 0.58; J': 0.66), followed by spring (d: 0.41; H: 0.56; J ': 0.61), and finally by winter (d: 0.38; H: 0.5; J': 0.52). The Margalef index detected significant differences between the winter station and spring-summer stations (ANOVA, F: 4.25 & $p < 0.05$). For the remaining two indices, H' (ANOVA, F: 4.08 & $p < 0.05$) and J'(ANOVA, F: 3.67 & $p < 0.05$), there were only significant differences between the winter and summer periods.

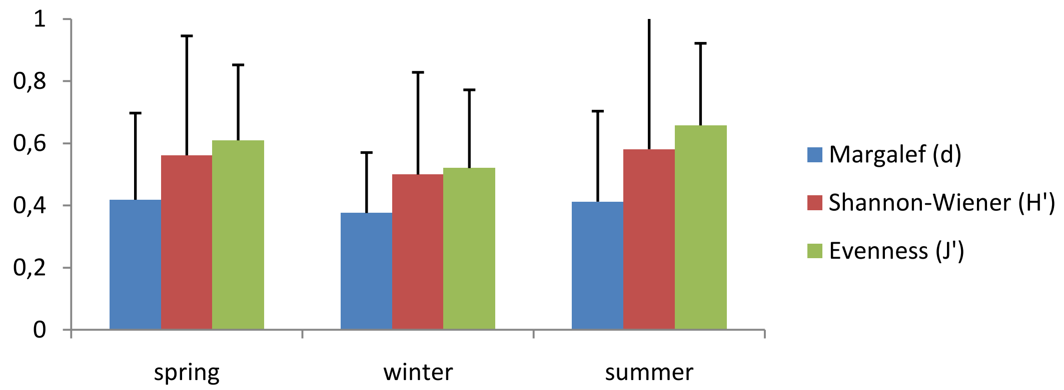

Fig. 3.3 The average of diversity (Margalef (d) and Shannon-Wiener (H')) and Evenness (J') index for samples obtained from different seasons during this study.

Based on the observation of the similarity index it was not possible to identify differences between samples; spring, winter and summer seasons were too disperse in the MDS distribution (Stress value: 0.11) (Fig. 3.4). Thus the test significances of similarity analysis (ANOSIM) indicate that in general, between seasons (spring, winter and summer), there are no significant differences (ANOSIM: R: 0.017 & p> 0.05).

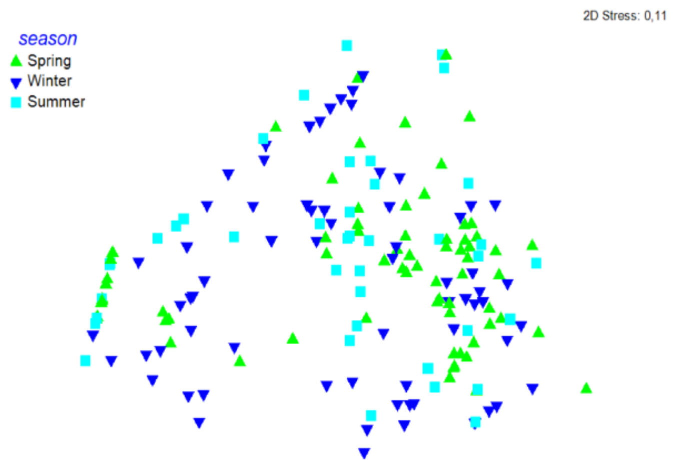

Fig. 3.4 MDS (Multidimensional Scaling analysis) showing similarities between seasons (spring, winter and summer), the stress level was 0.11.

b) Type of port

The percentages of abundance estimated between seasons were similar according to the type of port: recreational and fishing (Fig. 3.5).*Chthamalus montagui*, *Amphibalanus* sp. and *Amphibalanus amphitrite* were once again the most abundant ones in the recreational and fishing ports. *C. montagui* obtained a maximum of 82% while *Amphibalanus* sp. and *A. amphitrite* showed values of 13% and 4% respectively, in fishing ports (Fig. 3.5). In recreational ports, the native *C. montagui* again presented the higher percentage value of abundance (54%); however, the values of *Amphibalanus* sp. and *A. Amphitrite* in the recreational port were quite close to each other (24% and 20%, respectively) and account for almost half of the abundance of the native *C. montagui* (54%)(Fig. 3.5; ANNEX II).

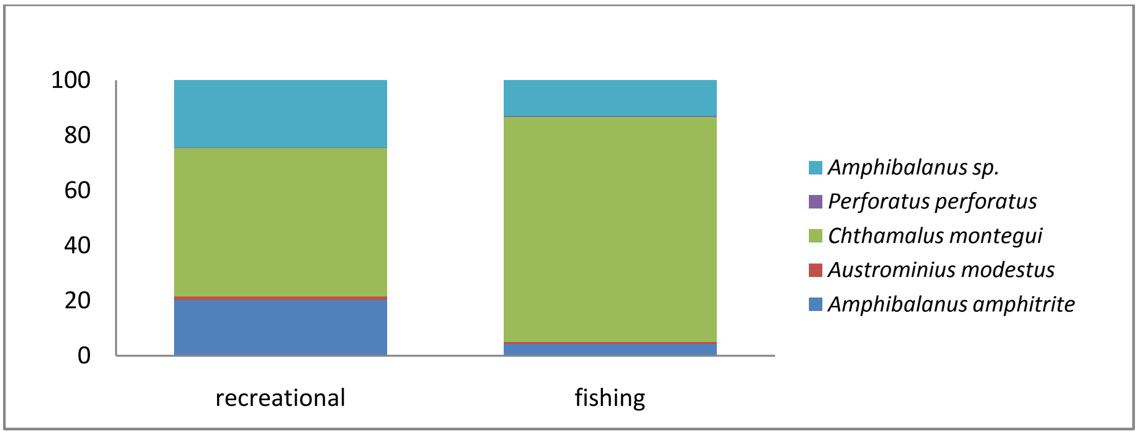

Fig. 3.5 The average percentage abundance of type of port for barnacle species found in the sampled grids.

A. amphitrite showed the highest values in recreational ports, while *C. montagui* presented larger abundances in the fishing ports, being these results significant (ANOVA, P <0.05). *Amphibalanus* sp. appeared to remain constant in both types of ports (ANOVA, P> 0.05). While *Austrominius modestus* had the lowest contribution, not exceed 2 %, in both types of ports (Fig 3.5; ANNEX II).

By comparing the frequencies of occurrence between types of ports (Fig. 3.6), invasive *A. amphitrite* was found to be commoner in the recreational ports (87%) while the native *C. montagui* appeared in greater numbers in samples from the fishing ports (79%) (Fig 3.6; Annex III). *Amphibalanus* sp. showed frequently in recreational and in fishing ports (76% and 78% respectively); in all these cases, the frequency of occurrence obtained for those species was classified as common. Similarly, the invasive *A. modestus* showed a slightly higher percentage in recreational ports (51%) than in fishing ports (44%). Finally, the native *Perforatus perforatus* presented a similar frequency in both ports (10%), which is quite a high occurrence for this species. But still the last two species showed again uncommon and rare occurrence respectively.

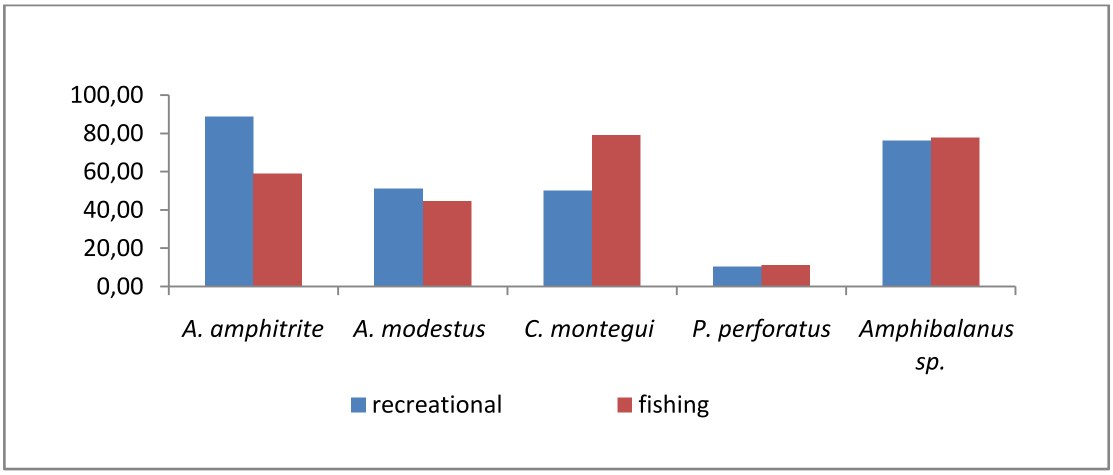

Figure 3.6 Frequency of occurrence of barnacle species in different types of ports: recreational and fishing.

Afterwards, the diversity indices were calculated and analyzed, showing higher values in recreational ports than in fishing ports (Fig.3.7). Margalef index (d) was the one which showed the closest values between types of ports (0.43 in recreational and 0.38 fishing), these differences not being significant (ANOVA, F: 1.81 & $p > 0.05$). Evenness diversity index showed higher values for the recreational ports (ANOVA, F: 23.76 & $p < 0.05$).

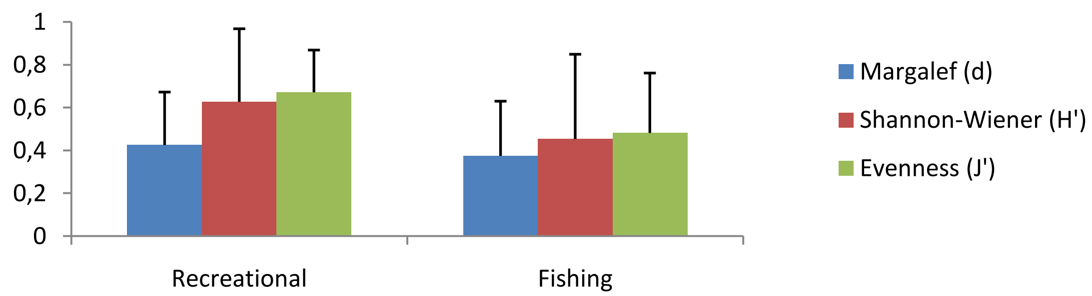

Fig. 3.7 The average of diversity (Margalef (d) and Shannon-Wiener) and Evenness index (J') obtained from samples for the different type of ports studied: recreational and fishing.

MDS analysis comparisonbetween fishing and recreational ports showed a large spread between the samples analyzed. (Fig. 3.8), Using the statistical analysis of similarity ANOSIM, small differences between the type of ports were found but these differences were significant (ANOSIM: R: 0.14 & $p < 0.05$).

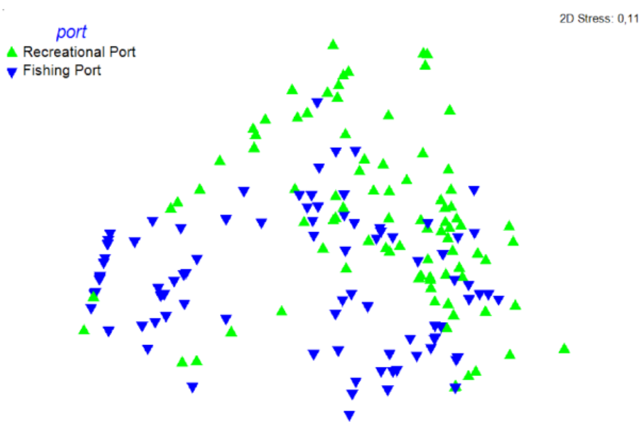

Fig 3.8 MDS (Multidimensional Scaling analysis) showing the similarities between type of ports (sheltered and unsheltered), the stress level was 0.11.

As there were significant differences, the SIMPER analysis was carried out for determining the species which have contributed the most to the dissimilarity between samples. In this comparison *C. montagui* presented the highest contribution to dissimilarity with almost 50% of the differences, followed by *Amphibalanus* sp. with 24% and finally *A. amphitrite* with 19% of contribution. In addition to all these species, a high consistency through samples was observed (Diss/sd> 1).

c) <u>Port location</u>

After the analysis of the percentages of abundance, the native species appeared as the most common for sheltered and unsheltered ports with approximate 75% and 69%, respectively (Fig. 3.9). No significant differences in terms of port location were found (ANOVA, $P > 0.05$). For sheltered ports, *Amphibalanus* sp. presented a value of 21% per sample and *Amphibalanus amphitrite* showed a percentage of abundance below 4%. In the case of unsheltered ports, *A. amphitrite* was the second most abundant species (20%) per sample, followed by *Amphibalanus* sp. (10%) and finally the *Austrominius modestus* with a value a slightly higher than 1% (Fig. 3.9).These two species of *Amphibalanus* showed significant differences between sheltered and unsheltered ports (ANOVA, $P < 0.05$).

Chthamalus montagui and *Amphibalanus* sp. presented higher abundances in sheltered ports while *A. amphitrite* showed the highest percentage in unsheltered ports (Fig 3.9; ANNEX II).

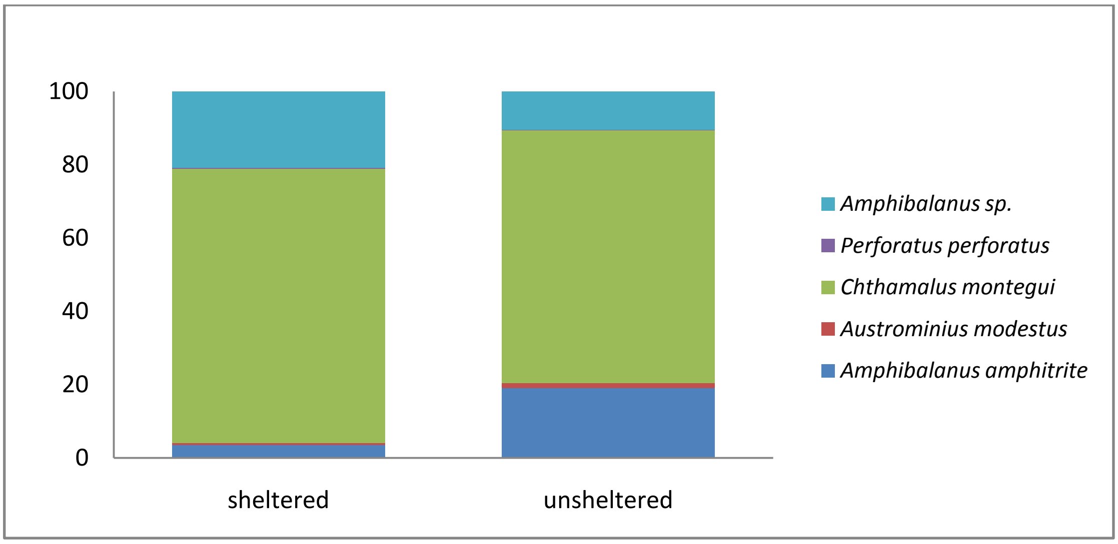

Fig. 3.9 The average percentage abundance of port location for barnacle species found in the sampled grids.

In sheltered ports, when the frequency of occurrence (Fig. 3.10) was analysed, *Amphibalanus* sp. was the most dominant species, with an occurrence of 83%, followed by *A. Amphitrite* with 61% and *C. montagui* with 50%. In unsheltered ports, the invader *A. amphitrite* was the most frequent species (90%), followed by the native *C. montagui* (84%). The invasive *A. modestus* presented an occurrence of 63% in unsheltered ports; it was a higher frequency than *Amphibalanus* sp (59%). Finally, *Perforatus perforatus* was again the species with the lowest frequency of occurrence (7%) for sheltered ports and 15% for unsheltered ports (Fig. 3.10). The most frequent species *C. montagui*, *A. amphitrite*, *A. modestus* and *Amphibalanus* sp. showed as common, while *P. perforatus* presented a rare occurrence. All the species are more frequent in the unsheltered ports except for *Amphibalanus* sp.

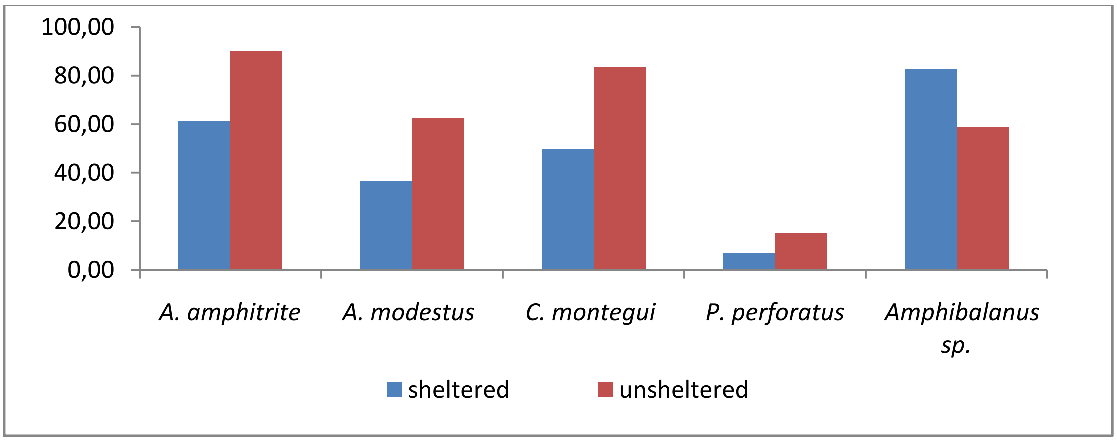

Figure 3.10 Frequency of occurrence of barnacles species, showing the differences in port location (Sheltered and unsheltered).

In Fig. 3.11, the diversity indexes are presented for the different port locations, showing the highest values of diversity and Evenness index in the unsheltered ports. This index showed similar estimated values for both types of port (0.53 for sheltered and 0.64 unsheltered ports), while the diversity index of Shannon-Wiener presented a greater difference (0.4 and 0.71 respectively). In general, it can be said there were significant differences between all index in relation with the port location (ANOVA, $P < 0.05$).

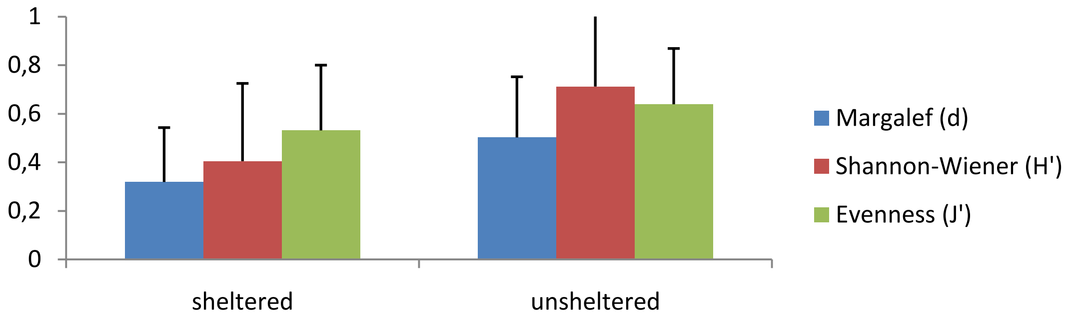

Fig. 3.11 The average of diversity (Margalef (d) and Shannon-Wiener (H')) and Evenness index (J') obtained from the samples for the different port locations studied: Sheltered and unsheltered.

When the MDS was analyzed between sheltered and unsheltered ports, two different dispersed groups were found, showing a tendency for each port location (Fig. 3.12). Dispersion among samples was high, showing that unsheltered ports presented higher dispersion than sheltered ports (stress level: 0.11). Through the similarity test ANOSIM, it was confirmed that there were significant differences when the port locations were compared (ANOSIM: R: 0.174 & $p < 0.05$).

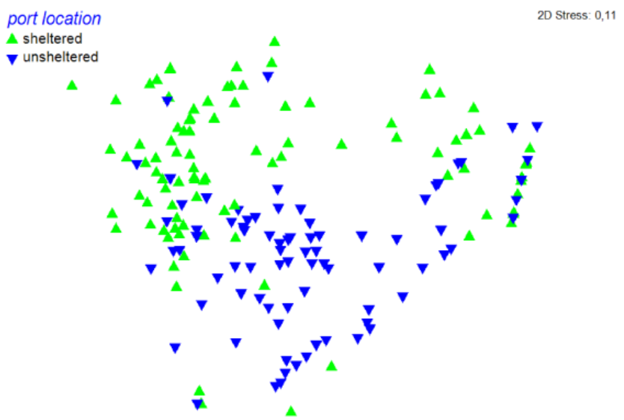

Fig 3.12 MDS (Multidimensional Scaling analysis) showing the similarities between port location (sheltered and unsheltered), the stress level was 0.11.

As significant differences between ports locations were observed, SIMPER analysis was carried out, highlighting *C. montagui* with 46%, *Amphibalanus* sp. with 24% and *A. amphitrite,* with a percentage of 22%, as the species that contributed the most for the 92% of dissimilarity observed between sheltered and unsheltered ports. A high consistency of these species along samples (Diss/sd> 1) was found.

d) Geographic location

Taking into account the different geographic locations of the ports of study (west, east and centre), average percentages of abundance were estimated (Fig. 3.13). Again, *Chthamalus montagui*, *Amphibalanus amphitrite* and *Amphibalanus* sp. were the ones with the highest percentages; in the central ports, the native *C. montagui* was the most abundant (79%) while in western ones it slightly decreased (75%) in abundance. In western ports *Amphibalanus* sp. was the second most abundant species (21%) followed by *A. amphitrite* with a contribution of 4% in abundance. In the case of central ports *A. amphitrite* was the second species with the highest percentage of abundance (13%) followed by *Amphibalanus* sp. with 7% per sample. In, the eastern ports the highest percentage of abundance for invasive *A. amphitrite*was 41%, followed by *C. montagui* (35%) and *Amphibalanus* sp. (21%) (Fig 3.13; ANNEX II).

C. montagui dominated the western and central ports while in the eastern ports it occupied a second position; however, differences in the abundance of this species may be considered as non-significant (ANOVA, P> 0.05). On the other hand, *A. amphitrite* appeared to dominant the eastern ports and showing a lowest percentage (< 4%) in the western ports; here the existence of significant differences in abundances of this species were tested (ANOVA, P <0.05). As for *Amphibalanus* sp., this species appeared to remain more or less constant, with a high abundance in western and eastern ports and with a lower value of abundance (7%) in the central ports. Only the western ports showed significant differences (ANOVA, P< 0.05) when compared to the other two locations (east and central).

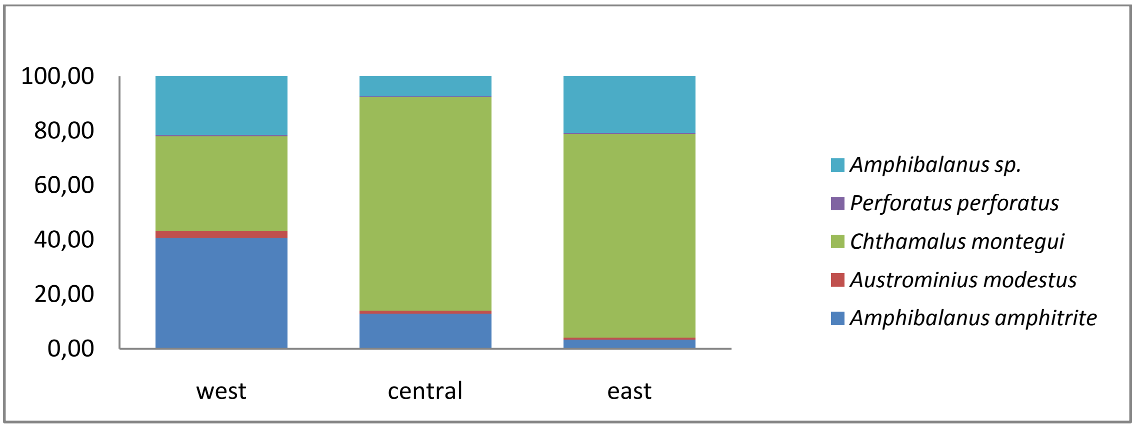

Fig. 3.13 The average percentage of abundance for the barnacle's species found in the grid, showing the differences between geographic locations: west, east and central.

Analyzing the frequency of occurrence based on geographic location (Fig. 3.14), *Amphibalanus* sp. and *A. amphitrite* were the most frequent (100%). Both species showed a very frequent of occurrence. *Amphibalanus* sp was also found as the most frequent species in eastern ports with 83%of occurrences. In western port were A. amphitrite and *C. montagui* with 86% and 82% the ones showed higher frequency. Here the frequency can be considered as common. *Austrominius modestus* (77%) and *Perforatus perforatus* (27%) were also more frequent in the central ports than in the western and esatern ones (Fig 3.14; Annex III). *P. perforatus* again showed rare frequency while *A. modestus* presented a common. Again there is the same pattern: all species are more frequent form west to east except for *Amphibalanus* sp.

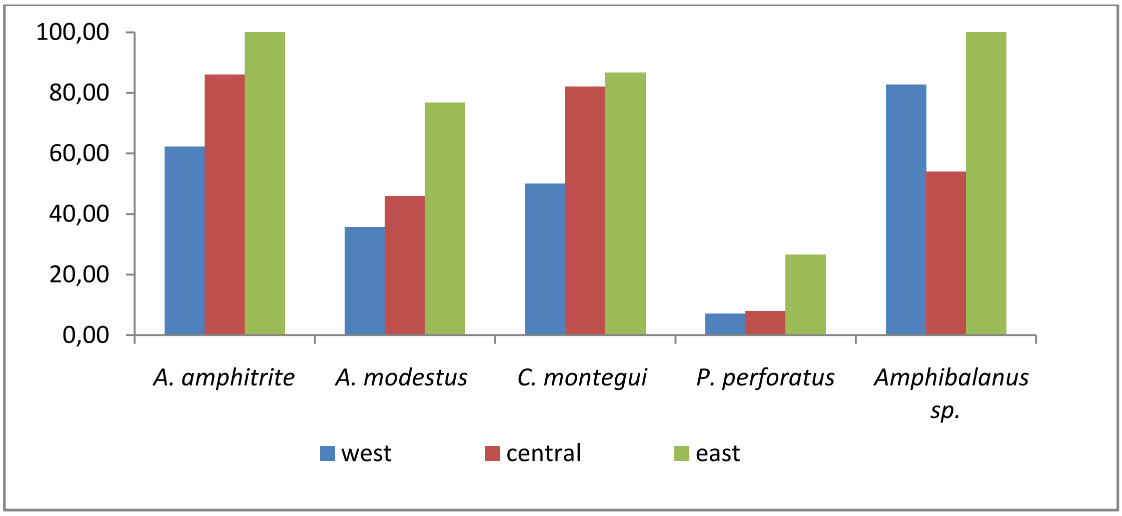

Figure 3.14 Frequency of occurrence of barnacles species, showing the differences by geographic location (west, central and east).

The Diversity and Evenness indices showed major differences depending on the geographical location of each port (Fig. 3.15). Samples from the eastern ports were the ones with the highest values for the Diversity and Evenness index calculated (d: 0.66; H: 0.98; J ': 0.73), showing almost double value than the other two geographical locations. After to apply the ANOVA test significant differences for all the index of the eastern ports compared with western and central were observed (ANOVA, P <0.05), but there were not significant differences when western and central ports were compared (ANOVA, P> 0.05), for that reason this ports showed similar values in all index calculated d: 0.32 and 0.1; H': 0.4 and 0.54; J': 0.53 and 0.57 respectively (Fig 3.15, Annex IV). The western ports have more diversity than the other two.

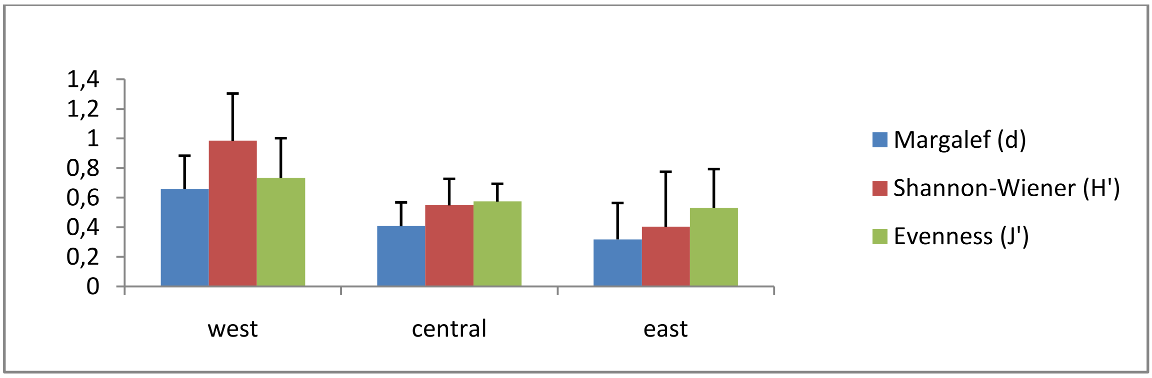

Fig. 3.15 The average of diversity and evenness index obtains from the samples for the different geographic location: West, central and east.

The geographic locations of the ports were also studied through the analysis MDS, where it was showed a large scatter in the data (Fig. 3.16). A clear differentiation between geographical locations of the ports was not visible, but the ports of western and central were those with the largest difference due to the distance between them (stress level: 0.11).

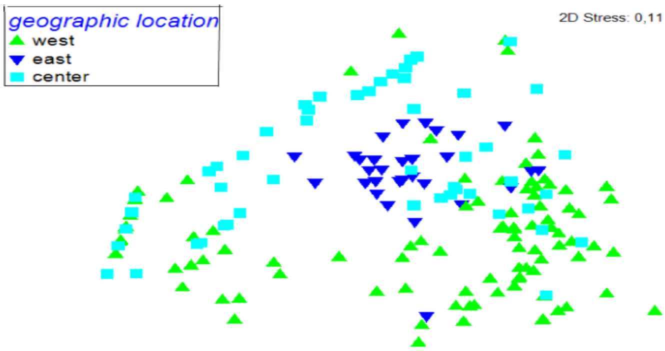

Fig 3.16 MDS (Multidimensional Scaling analysis) showing the similarities between geographic location: West, east and central. The stress level was 0.11.

This was demonstrated by conducting the test ANOSIM that in a globally context showed significant differences (ANOSIM, R: 0.091 & $p < 0.05$) and when it was compared by peers there were only significant differences between central and west ports, for the two other two comparisons no significant differences were found (table 3.4).

To determine what species contributed the most for significant differences between the western and central ports a SIMPER analysis was conducted; the other two comparisons were not calculated because nosignificant differences were found. All these percentages of contribution to the dissimilarity encompassed more than 93% of the total (48% *C. montagui*, *Amphibalanus* sp. with 26% and *A. amphitrite* with 19%). All these species showed a high consistency in the samples (Diss/sd> 1).

Table 3.4R number and significance level (%) of ANOSM (similarity analysis) for the factor of geographic location: West, east and central.

Factor	**R number**	**Significancelevel (%)**
west x east	0.064	6.7
west x central	0.142	0.1
east x central	-0.002	48.4

e) Sampling localities

The average percentage of abundances was also estimated for the different studied localities individually. Here, a high heterogeneity of abundance was observed within the different species (Fig 3.17). The native *Chthamalus montagui* was the most dominant in Faro and Quarteira-Vilamoura localities. In Faro this species showed the highest percentage of abundance (89%), followed by *Amphibalanus* sp. (8%) and *Amphibalanus amphitrite* present (4%). In the Quarteira-Vilamoura locality, native *C. montagui* showed an abundance of 79%, followed by *A. amphitrite* and *Amphibalanus* sp. with average percentages of 13% and 7%, respectively.

On the other hand, native *C. montagui* was not the most abundant species in the localities of Olhão and Lagos. In Olhão *Amphibalanus* sp. was dominate (50%) followed by *C. montagui* (43%) and *A. amphitrite* (6%). On the other hand, in Lagos, invasive *A. amphitrite* howed a the higher percentage (41%), followed by *C. montagui* (35%) and later by *Amphibalanus* sp. (21%)(Fig. 3.17).

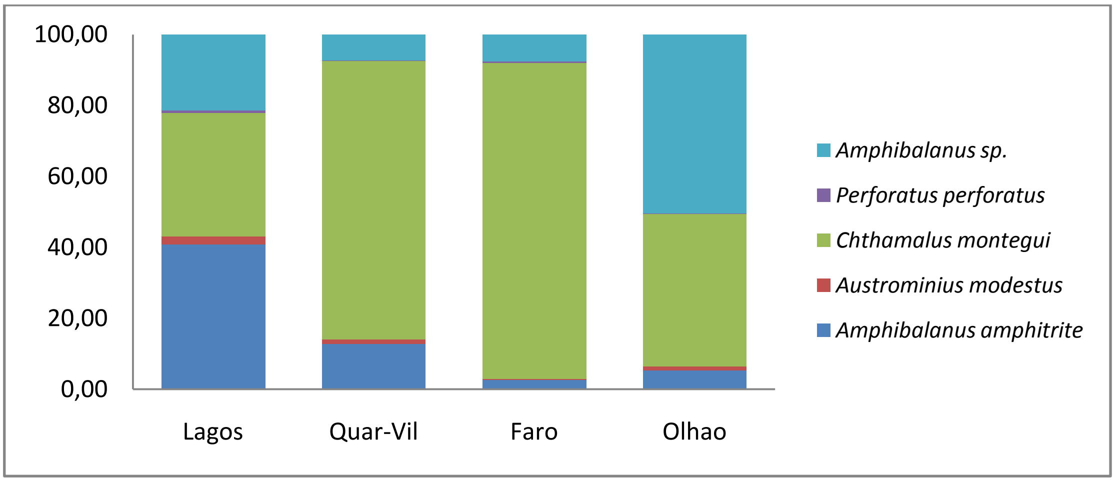

Fig. 3.17 Average percentage of abundance of barnacles species found in studied grids, showing the differences between localities: Lagos, Quarteira-Vilamoura Faro and Olhão.

The native *C. montagui* and the invasive *A. amphitrite* were the ones that showed significant differences in their abundances when Faro and Olhão were compared with the localities of Quarteira-Vilamoura and Lagos (ANOVA, $p < 0.05$). As for *Amphibalanus* sp., differences were only observed between Olhão and the other three studied localities (ANOVA, $p < 0.05$).

Amphibalanus sp. was the most common species in the localities of Lagos and Olhão with a frequency of occurrence of 100% and 94%, respectively. The highest frequency of occurrence found for invasive *A. amphitrite* (100%) occurred in the Lagos locality. Lagosrepresented the locality where the highest frequency of *C. montagui* was observed (87%), invader *Austrominius modestus* was also more frequent in this locality (77%) and finally *Perforatus perforatus* presented also its maximum occurrence by sample (27%) (Fig 3.18; ANNEX III).

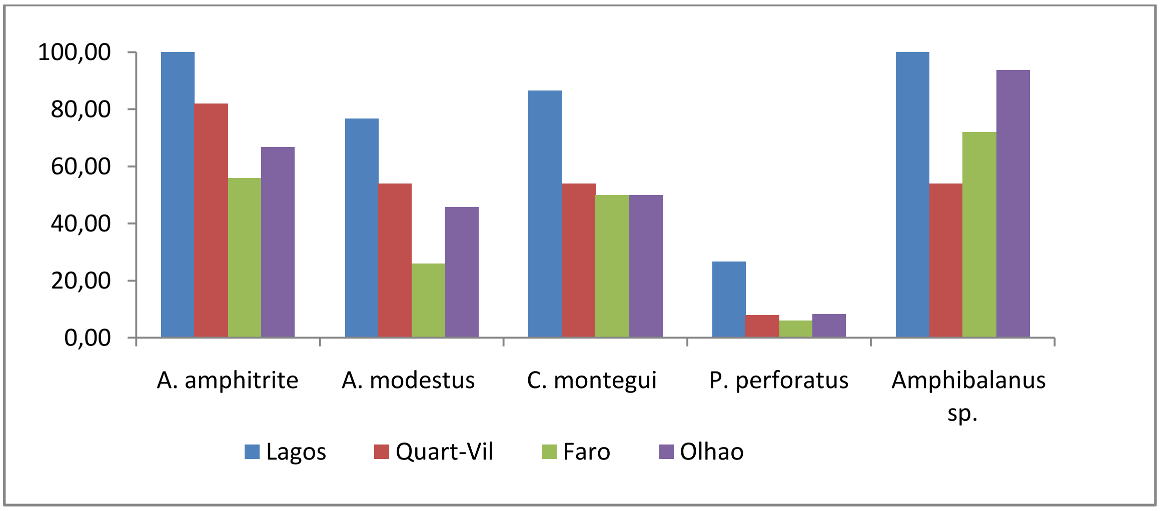

Figure 3.18 Frequency of occurrence of barnacles species, showing the differences between localities: Lagos, Quarteira-Vilamoura Faro and Olhão.

Diversity indices were also obtained for the different places of study (Fig 3.19), showing that in the Lagos localitya greater diversity among samples was found (d: 0.66; H: 0.98; J ': 0.73). On the other had Faro was represented by the lowest values (d: 0.26; H: 0.37). However, using the evenness index, Olhão was represented by the lowest value (J ': 0.49) and Lagos represented with the highest (J ': 0.73) (Fig 3.19, Annex IV).

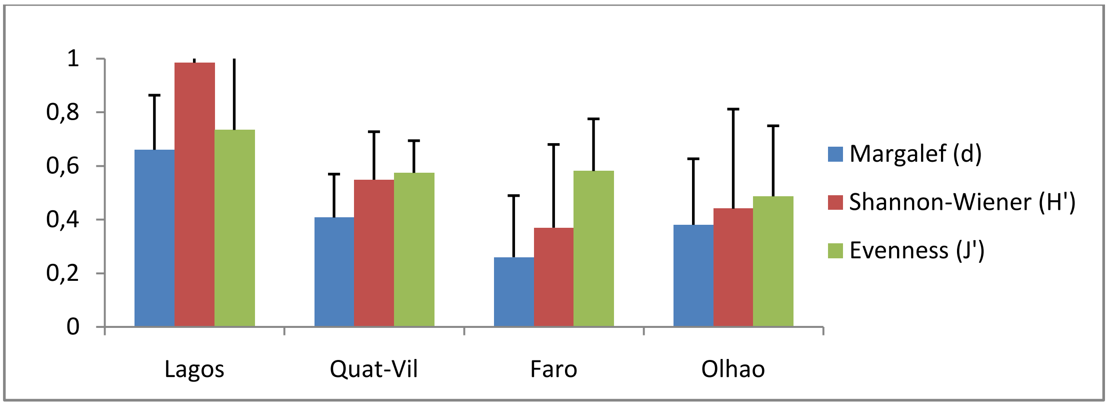

Fig. 3.19 The average of diversity (Margalef (d) and Shannon-Wiener (H')) and evenness index obtained from the samples for the different localities studied: Lagos, Quarteira-Vilamoura Faro and Olhão.

The evenness index between localities did not show many differences. The only estimated differences were obtained when Lagos was compared to Olhão and Quarteira-Vilamoura (ANOVA, $p<0.05$). Differences were found between all localities using the Margalef index (ANOVA, $p<0.05$), the only exception was between Olhão and Vilamoura-Quarteira (ANOVA, $p> 0.05$). The estimations using the Shannon index were similar to that of Margalef, however, here, the locality of Olhão did not show significant differences (ANOVA, $p> 0.05$) with Faro andQuarteira-Vilamoura.

When the MDS was used to look for differences between the studied localities there was considerable scattering in Faro, Olhãoand Quarteira-Vilamoura but for Lagos, the distribution of the samples less disperse (Fig.3.20). The ANOSIM statistical test showed that globally, there were significant differences between the studied localities (ANOSIM, R: 0.201 & $p <0.05$).

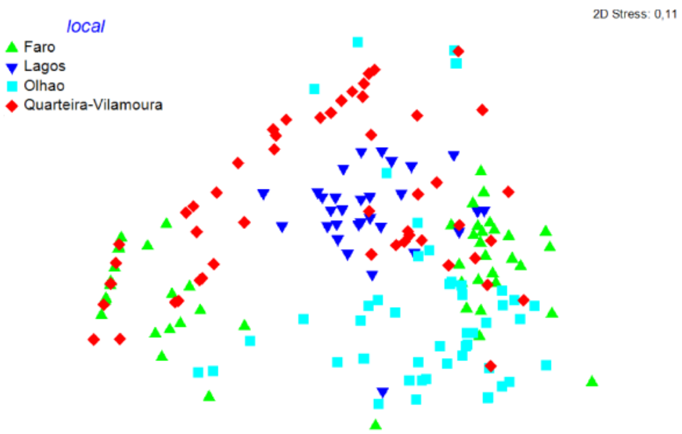

Fig 3.20 MDS (Multidimensional Scaling analysis) showing the similarities between localities (Faro, Lagos, Olhao and Quarteira-Vilamoura), the stress level was 0.11.

Significant differences were also found when comparing all localities between each other (table 3.5). The only exception happenedbetween Lagos and Quarteira-Vilamourawhere no significant differences were detected (ANOSIM, R: 0.002 & p> 0.05).

Table 3.5 R number and significance level (%) of ANOSM for the factor of port locations: Faro, Lagos, Olhão and Quarteira-Vilamoura.

Factor	**R number**	**Significance level (%)**
Faro x Lagos	0.176	0.2
Faro x Olhão	0.204	0.1
Faro x Quarteira-Vilamoura	0.088	0.2
Lagos x Olhao	0.442	0.1
Lagos x Quarteira-Vilamoura	-0.002	45.4
Olhão x Quarteira-Vilamoura	0.344	0.1

Through SIMPER, the species that most contributed to the dissimilarity between samples was the native *C. montagui* followed by. *Amphibalanus* sp. and *A. amphitrite*. These results were obtained for all localities studied with the exception between the comparison between Lagos and Olhão where invasive *A. modestus* appeared with a contribution rate close to 8%. As with the other factors, through the product Diss / sd it was demonstrated that all the species had a high consistency in the samples analyzed (Diss / sd> 1).

3.2.2 Sea squirts identification and counting.

During the Sea squirt sampling, a total of 3,900 individuals were counted in the different ports of the Algarve. In this case ropes, boxes and other floating objects found in the ports were analyzed. A total of 9 species of solitary sea squirts were found and identified (*Ascidiella aspersa, Molgula occidentalis, Ciona intestinalis, Phallusia mammilata, Microcosmus squamiger, Styela plicata, Styela canopus, Corella* cf. *eumyota* and *Ascidia* cf. *mentula).* The colonial sea squirts were not taken into account as part of the counting procedure (*Botryllus leachii*, *Botryllus schlosseri* and *Didemnum* cf. *vexillum*). In general, the species which showed a higher percentage of abundance and frequency of occurrence in all the samples were the invasive species *S. plicata* and *M. squamiger*.

a) Season

After obtaining the average percentage of abundance for the seasons studied, two species (*Styela plicata* and *Microcosmus squamiger*), showed a high dominance over the rest (Fig. 3.21). During the spring season the percentage of abundance for these species was 48% and 47%, although for summer lower values were found (34% and 41%, respectively). The other species found were native, in spring *Phallusia mammilata* with 2% and *Ciona intestinalis* with 1% and in summer *Molgula occidentalis* with 14%, *C. intestinalis* with 7% and *Ascidiella aspersa* with 2% (Fig. 3.21). All the species presented significant differences of abundance between seasons when the T-test was applied (T-test, $p < 0.05$).

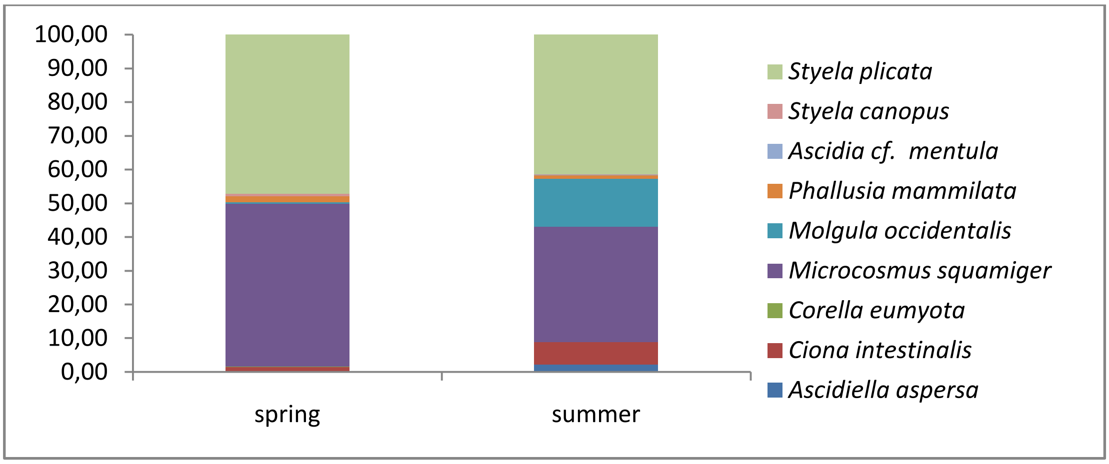

Fig. 3.21 The average percentage of abundance for the sea squirts species found in the sea squirts counting showing the differences between seasons: spring and summer.

In terms of frequency of occurrence for the spring and summer, both *S. plicata* and *M. squamiger* were once again found to be the most common species in both seasons (Fig. 3.22). In spring *M. squamiger* was represented by the highest frequency of occurrence (91%), followed by *S. plicata* (86%), *P. mammilata* (37%) and *C. intestinalis* (29%). The remaning species, *M. occidentalis*, *S. canopus*, *C.* cf. *eumyota* and *A. aspersa*, were represented by rates of less than 9% of occurrence.

In the summer the dominant invasive *S. plicata* showed a frequency of 100% and *M. squamiger* of 92%. But other species, such as *C. intestinalis* (61%), *A. aspersa* and *M. occidentalis* both with 47% were also found in the spring. Furthermore, below 11% of occurrence were *P. mammilata*, *S. canopus* and *Ascidia* cf. *mentula*.

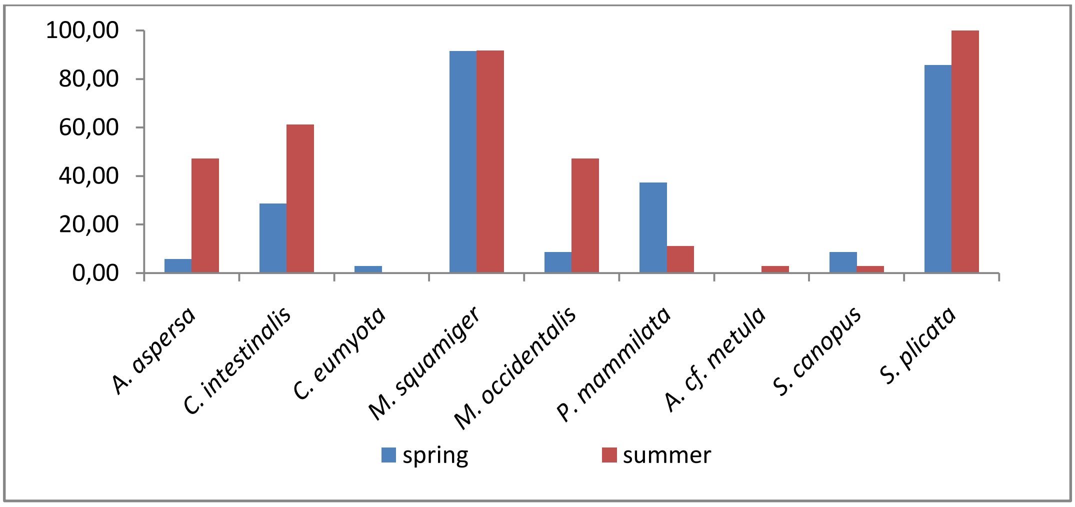

Figure 3.22 Frequency of occurrence of sea squirts species, showing the differences in season (spring and summer).

In relation to the degree of similarity between seasons, summer was the period with the highest dispersion of the samples, rather than spring (stress value: 0.14).However, both groups (spring and summer) are well identified in the distribution plotted in the MDS analysis (Fig 3.23). The ANOSIM significance test showed significant differences between both seasons (ANOSIM, R ': 0.159 & p <0.05).

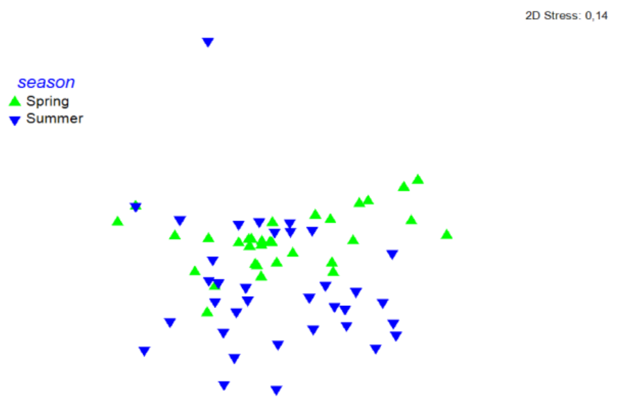

Fig 3.23 MDS (Multidimensional Scaling analysis) showing the similarities between seasons (spring and summer), the stress level was 0.14.

Using the analysis of dissimilarity percentages (SIMPER) the percentage of dissimilarity between spring and summer was 42%. *S. plicata* and *M. squamiger* were the species with the greater contribution to this difference, with 28% and 27% respectively. Also contributing to this percentage of dissimilarity were *M. occidentalis* (16%), *C. intestinalis* (13%) and *A. aspersa* (7%).

In terms of consistency, the species *M. squamiger, S. plicata* and *C. intestinalis* showed great consistency in the samples (Diss / sd > 1), while *M. occidentalis* and *A. aspersa* showed less consistency between samples (Diss / sd <1).

b) <u>Type of port</u>

As for the percentage of abundance based on the type of port (Fig. 3.24) two invasive species (*Styela plicata* and *Microcosmus squamiger*) appeared once again as the most abundant for both ports. The percentage of abundance presented by *S. plicata* was 73% for fishing ports and 47% for recreational ports. The values obtained for *M. squamiger* were 45% in fishing ports and 38% in recreational ports. These two species showed significant differences (T-test, $p < 0.05$) in the comparison between the different types of ports.

Other species such as *Molgula occidentalis, Ciona intestinalis, Ascidiella aspersa* and *Phallusia mammilata* also contributed to the total percentage of abundance. These species coincided in both fishing and recreational ports (Fig 3.24; ANNEX II) showing there were not significant differences between them (T-test, $p > 0.05$).

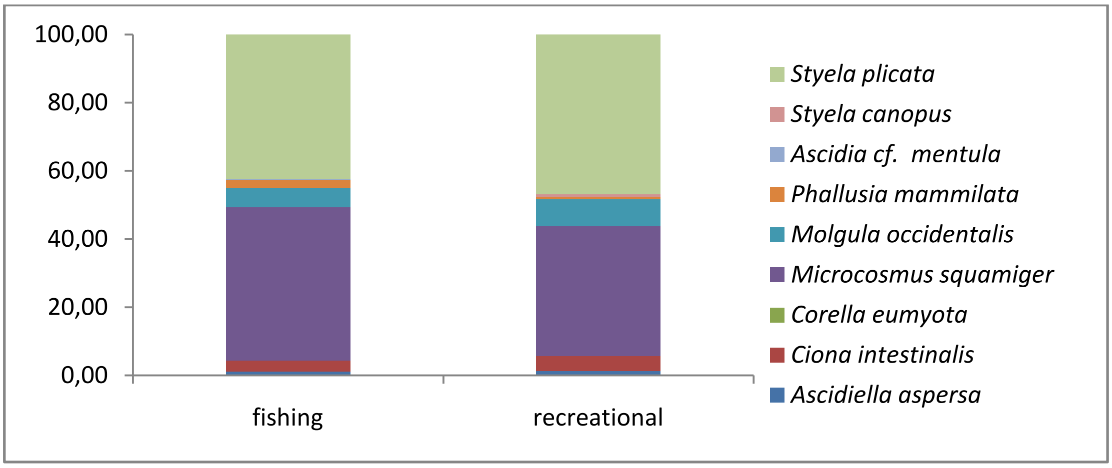

Fig. 3.24 The average percentage of abundance for the species found in the sea squirts counting showing the differences between port: fishing and recreational.

The frequencies of occurrence obtained indicated once again that the most common species were *M. squamiger* and *S. plicata* (around 90%), followed by the native *C. intestinalis* (between 40%-50%)(Fig. 3.25). In fishing ports *P. mammilata*, *M. occidentalis* and *A. aspersa* showed similar frequencies of around 30%. In recreational ports, the same 3 species were observed together with *Styela canopus* and *Corella* cf. *eumyota* with frequencies of 13% and 3% respectively (Fig. 3.24; Annex III).

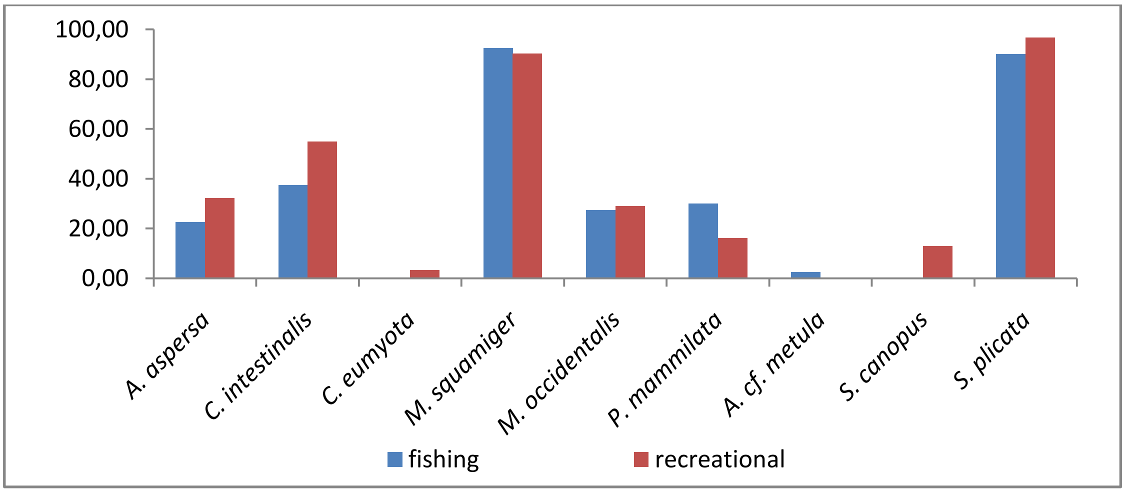

Figure 3.25 Frequency of occurrence of sea squirts species, showing the differences in port (Fishing and recreational).

The similarity estimated by MDS analysis showed no clear distributions between the different types of ports (Fig 3.26) (stress value: 0.14). The ANOSIM significance test also indicated that there were no significant differences between fishing and recreational ports (ANOSIM, R ': 0.007 & p> 0.05).

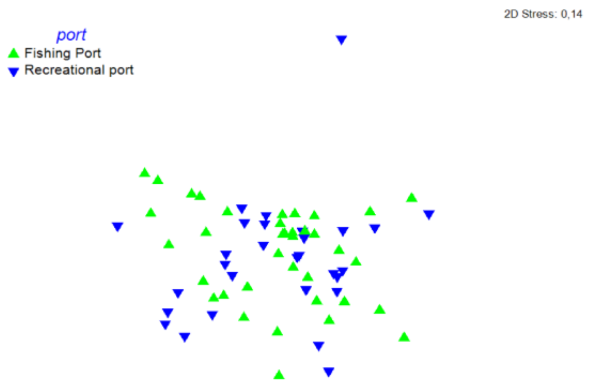

Fig 3.26 MDS (Multidimensional Scaling analysis) showing the similarities between type of ports (Fishing and recreational), the stress level was 0.14.

c) Port locations

The highest abundances for sheltered ports were for the invasive *Microcosmus squamiger* (49%) and *Styela plicata* (35%). The species that followed were *Molgula occidentalis* with an average percentage of abundance of 10%, and *Phallusia mammilata* and *Ciona intestinalis*, both contributing with 4% to the total percentage of abundance. In unsheltered ports *S. plicata* was dominant with an abundance of 58% followed by *M. squamiger* (36%). The native *C. intestinalis*, *Ascidiella aspersa*, *P. mammilata* and *M. occidentalis* contributed around 4% to the total percentage (Fig 3.27; ANNEX II). Significant differences between port locations were shown for the two native, *A. aspersa* and *M. occidentalis* (T-test, $p < 0.05$) and the other two did not present any significant differences in their abundances between port locations (T-test, $p > 0.05$). The main species did not show significant differences (T-test, $p > 0.05$).

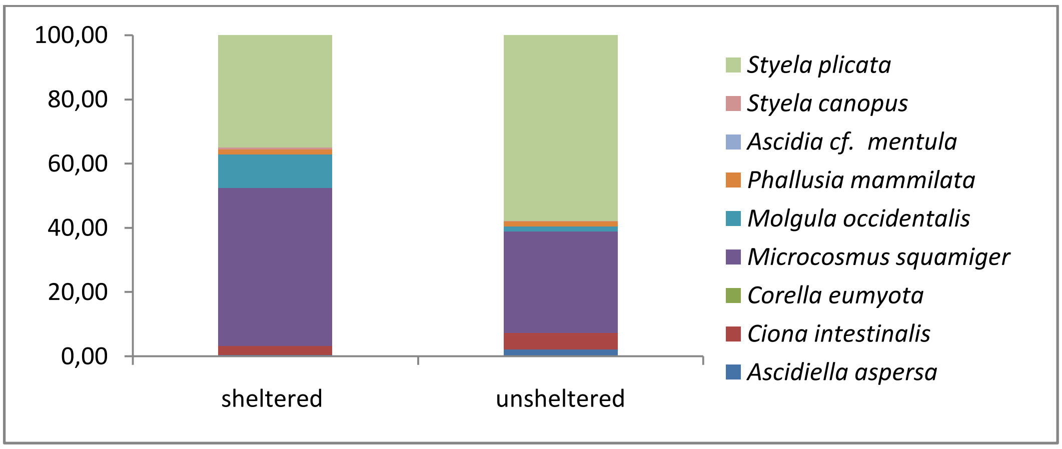

Fig. 3.27 The average percentage of abundance for the species found in the sea squirts counting showing the differences between port locations: sheltered and unsheltered.

By analyzing the frequency of occurrence for sheltered and unsheltered port *M. squamiger* and *S. plicata* were noted as the most common species, with similar percentages in the different port locations, 90% for each species (Fig 3.28). It was *C. intestinalis* and *A. aspersa* which presented different occurrence, being higher this frequency in unsheltered ports with 52% and 42% respectively while in sheltered were 40% and 15% for that species.

Besides the invasive (*M. squamiger* and *S. plicata*), the native ones (*P. mammilata* and *M. occidentalis*) also showed high frequencies (28% and 33% respectively) in sheltered ports. *Styela canopus* and Corellacf. *eumyota* also appear more frequently in sheltered locations (8% and 3% respectively) while *Ascidia* cf. *mentula* was only found in a very small percentage (3%) in unsheltered ports.

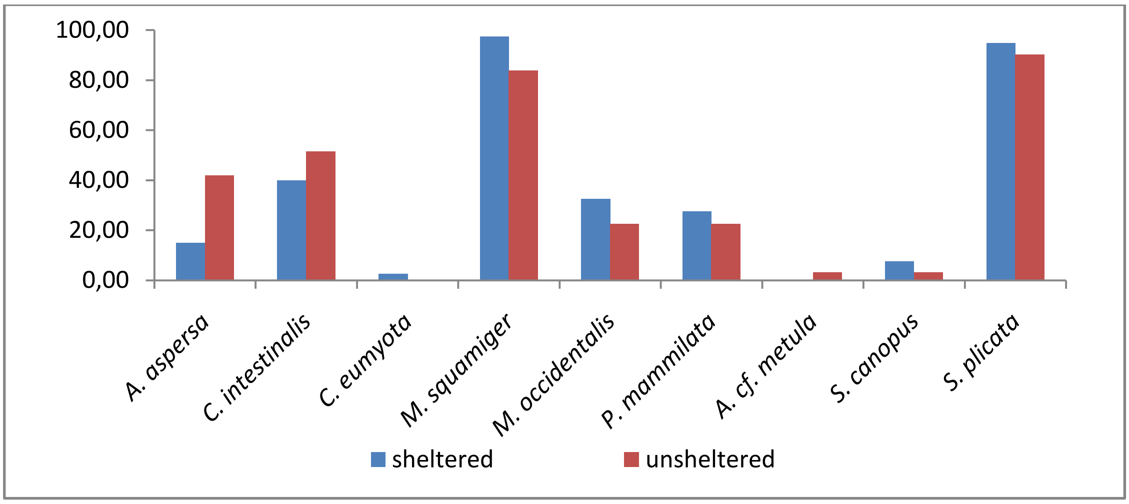

Figure 3.28 Frequency of occurrence of sea squirts species, showing the differences in port location (Sheltered and unsheltered).

In the similarity analysis of samples from sheltered and unsheltered ports through the MDS it was observed the two different groups and a large dispersion between samples were showed (stress value: 0.14) (Fig 3.29.). Through ANOSIM the differences between types of port were significant (ANOSIM, R ': 0.102 & p <0.05).

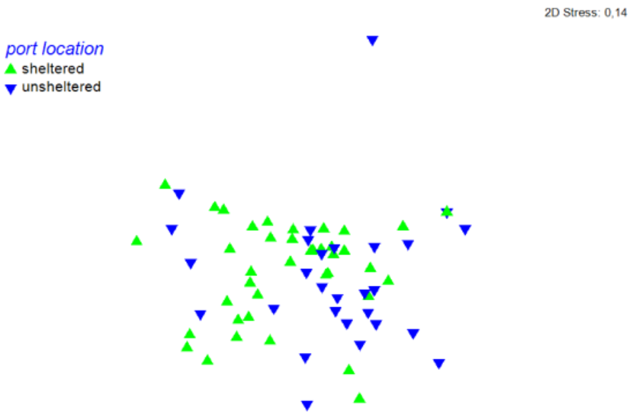

Fig 3.29 MDS (Multidimensional Scaling analysis) showing the similarities between port location (sheltered and unsheltered), the stress level was 0.11.

In terms of dissimilarities between port locations, by using SIMPER, *S. plicata* and *M. squamiger* were determined as the species which most contributed to the dissimilarity (both 28%). But the native *C. intestinalis* also presented a high percentage of contribution (13%). All these species showed a high consistency in the samples (Diss / sd> 1). Conversely, natives such as *M. occidentalis* and *A. aspersa* also contributed to the dissimilarities (14% and 7%) although showing less consistency in the samples (Diss / sd< 1).

d) Locals

Analyzing the abundances by localities the main dominant species found in Faro and Lagos was *Microcosmus squamiger* with 59% and 54%, respectively (Fig. 3.30). As for Faro, the species with highest abundances after *M. squamiger*, were *Styela plicata* and *Molgula occidentalis* both with 18% and on a smaller scale *Ciona intestinalis* and *Styelacanopus* were also found. In Lagos, the second most abundant species was also the invasive *S. plicata* (33%) and the native *C. intestinalis* (8%). Other species such as *Ascidiella aspersa* and *M. occidentalis* only contributed with a minimum percentage (both 4%) for the total abundance.

In the localities of Olhão and Quarteira-Vilamoura, *S. plicata* with an abundance of 57% and 64% respectively was the dominant species. The invasive *M. squamiger* with 36% and 26% respectively was presented as the second most abundant in both locations. However, in Olhão, native species such as *P. mammilata* and *C. intestinalis* presented an abundance of 3%. While in Quarteira-Vilamoura, *C. intestinalis*, *P. mammilata*, *M. occidentalis* and *A. aspersa* showed lower contribution to the total abundance (Fig 3.30; ANNEX II).

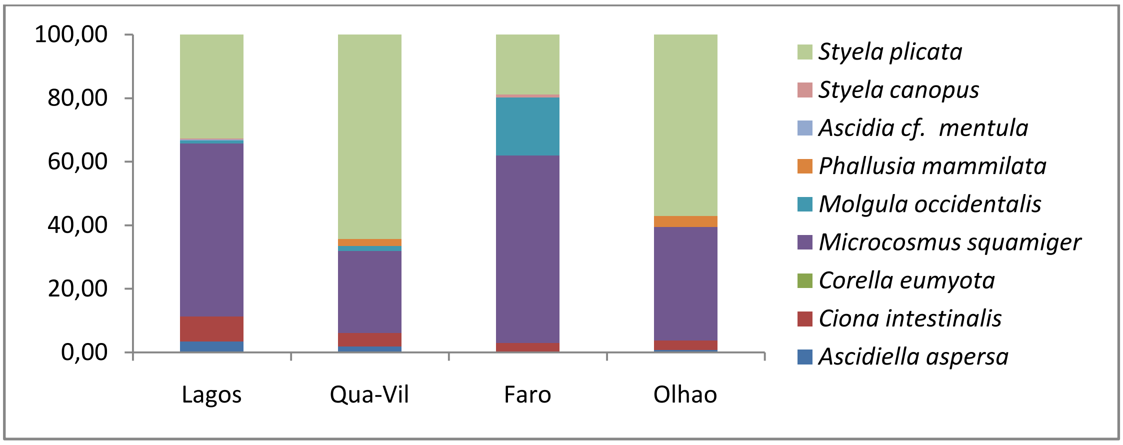

Fig. 3.30 The average percentage of abundance of Sea squirts, showing the differences between localities: Lagos, Quarteira-Vilamoura (Qua-Vil) Faro and Olhão.

S. plicata was the only species that presented significant differences between all localities studies (ANOVA, $p < 0.05$) except for the comparison between Lagos and Faro, where the observed differences were not significant (ANOVA, $p > 0.05$). *M. squamiger* and *M. occidentalis* presented significant differences when Faro was compared with the other three localities (ANOVA, $p < 0.05$). In a global context, significant differences appeared in terms of abundance for the native species *A. apersa*, *S. canopus* and *P. mammilata* (ANOVA, $p < 0.05$) but due to the higher variability between samples through Tukey's test, it was difficult to determine in which localities these differences were present.

Focusing on the frequency of occurrence, the invasive species *M. squamiger* and *S. plicata* were again those which presented the highest values in all the localities (around 90% except for Lagos with 70%) (Fig 3.31). Other natives such as *C. intestinalis*, *M. occidentalis* and *A. aspersa* presented a high percentage of occurrences in all localities. *C. intestinalis* and *A. aspersa* showed their highest occurrence in Quarteira-Vilamoura (60% and 50%) and *M. occidentalis* inFaro with 65% of the total frequency of occurrence. Also in Faro and Lagos the species *S. canopus* appeared with an occurrence of 15%, while in Olhão and Quarteira-Vilamoura it was the native *P. mammilata* which made its appearance (50% and 35% respectively). With only a onetime occurrence, *C.* cf. *eumyota* contributed with 5% in Faro and *A.* cf. *mentula* 4% in the port Lagos with 9%.

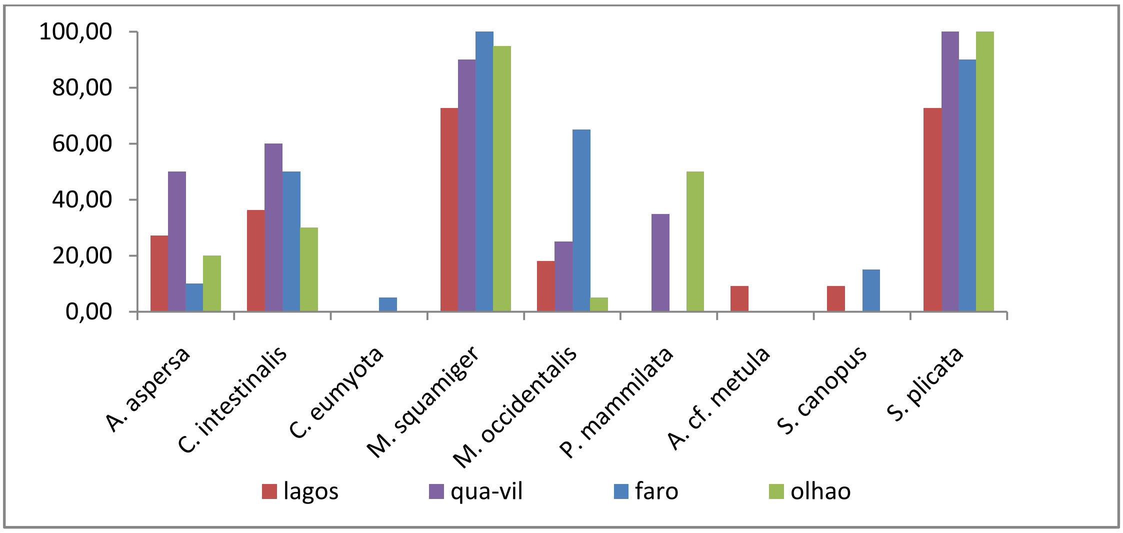

Figure 3.31 Frequency of occurrence for Sea squirts, showing the differences between localities (Lagos, Quarteira-Vilamoura (Qua-Vil) Faro and Olhão).

Regarding the degree of similarity between the different places of study obtained through MDS analysis, three distinct groups were observed. Although for Lagos the obtained distribution of samples was more dispersed than for the other locals (stress value: 0.14) (Fig 3.32.). Generally, through statistical ANOSIM, the existence of significant differences between localities was observed (ANOSIM, R: 0.363 & $p < 0.05$).

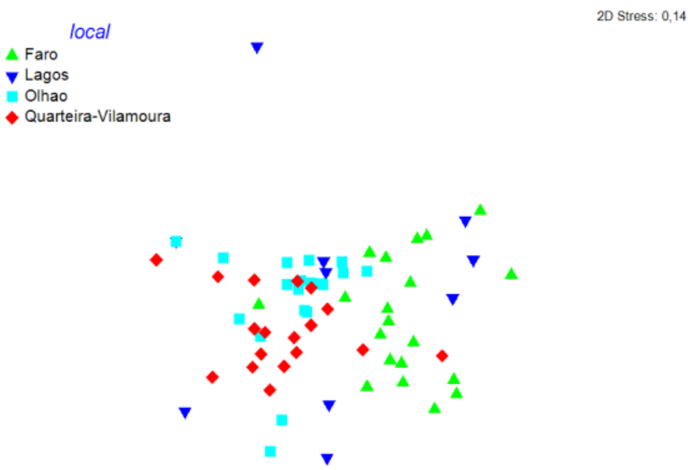

Fig 3.32 MDS (Multidimensional Scaling analysis) showing the similarities between localities (Lagos, Quarteira-Vilamoura (Qua-Vil) Faro and Olhão), the stress level was 0.14.

When comparing between different localities using ANOSIM the existence of significant differences between all localities studies was observed (Table 3.6).

Table 3.6 R number and significance level (%) of ANOSM for the factor of port locations: Lagos, Quarteira-Vilamoura Faro and Olhão.

Factor	**R number**	**Significance level (%)**
Faro x Lagos	0.347	0.1
Faro x Olhão	0.449	0.1
Faro x Quarteira-Vilamoura	0.449	0.1
Lagos x Olhão	0.394	0.1
Lagos x Quarteira-Vilamoura	0.452	0.1
Olhão x Quarteira-Vilamoura	0.124	0.4

When the different localities were compared through SIMPER it was noted that invasive species *M. squamiger* and *S. plicata* were the largest contributors to the dissimilarity between the compared samples. Only in the comparison between Faro and Lagos did native *M. occidentalis* appear as the second largest contributor to the dissimilarity, above *S. plicata*. Besides these two invasive species, other species also contributed to the dissimilarity, these were classified into 3 groups: 1- *M. occidentalis*, *C. intestinalis* and *A. aspersa* for comparin Faro with Lagos and Faro with Quarteira-Vilamoura; 2- *M. occidentalis*, *C. intestinalis* and *P. mammilata* for the comparison of Faro with Olhão; 3- *C. intestinalis*, *P. mammilata* and *A. aspersa* which contributed to dissimilarities between Lagos, and Olhão and Quarteira-Vilamouraand finally between Olhão and Quarteira-Vilamoura.

The contribution rates for each comparison were different. The comparison between Lagos and Quarteira-Vilamourashowed the highest degree of dissimilarity (49%) out of all the localities compared. In most of the comparisons almost all the species were consistent across samples (Diss / sd> 1), only a few of them showed low stability. The comparison between Lagos and Olhão was where the lowest consistency of species was found only showing consistency in the samples for the two invasive species (*M. squamiger* and *S. plicata*). As for the other species, the values presented in the samples were more random (Diss / sd<1).

After 3 seasons of observation, each port was assigned a category depending on the abundance of Sea squirts observed (table 3.7).

Table 3.7 Association of categories based on the abundance of sea squirts in the ports: 1, 2, 3 & 4 (1→ >1.000 individuals; 2→ between 1.000-10.000 individuals; 3→ between 10.000-100.000 individuals; 4→ >100.000 individuals).

Location	Type of port	Port abundance
Olhão	Fishing	4
Olhão	Recreational	3
Faro	Fishing	2
Faro	Recreational	3
Quarteira	Fishing	3
Vilamoura	Recreational	2
Lagos	Fishing	1
Lagos	Recreational	1

Finally, regarding the categories assigned related to the abundance of sea squirts presented in the ports, a high dispersion of the samples was observed through the MDSanalysis (stress value: 0.14) (Fig 3.33). On a global level, through statistical ANOSIM, significant differences between the different types of abundances were presented (ANOSIM, R: 0.144& $p < 0.05$).

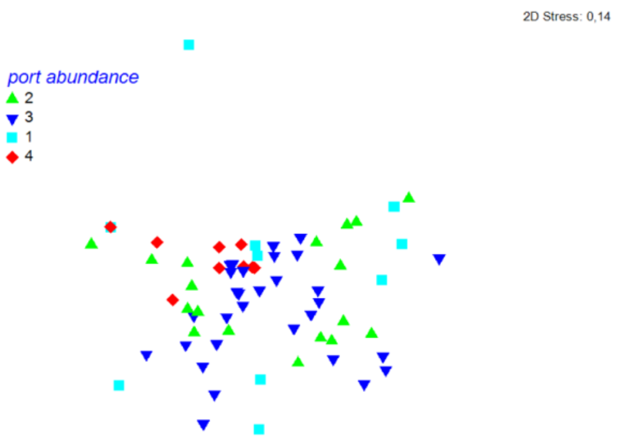

Fig 3.33 MDS (Multidimensional Scaling analysis) showing the similarities between port abundance: 1, 2, 3, & 4(1→ >1.000 individuals; 2→ between 1.000-10.000 individuals; 3→ between 10.000-100.000 individuals; 4→ >100.000 individuals), the stress level was 0.14.

When all the port abundances were compared through ANOSIM, significant differences were observed between ports with abundance 1 and the remaining ports (table 3.8).

Table 3.8 R number and significance level (%) of ANOSM for the factor of port abundance: 1, 2, 3 & 4 (1→ >1.000 individuals; 2→ between 1.000-10.000 individuals; 3→ between 10.000-100.000 individuals; 4→ >100.000 individuals).

Factor	**R number**	**Significance level (%)**
2 x 3	0,077	5,1
2 x 1	0,165	2
2 x 4	0,018	34
3 x 1	0,341	0,1
3 x 4	0,026	36,2
1 x 4	0,241	0,4

Through SIMPER dissimilarity analysis, the invasive species *S. plicata* and *M. squamiger* were found as the main contributors to the difference between all port abundances. However, other species also contributed to this dissimilarity. First, the comparison between port abundances 1 and 2 showed that *M. occidentalis* and *C. intestinalis* contributed 24% to the total dissimilarity. Second, the comparison between ports with abundances 1 and 3 showed that *C. intestinalis*, *M. occidentalis*, *A. aspersa* and *P.mammillata* provided around 40% of the dissimilarities between these two groups. And third, the species *P. mammilata*, *C. intestinalis* and *A. aspersa* contributed 25% to the total dissimilarity presented in the comparison between ports with abundances 1 and 4. Only the two invasive species (*M. squamiger* and *S. plicata*) presented a high consistency in all the samples (Diss/sd > 1), whilst the other species did not (Diss/sd < 1). When only comparing ports with abundances 1 and 3, the native *C. intestinalis* appeared with high consistency (Diss/sd > 1).

3.3.3 Case study

From the results of the case study, barnacles and sea squirts were separated from the remaining species found attached to the test devices in order to determine if the removal of invasive species would affected the community. For this purpose, the number of visits and surface type were taken into account as factors for in this case study.

1- Removal of invasive species vs. control.

a) Types of surface

Communities found attached were analyzed in order to determine the similarity between samples. Through the MDS plot the greatest similarity was observed between the PVC surfaces and cement plates. Dispersal of samples was low for PVC peaces and slightly higher for cement plates (stress value: 0.11) (Fig 3.34). This was obtained for all the devices including the control.

In contrast, closed wallets presented the highest dispersion between samples, proving the differences when compared with othertest surfaces. Statistical ANOSIM was also used to determine the existence of differences between communities, obtaining significant differences between surfaces on a general level (ANOSIM, $p < 0.05$).

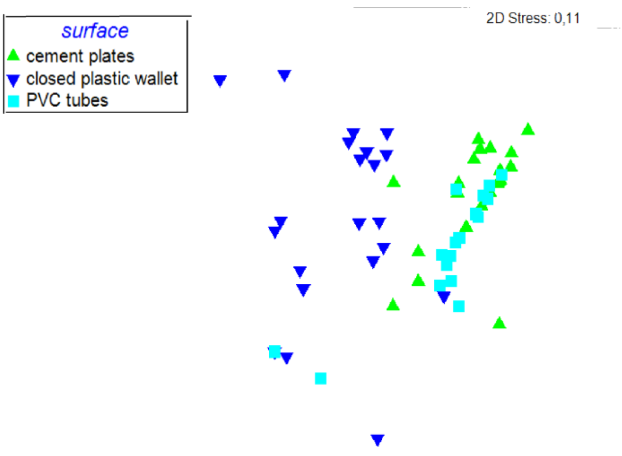

Fig 3.34 MDS (Multidimensional Scaling analysis) showing the similarities between types of surface (Cement plates, closed plastic wallet and PVC surfaces the stress level was 0,11.

When comparing between the different types of surface, significant differences were found between all cases studied (table 3.9). Matching with the distribution obtained in the MDS (Fig 3.34), the highest dissimilarity was presented between the wallet and the other two surfaces.

Table 3.9 R number and significance level (%) of ANOSM according to the type of surface: (Cement plates, closed plastic wallet and PVC surfaces).

Factor	R number	Significance level (%)
Cement plates vs. closed plastic wallet	0.44	0.1
Cement plates vs. PVC surfaces	0.2	0.2
closed plastic wallet vs. PVC surfaces	0.375	0.1

The invasive species *Amphibalanus amphitrite* was the largest contributor to the dissimilarity between all surfaces and was the only one to have a high consistency in the samples through SIMPER. Comparing between cement plates and the closed wallets, *A. amphitrite* contributed the most to the dissimilarity with 48%. Other species such as *Molgula occidentalis* and *Styela plicata* provided 20% to the total percentage, and a grupo composed by *Botryllus schlosseri*, *Ciona intestinalis*, *Molgula* sp., Ascidiacea n.id. and *Amphibalanus* cf. *eburneus*,contributed with another 20%. Comparing cement plates with the PVC piece, small significant similarities were shown highlighting that *A. amphitrite* was the one that contributed the most for the dissimilarity between the two surfaces (65%). In ascendingof contribution to the total dissimilarity *A.* cf. *eburneus, Perforatus perforatus, B. schlosseri, S. plicata* and Ascidiacea n.id.also played their part.

Finally comparing the closed wallets with the PVC piece, greater dissimilarity between the surfaces were observed. *A. amphitrite* contributed to this dissimilarity 34%, *M. occidentalis* and *S. plicata* presented a high contribution of 30% between the two. The last portion of dissimilarity was shown by *C. intestinalis, B. schlosseri, Molgula* sp. and *Ascidiacea* n.id with 24%.

b) Times of visits

The effect of the removal invasive species from the test devices along time was compared to the control devices where the removal was not performed until the end of this study. Testing for differences and comparing samples, group 4 and the control, showed a great similarity with a medium degree of variability between samples (stress value: 0.11) (Figure 3.35). Less similarity was obtained for groups 1 and 2 that when compared showed greater variability between them. Group 3 was a mixture of the others, showing some similarity to group 4 and the control but is still represented by a large dispersion as seen in groups 1 and 2. On a general note, through statistical

ANOSIM, significant differences were found between the times of visits (ANOSIM, $p < 0.05$).

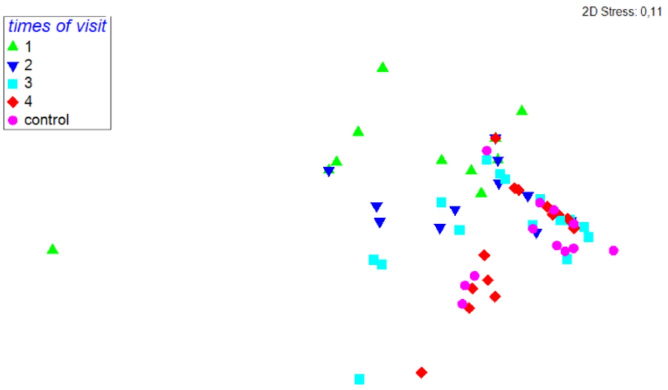

Fig 3.35 MDS (Multidimensional Scaling analysis) showing the similarities between times of visits (1- first observation, 2- second observation and first removal, 3-third observation and second removal, 4- last observation and final removal and Control- untouched during the entire experience), the stress level was 0,11.

Differences between the time of visit 1 and the other groups (2, 3, 4, control) and between the time 4 with the times 2 and 3 were presented (table 3.10). The control and the time 4 did not show significant differences.

Table 3.9 R number and significance level (%) of ANOSM for the factor times of visit (1- first observation, 2- second observation and first removal, 3-third observation and second removal, 4- last observation and final removal y Control- remain all the experience in the water not removal).

Factor	**R number**	**Significance level (%)**
1 x 2	0.141	2.7
1 x 3	0.254	0.4
1 x 4	0.381	0.1
1 x control	0.444	0.1
2 x 4	0.136	1
3 x 4	0.085	4.2

The species that contributed to the dissimilarity in the comparison between groups 1 and the other groups was invasive *Amphibalanus amphitrite*. This species was the largest contributor to the dissimilarity (an average of 45% of the dissimilarity was due to this species) and was the only species that presented consistency between samples. The other species that also contributed to these dissimilarities between groups were the same in all 4 comparisons: *Molgula occidentalis, Botryllus schlosseri, Ciona intestinalis*, *Molgula* sp, *Ascidiacea* n.id and *Amphibalanus* cf. *eburneus*. Only 2 species contributed to the dissimilarity but not in all comparisons, as is the case of *Styela plicata* which contributed to all except between time 1 and 4. The other species was *Perforatus perforatus* which appeared in the comparisons of time of visit 1 with 2 and 3.

Finally, group 4 compared to groups 2 and 3 again showed *A. amphitrite* as the largest contributor to the dissimilarity with an average percentage of 44%, followed by *M. occidentalis* with an average of 18% and *S. plicata* with an average of 9%. Besides *C. intestinalis* and *Molgula* sp. also contributed in both comparisons. Although *B. schlosseri* only appeared when groups 2 and 4 were compared; for groups 3 and 4 *P. perforatus* and *A.* cf. *eburneus* were the last to contribute to the total percentage of dissimilarity.

1- Recruitment related with surfaces and removal procedure.

For the three invasive species found (*Styela plicata*, *Amphibalanus* cf. *eburneus* and *Amphibalanus amphitrite*) data was analyzed through statistical two way factorial ANOVA, determining the existence of differences in their recruitment. So it was taken into account if the invasive species had been removed previously (when the device was picked up after several removals) and if they had not been removed (control group remain all the experience in the water without any removal).

Comparisons were carried out in relation to type of surface, and the number of times that invasive species had been removed, or the combination of both factors. For *S. plicata* and *A.* cf. *eburneus* no differences in the recruiting were shown (ANOVA, $p > 0.05$).But there were differences in recruitment between surfaces in the case of *A. amphitrite* (ANOVA, p showed <0.05) but not between times of visits (ANOVA, $p> 0.05$).

Combining both factors, small significant differences were found for *A. amphitrite* between the control cement plates and the control wallets and after the removal treatment (after the forth visit) (ANOVA, $p <0.05$). The differences that were found were small but significant. These differences showed are related with the preference of invasive species for cement surfaces.

4.DISCUSSION

During the sampling period, the percentage of invasive species found for the two groups of interest was higher than that of native species, showing a rate of 60% for barnacles and 58% for sea squirts. This seems to be related to the ability that the invasive species have over native species of adapting to new conditions (Essink& Dekker, 2002). Invasive species have characteristics that enable them to become major competitors for the native fauna, generally causing changes to the biodiversity, but in turn also having a social and economic impact (Bax *et al*. 2003). This said, according to Essink& Dekker (2002) the characteristics presented by invasive species that provide these advantages over the native species are: "successful in colonizing new areas, namely environmental tolerance, high genetic variability, short generation time, early sexual maturity, high reproductive capacity and broad diet", beside there are important vectors in place to introduce this invasive species.

According to Bassindale (1936), Katz (1983) and Lemaire (2011) the two main groups of interest in this study are organisms that have mobile larval stage and which become sessile organisms when reaching maturity. The free-moving stage makes them optimal organisms to move with ballast waters, whereas the adult sessile organisms find their best form of travel via biofouling on the hulls of boats. According to Ruiz *et al*. (2000), both biofouling and ballast water are considered the main mechanisms of introduction for invasive marine invertebrate species. Fouling organisms that are mainly transported by these dispersal vectors are usually accidentally introduced due to the inability to establish any kind of control over them. Aquaculture also plays an important role in the introduction because many invasive organisms are attached to the targeted species which are then used in aquaculture (Afonso, 2011).

Another species found while performing the case study was *Amphibalanus* cf. *eburneus*. This invasive barnacle originates from the American Atlantic coast and has only been found colonizing the archipelago of the Azores (Southward, 1998) and Mediterranean (Southward, 2008). This is first time that the species is recorded in mainland Portugal. This invasive species is currently being studied in more detail for a future publication. Finally, a large number of individuals of the invasive sea squirt *Microcosmus squamiger* were reported in the ports of the Algarve. This is the first confirmation for its occurrence in southern Portugal. To date, in Portugal, this species is only known from the archipelago of the Azores and Cascais (Ruis *et al.*, 2009) and has never been described for the Algarve region. On the other hand there are other species that were introduced years ago and that are now established in the ecosystem. According to Carlton (2003) a certain amount of time must be established between the species being introduced and colonizing the area, and it being considered as an established or naturalized species. This is the case of the chiton *Chaetopleura angulata* reported by Hidalgo (1916) and the Pacific oyster *Crassostrea gigas* mentioned by Edwards (1976) for Portugal. Both species are now considered part of the indigenous fauna due to the long period of time that has passed since they were introduced.

Using the grid methodology for barnacle counting, two invasive species (*Amphibalanus amphitrite* and *Austrominius modestus*), two native (*Chthamalus montagui* and *Perforatus perforatus*) and one species of unknown origin (*Amphibalanus* sp.) were found in the ports of Algarve during the sampling period. Invasive *A. amphitrite* showed greater abundance and frequency of occurrence on the grids. According to Southward (2008) this species is a common fouling barnacle found all over the world, mainly in port areas which present warm and sheltered conditions. It can generally be found in the intertidal and sub littoral areas. Furthermore, its reproduction period is between the months of March and September (Southward, 2008) coinciding with the sampling period. According to Crisp & Patel (1960) after studying their reproduction in laboratory, found an optimum temperature for reproduction around 15 ± 2°C.According to the Portuguese Hydrographic Institute, the coastal water temperature during the sampling period was between 14-20°C, probably with slightly higher values in the port waters, therefore establishing these temperatures as the optimal values for the development of the species. This species also presented a high consistency in the samples thus facilitating the future monitoring of this invasive species. On the other hand, invasive *A. modestus* did not present very high values of abundance. This observation seems to be related to the reproductive cycle of this species that according to Southward (2008) takes place throughout the year, showing a decrease from mid-winter to spring reaching maturity during the summer months. Native *Chthamalus montagui* showed higher abundances throughout the entire sampling period. According to Southward (2008) this barnacle presents a surprisingly high number of individuals in areas such as the south-west coast of Europe, especially in Portugal, Spain and the Atlantic part of Morocco. This high abundance could be related to the upwelling that occurs in this area (Arístegui *et al.* 2004; Chicharo, 2004) which brings a high amount of plankton providing favourable conditions for the settlement of this species (O'Riordan *et al.* 2004; Southward, 2008). Furthermore, the large abundance of this species in this area could be related with its rapid fixation/colonization. This rapid fixation/colonization may obstruct the fixation of other species (invasive and native). In the field a great fixation for this species was observed and, according to O'Riordan *et al.* (2004) and Southward (2008) they are found in a large number. It seems that native *C. montagui* is not affected by other invasive barnacle species cohabiting in its optional ecological niche, being a strong competitor, at least in this particular geographical area. As for native *Perforatus perforatus*, it showed low abundances in all studied areas. This seems to be related with its distribution, a dominant species of the sublittoral zone, where sampling was rarely carried out (Southward, 2008). Also according to Southward(2008), the reproduction period for this species is between June and September, reaching the settlement period in August and September. Therefore this could also be a factor contributing to its low abundance during the sampling period. The fifth species *Amphibalanus* sp., not yet fully identified, appears to have a combination of the characteristics of native *C. montagui* and the invasive *A. amphitrite*, so the hypothesis of a possible hybridization between both species is proposed. To test this hypothesis, further morphological and genetic studiesare proposed to determine if we are in fact dealing with a hybrid of the two mentioned species, a different species or even a subspecies. According to Tsang (2008), hybridization has occurred between two different subspecies (*Tetraclita japonica japonica*and *Tetraclita japonica formosana)* in the north-western Pacific.

Settlement period seems to be related with the differences between seasons, being in spring when the peaks of maximum reproduction for most species take place (Southward, 2008). In relation with our results, there were not significant differences in terms of abundance. However, the two invasive species appeared quite frequently in the samples analysed, 70-80% (*A. amphitrite*) and about 40-50% (*A. modestus*). These differences in the settlement period, can explain why the frequency of occurrence was more equitable during the spring season (Southward, 2008). As referred to above the only exception is native *P. perforatus* with a reproduction period outside of this season, hence its abundanceand frequency always being the lowest. Besides, during the winter season, there was a lower diversity in comparison with the other two seasons. Whereas, in the case of species richness and diversity, were winter and summer the seasons that showed differences in the specific distribution. The short duration of sampling (from February to July) may be one of the reasons why no differences were found among the samplesat community levels. A wider sampling period, extended to at least one year could eliminate the error of not having encompassed all the reproductive cycles of thestudied species of barnacles and this way produce better results.

The input of marine invasive species is related with the transit of international vessels. According to Johnson *et al*. (2001), the recreational boats are related with the transport of larva and adult life individuals. The increasing of the transport networks has favoured this transport of invasive species by humans (Ladd *et al.* 2001; Ashton *et al.* 2006; Hulme, 2009). Also according to Murray *et al*. (2011) and Ashton *et al.* (2006) recreational boats are the major vector of introduction for invasive species contributing to the spread of those organisms. All this was reflected in the results obtained from this study when type of ports and localities were compared. In the comparison between types of ports some differences were obtained between fishing port and marinas. Both invasive species were most frequent in recreational ports, *A. amphitrite* (87%) and *A. modestus* (51%), while the native specieswere more frequent in fishing ports, *C. montagui* (79%). For the other native species *P. perfuratus* (10%) and for the unknown origin species *Amphibalanus* sp. (77%) there were no significant differences between types of ports. Also the highest abundance of the invasive species *A. amphitrite* (20%) was found in recreational ports, in contrast with the native, *C. montagui* (82%) which showed the largest abundance in the fishing ports. Besides, the greatest diversity and heterogeneity of the samples was found in recreational ports (d: 0.43 and J': 0.67). In the case of fishing ports less heterogeneity was observed, probably due to the high abundance of native *C. montagui*, causing great dominance over the rest in the distribution of species in the samples. There were also differences found between both types of ports in terms of community. The native *C. montagui* contributed to half of the percentage of these dissimilarities. The main reason for this is the difference in the abundance of the species from one port to another, with values nearly doubled in the fishing ports in comparison with the marinas. On the contrary, *A. amphitrite* contributed in a fairly smaller percentage to the total dissimilarity (19%). Although there were differences between the abundances of this invasive species (*A. amphitrite*), this was not reflected on the dissimilarities between ports in terms of community.

Also in the comparison between localities, a larger input of invasive species was reflected in the localities where the two largest marinas were located. In terms of abundance, *A. amphitrite* was found as the most abundant for the Lagos area with 41% of the total. Quarteira-Vilamoura also presented high values of abundance (13%) for this species (*A. amphitrite)*, however the highesttotal abundance observed for that locality was estimated for *C. montagui* (79%). The great abundance of the native species (*C. montagui)* in Quarteira-Vilamoura location could be explained by the fact that these ports occur more or less in the same geographical area. In the fishing port of Quarteira, *C. montagui* presented very high abundance values probably overlapping the abundance values of *A. amphitrite* for the Vilamoura recreational port. Also note that in marinas, cleaning tasks for the maintenance of the facilities are carried out. This maintenance is regularly done in the Vilamoura marina and may have influenced the total abundance of *A. amphitrite*, found mainly on the pontoon poles that were normally scraped for cleaning. The native *C. montagui* presented its maximum abundance in Faro with 89% of the total abundance followed by Quarteira-Vilamoura. It was expected that this species would also be the most abundant in Olhão, as the fishing port there is quite crowded but, in this case, *Amphibalanus*sp. dominated with half of the total abundance. These results may be related to differences in the environmental conditions found between these ports. Here it would also be interesting to know if the potential hybrid between *C. montagui* and *A. Amphitrite* has managed to adapt and develop in this port, being so common here.

When the frequencies were studied for Lagos, a greater frequency of invasive *A. Amphitrite* and *A. modestus* was found (100% and 77% of occurrence respectively), followed by Quarteira-Vilamoura area (82% and 54%) where the largest marinas are located. Also in Lagos, the native *C. montagui* was present with the higher frequency (87%) in contrast with the lower abundance that this species showed in this area (this species has a high occurrence but in very low numbers of individuals). The weaknesses of the abundance of this native species lets other species grow more abundantly and frequently in Lagos. Besides the other two species *P. perforatus* and *Amphibalanus* sp. also showed their great frequency in Lagos locality (27% and 100% respectively). Both species furthermore presented their second highest frequency of occurrence in the locality of Olhão. Therefore the greater diversity among the samples corresponded to the Lagos area; however the diversity was practically the same in the other three port localities probably related to the abundance of *C. montagui* in these ports. Samples from Faro and Olhão showed a great heterogeneity, followed by Quarteira-Vilamoura and finally by Lagos. In conclusion, dissimilarities between all localities studies were found, with the exception being the comparison between Lagos and Quarteira-Vilamoura presenting similarity in their communities. The species that contributed most to this dissimilarity was again the native *C. montagui* for its great variability in sample distribution. *A. amphitrite* appeared more frequent among samples so it is presented in a more homogeneous distribution in the samples. The other invasive *A. modestus* also participated in the total dissimilarity when Lagos and Olhão were compared, due to its greater abundance (2%) and occurrence (77%) in the Lagos area and its lower abundance (almost half) and occurrence in Olhão ports.

The degree of protection of the ports may cause some differences in the comparison between geographic and port locations. According to Ashton (2006), the marinas in Scotland seem to act like refuge for invasive species, providing a perfect habitat for this species. These differences could be related with the higher protection and less turbulence found inside the Ria Formosa lagoon. In this area, waters are consequently more turbid and richer having higher turbidity due to the elevated load of organic matter carried in the water renewal. These ports are the recreational and fishing portsof Faro and Olhão. On the contrary, less protected ports are those that are directly influenced by the ocean, showing greater clarity of the water and therefore greater renovation of the water. The recreational and fishing ports of Quarteira-Vilamoura and Lagos were listed as less protected ports. For the comparison between sheltered and unsheltered ports, some differences were found in terms of abundance. For that reason, invasive *A. amphitrite* and *A. modestus* both appeared more abundant (20% and 1.5%) and frequent (90% and 63% respectively) in unsheltered ports. The great abundance of *A. amphitrite* may be related to the high content of minerals in the water of unsheltered ports (20% abundance). Those minerals are used in the formation of the shell and calcareous base (Southward, 2008). The calcareous base of the invasive *A. amphitrite* helps to resist the incoming flow in ports, making their fixation stronger than that of *A. modestus* or *C. montagui* which form a membranous base (Southward, 2008).In sheltered harbours, the native *C. montagui* reached its maximum abundance at 75%, but there were not large differences in relation with unsheltered (69%). It was just the unidentified species *Amphibalanus* sp. and the native *P. perforatus* which showed their greatest abundance (21% and 0.29%) in sheltered ports. When the frequency of occurrence was compared, it was found that the natives *C. montagui* and *P. perforatus* showed greater frequency in unsheltered ports (84% and 15%) than in sheltered (50% and 7%). Also the two invasive (*A. amphitrite* and *A. modestus)* presented their greatest occurrence (90% and 63% respectively) in unsheltered ports. Instead, the unidentified species *Amphibalanus* sp. showed its greatest occurrence (83%) insheltered ports. The stated characteristics of the ports in relation with their location also influenced the diversity and evenness index of the samples. The higher values were shown for unsheltered ports implying a greater diversity of species in the samples. The opposite situation occurs in the sheltered ports because both diversity and evenness are lower referring again to the remarkable abundance of native *C. montagui*. The community also presented differences when their locations as sheltered and unsheltered areas were compared. 46% of these differences are associated with the native *C. montagui* although, in this case, there was no difference between the abundances. However, the distribution of this species (*C. montagui*) in the samples seems to show differences between them, probably in relation with the differences between the frequencies of occurrence. *Amphibalanus* sp. and *A. amphitrite* also contributed to the dissimilarity with 24% and 22% respectively, showing greater variation in terms of abundances. The distribution of thesespecies among the samples will probably be homogenous; therefore its rate of contribution to the total dissimilarity is lower than the native *C. montagui*.

It is very probable that many of the invasive species that are introduced into the Ria Formosa lagoon, find their ideal conditions to settle without having to search for cover in the confined water inside ports. By contrast, the new species which arrive in unprotected areas may be forced, according to the hydrodynamics of the area, to find more sheltered areas, at least until their stabilization and colonization of the area, and consequent reproduction.

The geographic locations (west, central and east) are related to the port characteristics. The ports on the east coincided with the ports considered sheltered, which are located within the Ria Formosa lagoon. According to Abecasis *et al.* (2006) the Ria Formosa has similarities to Mediterranean areas, sharing features in protection and in properties of the waters. Therefore Mediterranean species can easily find optimal conditions for their development and growth. The western ports, however, refer to the fishing port and marina of Lagos, which are considered as unsheltered ports. These western ports have more Atlantic characteristics (similar to open sea) and more influence by specific oceanographic conditions, such as upwelling (Sanchez and Relvas, 2003). Finally the central ones were considered as transition ports, showing a mixture of Mediterranean and Atlantic features. These ports are associated with the fishing port of Quarteira and the marina of Vilamoura. It was expected that the invasive *A. amphitrite* and *A. modestus* would show greater abundance and frequency of occurrence in the western and central ports in relation with the large circulation of foreign ships. Nevertheless it was only in the western port where the invaders *A. amphitrite* and *A. modestus*were found as the most abundant with 41% and 2% respectively. Whereas the native *C. montagui* had the highest abundance in central ports but no significant differences were observed in comparison with eastern ports. The great abundance of the native species (*C. montagui*) in central and eastern ports could be explained again by the more Mediterranean characteristics that these ports present. The unidentified species *Amphibalanus* sp. showed its greatest abundance in western and eastern ports (21% in both). On the other hand, the frequencies of occurrence did not meet expectations, and the invasive species (*A. modestus* and *A. amphitrite*) were found more frequently in eastern ports (77% and 100% respectively) than in western ports (36% and 62%). For the central and eastern ports no significant differences were observed in their diversity indices and evenness, this may be explained by Abecasis *et al.* (2006) with more similar Mediterranean features. These ports have greater homogeneity between samples, probably related to the high abundance of *C. montagui* which overlaps the other species.In terms of community, western ports did not seem to present differences from the other two, and this could be related to the low number of samples obtained for these ports. Besides this, the ports showed greater similarity in relation to the abundance, occurrence and diversity, but in terms of species composition trough the samplesa greater variability in this distribution among samples was observed.

As for the sea squirts found during the sampling period, two invasive species were presented clearly as the dominant species in all the ports. These were *Styela plicata* and *Microcosmus squamiger*, two species that according to Naranjo (1996) are distributed globally. These species prefer places with calm conditions and high presence of organic matter, i.e. they are organisms that have affinity for port areas where water turbidity is high. The invasive species of sea squirts that exist in the Algarve have the tendency to form clusters (Ramos, 1991; Lowe, 2002), so when counting was carried out on ropes and other objects, if one specimen appeared it was usually accompanied by a large number of individuals of the same species. These clusters are reflected on the study of the community, showing the main cause of differences in the distribution of species among all the samples. Besides, this sustains the great abundance of these two invasive species (*Styela plicata* and *Microcosmus squamiger*) in all the ports studied. For the counting procedure, external morphology allied with a specific identification key was used in the identification of the sea squirts. All the individuals dissected after the previous external identification were reconfirmed, meaning that the methodology used above proved to be quite successful. According to Ramos (1991), few species of sea squirts can be identified via external characters; but because most species found during our study belong to different genus, a clear differentiation between them was possible. Therefore this can be considered as a new approach for the *in situ* identification of this taxonomic group.In the internal identification process, *M. squamiger* mayoften be confused with *Microcosmus exasperatus* and the internal syphonal spines are what mark the differences between them (Kott, 1995). These spines can have blunt shapes, as those of *M. squamiger*, or pointed shapes, like *M. exasperatus*. After the dissection and observation of several specimens, it is certain that the species found in this area can be classified as *M. squamiger*. According to the study conducted by Ruis *et al*. (2009), most specimens of *M. exasperatus* reported in the Mediterranean are actually *M. squamiger*. This species (*M. squamiger*) has its optimum temperature between 12 and 25 °C which is within the temperature variation observed during the sampling period (Portuguese Hydrological Institute, 2015). In laboratory Ruis *et al*. (2009) also determined that at 20ºC is when the settlement occurs for this invasive species. For the other invasive *S. plicata*, its internal and external identification process is much easier, as it does not present great similarities with other species. According to Fisher (1976) in North Carolina this species presents its recruitment peaks when the water temperature is close to 20 °C. And when the temperature ranges between 5 and 15 °C or above 25 °C the inhibition of the settlement is produced (Fisher, 1976).

Differences in water temperature were shown during our sampling period (Portuguese Hydrological Institute, 2015) and that could affect this species. According to Fisher (1976), *S. plicata* found its best settlement temperature as well as *M. squamiger a*ccording to Ruis *et al*. (2009) within the above mentioned temperature range. For some native species the optimal temperature of reproduction was also found in this range. An example is *C. intestinalis* (Katz, 1983) at around 18ºC. A possible hypothesis to explain the differences between seasons is that it is connected to the reproductive period of invasive and nativesea squirts or even the sea water temperature.In the spring and summer seasons the two invasive species *S. plicata* and *M. squamiger* were those that presented greater abundance. In the spring season it was consistent with almost 95% of the total abundance and both species also showed a high frequency of occurrence in the counting (86% and 91% respectively). The remaining 5% of abundance was divided between native *Phallusia mammilata* and *Ciona intestinalis*, these species appeared with an intermediate frequency in the samples (29% and 37% respectively) although with low percentage in terms of abundance. There were some differences between the seasons in terms of abundance and frequency of occurrence. The differences showed that these dominant species (*S. plicata* and *M. squamiger*) seem to be the main cause of the differences presented between samples in relation with the sea temperature. As it was said before, invaders contributed largely tocommunity dissimilaritiesin relation with the clustering. This is due to the decrease presented during the summer in terms of abundance. Some native species also contributed to this total dissimilarity value, i.e. *M. occidentalis* and *C. intestinalis* showed higher frequency of occurrence (47% and 61%) and abundance (14% and 7% respectively) during the summer than during spring. This composition of species in different seasons in abundance and frequency contributes to the differences in the community.

As for the barnacles, the higher input ofthe invasive species is probably related to the foreign vessels. Therefore this could be the form of entry for invasive species via ballast water and biofouling which seems to be more frequent in areas where there is an intense transit of vessels. This could be observed in terms of abundance and frequency of occurrence when types of ports were compared, but not in community distributions. Considering the different types of ports, higher abundances of invasive the species *S. plicata* were observed in recreational ports (47%) with a slight increase in its frequency of occurrence (97%). In terms of protection and availability of organic matter, as occurred in the barnacle comparison, some differences were found. Furthermore, the calm water within the Ria Formosa, facilitates the fixation of the invasive species. According to Gamito (1997), there are extensive aquaculture activities inside the Ria

Formosa, therefore this is another invasive species input to take into account. These differences were shown when sheltered and unsheltered ports were compared. Besides, when the localities were compared, other differences were shown in relation with what was explained above. By grouping ports into sheltered and unsheltered ports, the maximum total abundance of invasive *M. squamiger* (50%) was reached, while in the unsheltered ports the invasive *S. plicata* dominated (60%). Also native *M. occidentalis*, which appeared in sheltered ports, and native *A. aspersa*, which appeared in unsheltered ports, contributed to the differences in abundances between ports. As for the frequency of occurrence found for each type of port, some differences were also observed in the comparison. It was higher in sheltered ports for all the invasive (*M. squamiger* (98%), *S. plicata* (95%), *S. canopus* (8%) and *C.* cf. *eumyota* (3%)) and some native species (*M. occidentalis* and *P. mammilata*), whereas in unsheltered ports two native species were present (*A. aspersa* and *C. intestinalis*). The aquaculture activity performed in the Ria Formosa (Gamito, 1997) could be related with the sporadic appearance of the invasive species *S. canopus* and *C.* cf. *eumyota*. All these differences in abundances and frequencies between port locations contributed to the existence of dissimilarities between communities, marked mainly by invasive species *M. squamiger, S. plicata* and native *C. intestinalis*. These species appeared consistently in samples although with certain variations in their distribution. Other species which contributed to the dissimilarities were native *M. occidentalis* by presenting consistency only in sheltered ports and native *A. aspersa* in unsheltered ports. The distribution through samples probably produced these differences in the community level. When port localities were compared, the species that stood out in terms of abundance in Lagos and Faro was the invasive *M. squamiger* with a percentage of 59% and 54%, respectively. In terms of community, there seem to be differences between all ports localities studied, probably connected to differences in the abundances presented. The invasive *S. plicata* and *M. squamiger* were the species that contributed to the dissimilarities in all comparisons. Emphasising the comparison between Lagos and Faro, where the native *M. occidentalis* contributed with the second highest percentage of dissimilarity. Some differences were shown in the sample's distribution of this species for Lagos and Faro. Greater similarities between the Quarteira and Olhão localities were expected, because these ports had very high abundance of sea squirts. However, small significant differences were found. This comparison showed the lowest percentage of dissimilarity (31%). Lagos was the locality which presented the highest percentage of dissimilarity in comparison with the other localities. For Lagos, the total amount of sea squirts was very low. This also had to be taken into account when the comparisons were carried out because the frequency and abundance might have been overestimated. Greater similarity was also expected between the ports of Faro and Olhão in relation to the availability of food and hydrodynamic conditions, as both are located within the Ria Formosa lagoon. This comparison presented the second lowest level of dissimilarity (42%). Between Quarteira-Vilamoura and Faro there was also a high dissimilarity. This dissimilarity may be related with the study carried out by Chicharo (1998), where it is explained how there is a large supply of nutrients from the upwelling produced in the western coast. This may indicate that there are no significant differences in productivity between the Ria Formosa (the most eastern) and the western areas (typically Atlantic coast). The hypothesis that the differences between Faro and Olhao *versus* Lagos and Quarteira-Vilamoura, were related to a greater fixation of sea squirts inside the Ria Formosa by hydrodynamic conditions was also considered. Nevertheless there have been no major differences in this respect, showing the same capacity of fixation to any substrate in the port facilities. By establishing a category for each localitiyin the study based on the abundance observed in ports, significant differences were also found. These differences arose between the Lagos locality and other port localities; the main reason seems to be related to the low abundance of sea squirts observed during the entire sampling period. Therefore the specific composition of the samples did not show great similarities. The main contributions to the dissimilarities when carrying out all of these comparisons were the invasive *S. plicata* and *M. squamiger*.

All this leads us to believe that, based on the abundance of sea squirts; the categories 2, 3 and 4 could have been classified as the same one. Species composition did not show differences in terms of the total abundance of sea squirts presented in ports with abundance 2, 3 and 4.

The case study carried out during the sampling period showed interesting results in relation to the best attachment surfaces for barnacles and sea squirts. The main differences found during the interpretation of the MDS plot was between closed wallets and the other two surfaces. Communities presented significant differences between all the surfaces. The highest variability was presented by the wallets, followed by the cement plates and finally the PVC surfaces showed the lowest. This variability seems to be related with the heterogeneity presented in the samples. The invasive *Amphibalanus* *amphitrite* was the main contributor to those dissimilarities, presenting high consistency in the samples. When the wallets were compared with the cement plates, the invasive species *A. amphitrite* contributed to 50% to the total dissimilarity. This is explainable because this species of barnacle was mainly associated with the cement plates, being much rarer in wallets. However, as mentioned above, the main difference shown was related to the extremely high amount of *A. amphitrite* colonising the cement plates. These observations indicate that the communities colonising PVC surfaces showed less heterogeneity between the samples during this study. This way, PVC surfaces were characterized by a lower number of species as well as a reduced abundance when compared to the cement plates and wallets. These differences seem to be related with the dimensionality of these surfaces. The wallets have a three-dimensional shape which facilitates the fixation of sea squirts, rather than a one-dimensional surface as seen for the other devices (PVC and cement plates) also used in the study. However, the one-dimensional surfaces of the PVC and cement plates were ideal for barnacle fixation. Besides, these results seem to contradict the ones obtained by Blum *et al.* (2007) using PVC surfaces in San Francisco, USA. According to this author PVC is used as a great surface for recruitment and to obtain the community's richness, but in our study this surface was the worst for that purpose showing the lowest community's richness and diversity.

During the case study the removal of invasive species from surfaces was under taken to determine if it affected to the community composition through time. Later, they were compared with a control panel containing the three studied surfaces. As expected, the main differences were found while comparing the first visit and the visits that followed *A. amphitrite* contributed 45% to the total dissimilarity in all comparisons. *M. occidentalis, B. schlosseri, C. intestinalis, Molgula* sp., Ascidiacea n.id. and *A. eburneus* also contributed in all comparisons. Comparing the first visit to the second and third ones, *P. perforatus* showed the smallest contribution. Finally the invasive *S. plicata* was a contributor to the dissimilarity except when comparing the first visit with the fourth. Logically this is related to the fact that in the first visit the species was only observed (no removal was made), due to the small size of the organisms, making complicated to identify them.

When the second and third visits (coinciding with first and second removal) were individually compared with the fourth visit (final collection) some differences were observed. The species which contributed to the differences in both cases (between 2 and 4 and between 3 and 4) were the same, *A. amphitrite*, followed by *M. occidentalis* and finally *S. plicata*. For the same comparisons but with a lower percentage of contribution, *C. intestinalis* and *Molgula* sp. were also found. Between visits 2 and 4, *B. schlosseri* also contributed with 6% of the dissimilarity, while between visits 3 and 4, the other species found were *A.*cf. *eburneus* and *P. perforatus*. These differences in the removal procedure seemed to be temporarily affecting communities. Only invasive species were removed and after a short period of time they reappeared on the device. Therefore high abundances of these species could be related with their establishment in that area. Furthermore these species presented a high capacity of expansion and an apparent stable population composed by adults with high reproductive capacity. This may indicate that at least two of the invasive species (*A. amphitrite* and *S. plicata*) may be considered as established in relation with their rapid reappearance on the tested devices. On the other hand, invader *A.*cf. *eburneus* presented a remarkable growth (highest of all barnacle species sampled) during the experiment, showing a high adaptation to environmental conditions, growing up to 15mm in diameter (maximum size listed as 25mm) in just four and a half months.

Finally, to determine what surface was the best for invasive species' recruitment, a comparison was carried out between the different surfaces, the removal or not of the invasive species, and the combination of both factors. The comparison was only performed between the control devices and the last sampling observation in order to detect recruitment differences of fouling fauna. Only three invasive species were compared, *S. plicata*, *A.*cf. *eburneus* and *A. amphitrite*. The first two did not present significant differences between surfaces nor between removals or not of invaders. There were no significant differences between the combinations of both either (But in the case of *A. amphitrite*, significant differences in recruitment between surfaces were shown. Wallets and cement plates were the surfaces which presented these differences. The removal or not of invasive species did not show significant differences. Therefore control and manipulated devices seem to be similar. Nevertheless, when the combination of both factors was compared significant differences were found. These differences were presented between control and manipulated wallets *versus* control plates of cement. Therefore it can be said that cement plates seem to be the best recruitment surface for this invasive species. Furthermore, as the other two invasive species did not present any kind of preference towards either of the surfaces it can be assumed that the cement plates will also be good for their recruitment. Hence if any of these species want to be studied in terms of recruitment using cement plates would be successful. Other surveys as Blum *et al.* (2007) and Marins *et al.* (2010) preferred to use PVC panels, but in our case study that surface was the one which presented the lowest diversity. The fact that the cement plates are the best recruitment surface for invasive species can be related to the larger amount of cement structures that can be found in port facilities. One solution to reduce the amount of invasive species in ports could be the use of non-polluting antifouling paints. This could possibly help to avoid the fixation of new invasive species. Furthermore the possibility of reducing cement areas in the port facilities appears to be more complicated, but could be a suggestion for the new constructions.

5. CONCLUSIONS

- Sea squirts identification through morphological external features is difficult. But the identification key described before can be considered a good tool for the sea squirts found during the sampling period. Although a reconfirmation by a dissection in the lab is also recommended.
- In relation with the high capacity of adaptation, high percentage of frequency and abundance for the invasive species, the necessity to establish preventive and control measures is evident. Maybe a greater number of technicians trained for this task in this area could help with future monitoring. This monitoring could be performed with the main objective to reduce the ecological (loss of biodiversity), economic (loss in aquaculture and vessels) and health impacts.
- Sessile invasive species mainly reach new places via ballast water and biofouling in relation with the intense circulation of international vessels currently produced in this area. Aquaculture activities can not be forgotten because it also promoted the arrival of invasive species.
- Invasive species present great flexibility to weather conditions, availability of nutrients and hydrodynamic conditions. But if they find warm, sheltered and eutrophic waters (such as port waters), a great affinity will be presented favouring settlement, expansion and reproduction of invaders.

- In general for sea squirts (solitary and colonial and even native and invasive) great affinity for three-dimensional surfaces (wallets) was presented; while barnacles preferred one-dimensional surfaces (cement plates and PVC surfaces). An astonishing amount of invasive barnacles was shown on cement plates.
- The removal of invasive species in the area studied, did not present big differences in the long term (recollection of devices). Although during the experiment small temporary differences were found. It will probably be related with the great abundance of invasive species and the possibility of being established.
- One of the invasive species found during the experiment presented surface affinity (cement plates) and the other invaders did not. This led us to believe that for any of these invasive species the use of cement as a recruitment surface guaranteed high rates of fixation. This is probably related with the high amount of cement infrastructure found in port facilities. Therefore, the decrease of these structures and the use of antifouling paints (no polluting) is highly recommendable.

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ANNEX I: Identification cards

SYSTEMATICS

Phylum Chordata

Subphylum Tunicata

Class Ascidiacea Nielsen, 1995

Order Aplousobranchia Lahille, 1886

Family Didemnidae Giard, 1872

***Didemnum vexillum* Kott, 2002**

(Figure 1)

COMMON NAMES

Carpet sea squirt (UK); Ascidia gelatinosa (PT).

DESCRIPTION

Didemnum vexillum is a species of colonial tunicate. They can form large masses which often have long, flexible leaf or flag like projections that are cylindrical and branched. Colonies are yellowish-cream in color with the yellow pigment observed in the gut, eggs, and embryos. Star shaped calcareous spicules are patchily distributed in the surface layer and in the cloacal cavity. The oral siphon is short with only six lobes. The atrial siphon is surrounded by large clumps of spicules around the opening. Color of conlonies yellowish-cream to grey. Zooids with common cloacal openings in circular or elongated channels; Zooids 1 mm long. Branchial siphons with 6 small projections. Stellate spicules in patches in surface tissue.

DISTRIBUTION & HABITAT

D. vexillum is believed to be native to the waters around Japan. It has been reported as an invasive species in a number of places in Europe, North America and New Zealand. Primarily, subtidal specie which may occur from lower intertidal zone to the continental shelf. Usually they can grow on hard natural and artificial substrates (may form long hanging, rope-like lobes, or beard-like colonies and they could be found in including docks, pilings, moorings, ship hulls, and rocks), and also on sea floor where they may form low undulate mats with short lobes on surface; they can also grow over other organisms when it needs more room to expand.

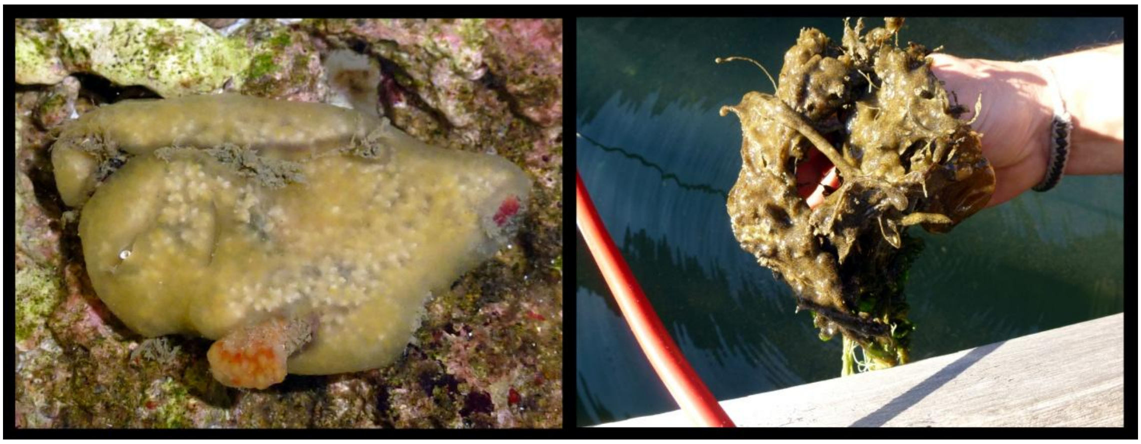

Figure.1 – *Didemnum vexillum* Kott, 2002 observed at the Port o Faro, in Southern Portugal in February 2015: left) small carpet colony; right) large massive like colony hanging from rope observed (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

The zooids are hermaphrodite, the sperm is liberated into the sea and some of it gets drawn into another zooid with the water current. It can be said that there is internal fertilization. The larvae have a short free-living stage just few hours then the metamorphosis phase starts and the larvae becomes a zooid to find a new colony. The new colony has grown by sexual reproduction and this growth occurs in a very quick way.

IMPACTES

Didemnum vexillum can quickly foul large areas of artificial and natural substrata. It is known to overgrow native *Mytilus edulis* Linnaeus, 1758, *Tubularia* spp., *Sabella* spp., *Fucus* spp., *Laminaria* spp and the invasive *Ascidiella aspersa* (Müller, 1776). They may be a threat to these and other epibiotic species. As a fouling organism, it grows over a variety of surfaces, altering marine habitats and threatening to interfere with fishing, aquaculture, and other coastal and offshore activities. It aggressively grows over bivalves and may smother them or interfere with their growth. It has no known predators.

REMARKS

Forms massive, thick or thin, sponge-like carpets; pendulous outgrowths originating as finger and flag-like surface lobes, reaching 1 m long; highly flexible, moving with surge or currents.

REFRENCES

http://www.marinespecies.org

http://eol.org/

http://www.salemsound.org/

http://invasions.si.edu/

http://www.marlin.ac.uk

Order Phlebobranchia Lahille, 1886

Family Corellidae Lahille, 1888

Corella eumyota **Traustedt, 1882**

(Figure 2 & 3)

COMMON NAMES

Orange-tipped sea squirt (UK); (PT).

DESCRIPTION

Grayish, semi-translucent tunic (exterior skin) revealing internal gut and gonads, but occasionally covered with debris. Rounded, oval or egg shaped body. Two prominent siphons: An oral siphon at top and an atrial siphon located 1/3 of the way down the side of the body. Often found adhering very tightly to one another in clumps. Typically grow to 4 cm in length.

DISTRIBUTION & HABITAT

Corella eumyota was originally described from Valparaiso in Chile and is native to the southern hemisphere, where it has a circumpolar distribution within the temperate and sub-polar regions (Chile, Antarctic Peninsula, South Africa, Australia and New Zealand). In 2002, this tunicate was discovered representing a range expansion into the northern hemisphere (France, North Atlantic Ocean in Spain and Portugal, UK Channel and Celtic Sea). Usually it is found in shallow, subtidal waters attached to docks, pilings, ropes, and other submerged structures. They prefer calm and protected waters.

Figure.2 – *Corella eumyota* Traustedt, 1882: top) small aggregation of individuals (adapted from National History Museum of the UK (**http://www.nhm.ac.uk/**); bottom) single individual collected Port of Faro, February 2015 (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

IOLOGY & ECOLOGY

C. eumyota is a brooding hermaphroditic tunicate which is capable of auto-fertilization because the sperm duct opening is located very close to the oviductal opening. The larvae are retained until they are competent to settle, so the free-swimming larval period is extremely short, the dispersion is limited and the settle on the parent tunic or adjacent adults helps with the formation of large clumps with different sizes of individuals. The breeding period in the southern hemisphere may last more than six months (fom mid-September to the end of March) but for the opposite in the northern the breeding is offset corresponding to the longer day length and warmer temperature.

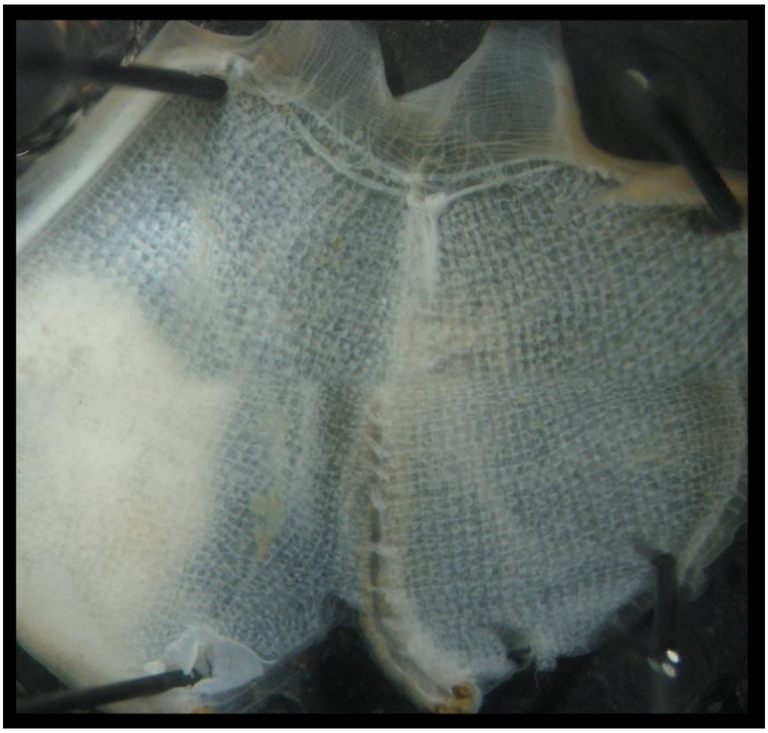

Figure.3 – Dissected specimen of *Corella eumyota* Traustedt, 1882 (adapted from Ecology & Evolution Lab of South Africa https://marcriusvil.wordpress.com).

IMPACTES

C. eumyota is considered as threat to shellfish colonies, as well as other sessile invertebrates.

REMARKS

The typical orange coloration of the siphons and the black U-shaped hind-gut are two of the main features to identify this specie. The mode of transport of *Corella eumyota* into the northern hemisphere, while unknown, is presumed to be anthropogenic by: hull fouling, transport of settled post-metamorphic individuals on floating debris in ballast water, or importation of contaminated mussels or oysters from the southern hemisphere.

REFRENCES

http://www.nhm.ac.uk/ (photo)

http://www.marinespecies.org

http://www.salemsound.org/

http://www.nhm.ac.uk/

https://marcriusvil.wordpress.com (dissection´s photo)

Order Stolidobranchia Lahille, 1887

Family Styelidae Sluiter, 1895

***Styela canopus* Savigny, 1816**

(Figure 4 & 5)

COMMON NAMES

Rough tunicate (UK); Mija-mija (PT).

DESCRIPTION

Styela canopus is a solitary tunicate but can grow in a dense cluster of individuals. The test is variable in thickness, tough and leathery with rough bumps and wrinkles. The color is usually grayish or yellowish becoming brown, purplish, or red, with four dark stripes on the siphons. Internally there are two gonads on the left side and 2-5 on the right. Specimens can grow up to 25-30 mm in total length.

DISTRIBUTION & HABITAT

This specie can be found in Atlantic Ocean (British Islands, France, Galicia, Portugal, Cape Verde Archipelago, Morocco, Senegal, Ghana, Bermudas, Florida and Massachusetts Bay, Panama, Cuba and Puerto Rico); in the Mediterranean Sea (Italy, Tunisia, Israel, France, Balearic Island, Cataluña, Levant, Almeria and Tunisia); Strait of Gibraltar (Gulf of Cadiz and Algeciras Bay), in the Adriatic and Red Sea.

P. canopus is coastal species normally inhabiting shallow water. The species has been observed in port areas, coral reefs and coastal detritus bottom from the low tide mark down to a 128 meters depth), attached to rock, in photophilic communities, in *Posidonia* meadows , Sea squirt bottoms and coastal lagoons.

Figure.4 – A claster of *Styela canopus* Savigny, 1816 adapted from NEMESIS (National Exotic Marine and Estuarine Species Information System, Smithsonian Reserch Center at: http://invasions.si.edu/nemesis).

BIOLOGY & ECOLOGY

S. canopus broodeds and fertilizeses its eggs within the atrial chamber and then released them into the water column upon hatching. The larvae settled after ~2 hours at 25 ^{0}C. Once settled, the tail is absorbed, the gill basket expands, and the tunicate begins to feed by filtering.

Figure.5 – Dissected specimen of *Styela canopus* Savigny, 1816 (Image by Sofia Tristancho, Algarve, Portugal, 2015).

IMPACTES

It is found on ships, buoys, piers, docks, mangroves and coral reefs, but no economic or ecological impacts have been documented for this species.

REMARKS

Its body shape varies from globular to elongate. Worm tubes, bryozoans and algae frequently grow on the test.

REFRENCES

http://invasions.si.edu/ (photo)

http://www.marinespecies.org

Naranjo, J.C.L (1995). **Taxonomía, zoogeografía y ecología de las Ascidias del Estrecho de Gibraltar. Implicaciones de su distribución bionómica en la**

caracterización ambiental de areas costeras. Memoria para optar al grado de Doctor en Biologia. Universidad de Sevilla.

Styela plicata (Lesueur, 1823)

(Figure 6 & 7)

COMMON NAMES

Pleated tunicates (UK); Mija-mija (PT).

DESCRIPTION

Styela plicata is a solitary tunicate, variable in shape, but roughly oval. It is fixed to the substrate by the posterior end of its body, usually without roots or stalks. Its tunic is firm and thick, slightly translucent, with deep, irregular, longitudinal furrows, and horizontal creases that form large, irregularly rounded lumps. Siphons coming in the front area, both siphons are short, with square apertures with rounded hump on each side. The oral tentacles and the dorsal lamina are smooth and the gills have 4 longitudinal folds. Individuals grow up to 10 cm in height

DISTRIBUTION & HABITAT

S. plicata is a native species from the Northwest-Pacific. The species can now also be found invading the East and Western Atlantic Ocean (British Islands, Morocco, Senegal, South Africa, North Carolina, Florida, Bermudas, Cuba, Puerto Rico, Río de Janeiro and Montevideo); Mediterranean Sea (Italy, Egypt, Israel, France, Balearic Island, Cataluña, Alicante, Malaga and Tunisia); Strait of Gibraltar (Gulf of Cadiz and Algeciras Bay); also in Adriatic Sea.

S. plicata is a coastal species that can be mainly observed from the lower level of the tide down to 40 meters depth (according to Vazquez, 1993 recorded between 0 and 180 meters depth). This species is usually associated with photophilic algae communities and is especially common in port areas as it supports well the high rate of sedimentation. It can also be found in detrital backgrounds, coastal lagoons, bivalves farming, muddy sands with *Caulerpa* and meadows of *Posidonia*.

Figure.6 – Specimens of *Styela plicata* (Lesueur, 1823) photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

Solitary ascidians are hermaphroditic, meaning that both eggs and sperm are released to the atrial chamber. Eggs may be self-fertilized or fertilized by sperm from nearby animals, but many species have a partial block to self-fertilization. Gonads on both sides (two on the left and 6-8 on the right) and consisting of numerous branched testicular lobes attached to mantle surrounding an ovary with elongated tube shaped. They are sessile filter feeders with two siphons, an oral and an atrial siphon. Water is pumped in through the oral siphon, where phytoplankton and detritus is filtered by the gills, and passed on mucus strings to the stomach and intestines. Waste is then expelled in the outgoing atrial water.

Figure.7 – Dissected specimen of *Styela plicata* (Lesueur, 1823) (Image by Sofia Tristancho, Algarve, Portugal, 2015).

IMPACTES

The species is may cause economic as well as ecological impacts. *S. plicata* is known to foul cultured bivalves, interfering with their growth. It also competes with native fouling species by dominating the substrate where it attaches.

REMARKS

Kott (1985) notes the great ability of this species to withstand high levels of pollution and low levels of salinity, being able to penetrate even in estuarine and fluvial areas. In the gills of some individuals, parasitic copepods were found as *Lichomolgus canui* Sars, 1917 and *Doropygus pulex* Thorell, 1859.

REFRENCES

http://www.sms.si.edu/ (photo)

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http://www.marinespecies.org

Naranjo, J.C.L (1995). **Taxonomía, zoogeografía y ecología de las Ascidias del Estrecho de Gibraltar. Implicaciones de su distribución bionómica en la caracterización ambiental de areas costeras.** Memoria para optar al grado de Doctor en Biologia. Universidad de Sevilla.

Botrylloides leachii **(Savigny, 1816)**

(Figure 8 & 9)

COMMON NAMES

Leach's Compound Ascidian (UK); Ascidia (PT).

DESCRIPTION

Botrylloides leachii is a colonial sea squirt and two different groups can be distinguished in relation with the growth form and colour:

a) - Colonies laminar and fouling reach from 1 to 2 cm up to 15cm of diameter. Also have a monochromatic coloration (orange yellow and bright orange). The zooids arranged in very elongated systems and next to each other. Very round sewage opening. The preserved colonies become greyish or brown very dark.

b) - Colonies of variable size. The smaller are discoidal, flattened (1-3 cm in diameter), the bigger (up to 20 cm) have large lobulations (up to 2 cm thick). The system is meandering but shorter than the other group and separated by more elongated openings sewage and also has portions of the tunic without zooids. Coloration of the tunic is orange or red more or less dark (burgundy), also have a white ring around the oral siphon.

DISTRIBUTION & HABITAT

B. leachii seems to be native throughout the Indopacific Ocean. It is considered as cosmopolitan in Mediterranean Sea, Atlantic Ocean and Indopacific Ocean. This specie has been introduced to Oriental Atlantic (Scandinavia, North Sea, British island, France, Galicia, Cantabria, Portugal, Morocco, Senegal and South Africa), Mediterranean Sea (Tunisia??, Egypt, Israel, France, Italy, Balearic Island, Cataluña, Alicante, Murcia and Granada), Strait of Gibraltar (Cadiz, Tarifa and Algeciras Bay) and Adriactic, Black, Red and Indic Sea. It is a littoral species that occurs from the tidal zone to 110 meters depth, usually by encrust.

Figure.8 – Colony of *Botrylloides leachii* (Savigny, 1816) sampled form the Port Lagos in January 2015 Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

This species is hermaphrodite, with a simple reproductive system. Fertilization is external, and after a time in the plankton the free-swimming tadpole larvae will settle and metamorphose. Zooids will be mature from May to July and the ovules form in April. Ascidians are known to be mucus filter feeders that can extract particles as small as 0.5 µm up to a limit set by the size of the oesophagus. The feeding currents are created by cilia in tracts located on either side of the stigmata in the walls of the branchial sac. Particles are collected on a mucus sheet that migrates by ciliary action across the internal surface of the branchial sac from where it is concentrated and directed into the stomach.

Figure.9 – *B. leachii verus B.schlosseri,* showing the zooids different shape sampled form the Port Lagos and Faro in January and June 2015 Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

IMPACTES

Fouling organisms. Ecological impact unstudied, but probably competes with other shallow-water invertebrates for space, especially in the fouling community.

REMARKS

The linear arrangement of its zooids is distinguishes from the start shape associated to the *Botrylloides schlosseri* zooids. It is the preferred food of Bean cowries *Trivia artica* (Pulteney, 1799) and *Trivia monacha* (da Costa, 1778)

To accurately confirm the the taxonomic validity of *B. leachii,* it is necessary to study a mature colony before the formation of eggs.

REFRENCES

http://www.marinespecies.org

http://www.marlin.ac.uk

http://www.corpi.ku.lt

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Ramos A.A.E. Ascidias litorales del Mediterraneo Ibérico (Faunistica, Ecología y Biogeografía). Universidad de Alicante.

Family Pyuridae Hartmeyer, 1908

***Microcosmus squamiger* Michaelsen, 1927**

(Figure 10 & 11)

COMMON NAMES

Sea squirts (UK); Ascidia (PT).

DESCRIPTION

Solitary tunicate that can also commonly occur in dense clumps or aggregations of individuals. The apertures are usually short and located about a third of the body length. In some ocassions the oral siphon is terminal with a long straight siphon, while the atrial siphon is short and about half-way along the body. Each tunicate can grow up to 50 mm in diameter. *M. squamiger* has a purple, leathery color.

This species is very often confused with closely related *Microcosmus exasperates* Heller, 1878. One of the few characteristics used to distinguish them is the shape of the internal siphonal spines. *M. squamiger* has very short, about 15-25 µm long, and shaped like fingernails with serrated rims, while *M. exasperatus* has longer pointed spines, about 40-50 µm long, which are posteriorly hooked.

DISTRIBUTION & HABITAT

It is native to the coasts of Australia and has established introduced populations on the West Coast of the United States, Mexico, the Mediterranean Sea (Balearic Island, Cataluña, Levant and Almeria), Straits of Gibraltar (Cadiz, Chipiona and Algeciras Bay), Atlantic coast of Spain, the Canary Islands, Red Sea and South Africa. *M. exasperatus* is a littoral specie found from intertidal zone to 20 meters depth. They form colognes in docks and breakwaters. And can be associated to mussels and barnacles covering sandy-muddy bottoms.

Figure.10 – Specimens of *Microcosmus squamiger* Michaelsen, 1927 photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

Internally, the most characteristical feature is the branchial sac, which is folded and with at least 8 folds on each side, but the right side usually has one or two more. Normally the most ventral fold is not complete and sometimes the next either. They have variable numbers of branched tentacles (more than 20). The gut lies on the left side and the intestine forms two narrow loops. The stomach is covered by the hepatic gland which has small papillae covering the surface. The gonads can be found in both sides and are clearly divided in four blocks in the right side and in three in the left gonad. In the left side the most distal gonad is located inside the primary curve of the intestine and the next one into the secondary loop. The larvae measure up to 1.3mm and usually have a well-developed tail. There is not ocellus in the larvae stage and just show one pigmented spot in the sensory vesicle that match with the statocyte.

Figure.11 – *Microcosmus squamiger* Michaelsen, 1927 dissected and prepared in laboratory (Photograph by Sofia Tristancho, Algarve, Portugal, 2015).

IMPACTES

Economic Impacts (damage to oyster culture racks, ropes, and shells through fouling), ecological impacts and competition (it outnumbers earlier invader and forms single-species patches at some locations, possibly crowding out other species in shallow water communities).

REMARKS

M. squamiger is considered a potential threat to the Mediterranean littoral communities, but further monitoring is needed to assess its impacts. Especially abundant in Algecira’s Bay covering columns and cement blocks of docks and breakwaters.

REFRENCES

http://invasions.si.edu/ **(photo)**

http://www.marinespecies.org

Ramos A.A.E. Ascidias litorales del Mediterraneo Ibérico (Faunistica, Ecología y Biogeografía). Universidad de Alicante.

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SYSTEMATICS

Phylum Arthropoda

Subphylum Crustacea

Class Maxillopoda Dahl, 1956

Order Sessilia Lamarck, 1818

Family Balanidae Leach, 1806

***Amphibalanus amphitrite* (Darwin, 1854)**

(Figure.12 & 13)

COMMON NAMES

Striped barnacle, purple acorn barnacle and Amphitrite's rock barnacle (UK); Craca (PT).

DESCRIPTION

A. Amphitrite (Darwin, 1854) is a medium-sized barnacle (basal diameter 19 mm), cone-shaped sessile barnacle. It has 6 calcareous wall plates surrounding the body. The walls are often smooth, with distinctive narrow vertical purple or brown stripes. Both the stripes and the white spaces between them are typically wider at the bottom and narrow toward the top. The plates have wide longitudinal ribs, narrowing to the tops of the shell plates. Also it has a diamond-shaped in the operculum, protected by a movable lid formed from two triangular plates.

DISTRIBUTION & HABITAT

A. amphitrite may be native throughout the West Pacific and Indian Ocean from Southeastern Africa to Southern China. It has been introduced to the Eastern Pacific (Panama to California), Northwestern Pacific (Korea, Japan and Russia), Southwestern Pacific (including New Zealand and possibly Southern Australia), Pacific Islands (Hawaii), Western Atlantic (Caribbean), and Northeastern Atlantic (Germany, united Kindgdom and France). *A. amphitrite* is typically found in the intertidal and shallow subtidal regions of sheltered marine waters, particularly harbors, and man-made structures, but is rare on open rocky coasts.

Figure.12 – One specimen of *Amphibalanus amphitrite* (Darwin, 1854) photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

A. amphitrite grows on a wide range of hard surfaces, including docks, ship hulls, logs, mangroves, rocks, oysters, and other shellfish. It is sensitive to cold temperatures, and in the northern limits of its range, it is most abundant in the warmest habitats, including thermal effluents. This barnacle prefers marine salinities between 30 and 40 ppt., but tolerates a range from 10 to 52 ppt. *A. amphitrite* juveniles and adults are filter feeders, sweeping the water with their long bristled appendages to gather phytoplankton, zooplankton, and detritus. Like many other barnacles, is hermaphroditic, but is capable of cross-fertilization. This barnacle produced 1,000 to 10,000 eggs per animal, generally increasing with body size.

Figure.13 – Typical *A. amphitrite* operculum photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

IMPACTES

A. amphitrite is one of the most abundant fouling barnacles in warmer is harbors worldwide. It is a major contributor to fouling of ship, navigational buoys, coastal power station intakes and harbor structures. Also they are frequent fouling organisms of cultured Pacific Oysters (*Crassostrea gigas* (Thunberg, 1793)) in warmer waters. *A. amphitrite* competed with the Eastern Oyster (*Crassostrea virginica* (Gmelin, 1791)) for settlement sites, and also affected survival and growth of oysters by settling on their shells. They also affect the composition of the fouling community, mainly by creating additional structure for the recruitment and colonization of motile species.

REMARKS

A. amphitrite belongs to a complex of species with very similar morphological characteristics. It is mostly confused with *A. improvisus*, *A.eburneus*, *A. reticulatus*, *A. subalbidus* and *A. variegatus.*

REFRENCES

http://www.sms.si.edu/

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http://www.marinespecies.org

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Southward A.J. (2008). Barnalces: Keys and Notes for the Identification of British Species. Shrewsbury, England, (United Kingdom). Field Studies Council (eds). 140 pp.

SYSTEMATICS

Phylum Arthropoda

Subphylum Crustacea

Class Maxillopoda Dahl, 1956

Order Sessilia Lamarck, 1818

Family Balanidae Leach, 1806

***Amphibalanus eburneus* (Gould, 1841)**

(Figure.14 & 15)

COMMON NAMES

Ivory barnacle (UK); Craca (PT).

DESCRIPTION

A.*eburneus (Gould, 1841*) is a medium-sized barnacle (basal diameter 20-30mm) with a white conical or tubulo-conical shell. The surface is very smooth. Usually those shells are covered with a yellow epidermis when the specimen is not corroded, but with the radii naked. Around the body have 6 calcareous wall plates which compose the shell.

The operculum is not central, is closer to carinal side, showing a large orifice, passing from rhomboidal to pentagonal shape.

DISTRIBUTION & HABITAT

A. eburneus is a commun sublittoral barnacle of the American Atlantic coast, occurring from Massachusetts to Caribbean coast. In the last century it has been spread in Mediterranean ports, Bay of Biscay and in north Spain to France. There is also a population in Azores Island and if the global warming continues it can be extend to Britain. Normally attached to ship, but it was also found attached to shells and floating wood (West India, Honduras and Venezuela). Sometimes this specie has association with *Megabalanus tintinnabulum*, *Amphibalanus Amphitrite* and *Amphibalanus improvisus.*

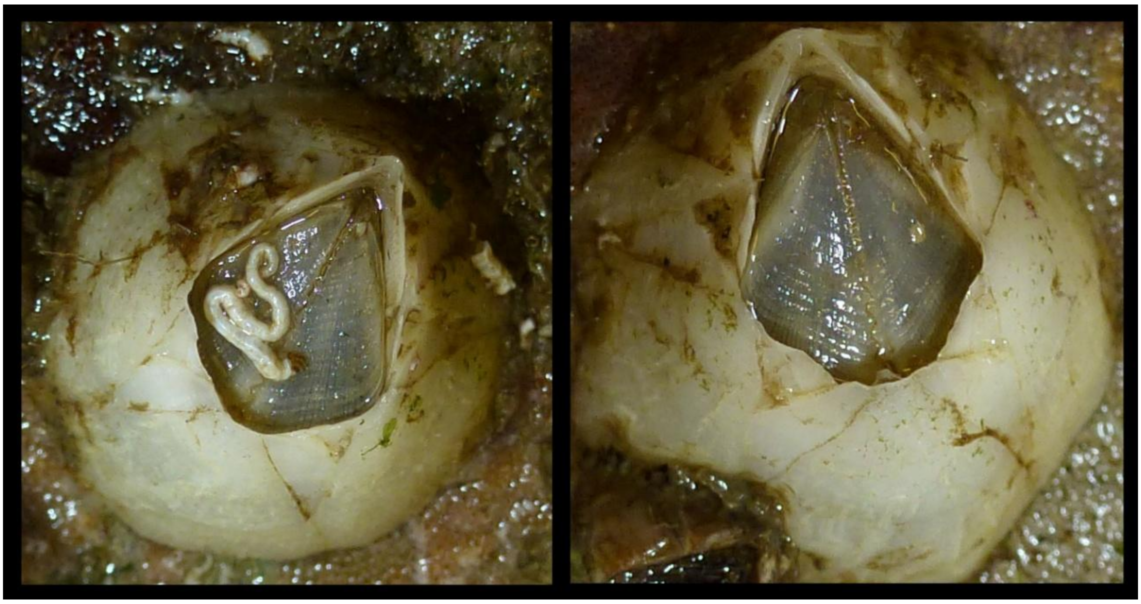

Figure.14 – One specimen of *Amphibalanus eburneus* (Gould, 1841) photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

A. eburneus grows on wide hard surfaces including rocks, oysters, mussels and other mollusks shells, pilings, seawalls and prop roots of the red moangrove Rhizophora mangle. Single and large aggregation of this specie can be found. The span can vary in relation of food availability and environmental factors. Like most of the free-living barnacles this specie is hermaphroditic but usually the population reproduction is via cross-fertization. The larvae pass through six naupliar stages and finally the cypris stage. The planktonic duration is around 7-13 days. It *A. eburneus* presents a quite large temperature and salinity range tolerance (the highest settlement is between 15-20ppt).

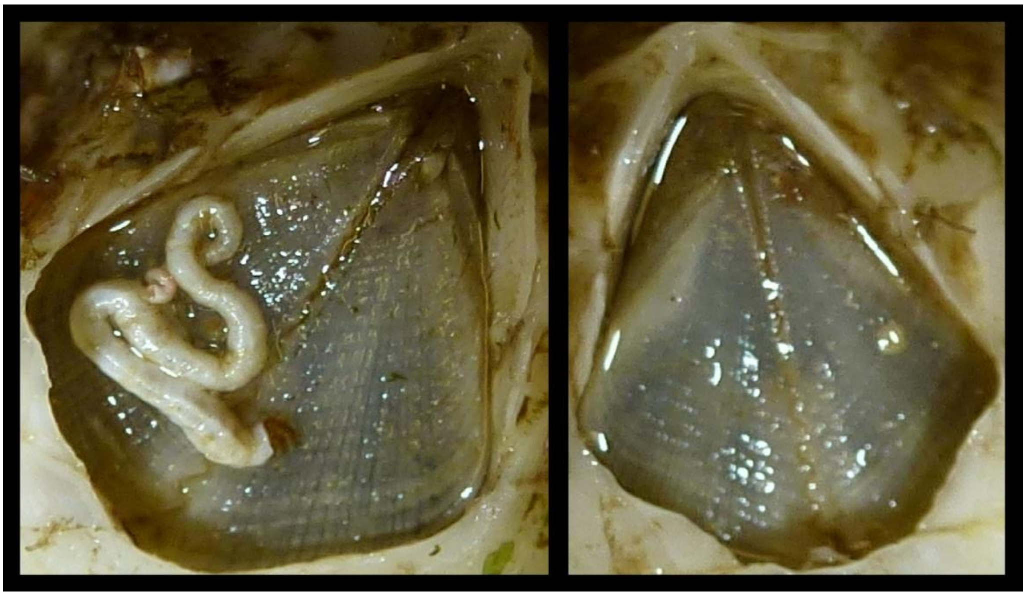

Figure.15 – Typical *A. eburneus* operculum photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

IMPACTES

A. eburneus is producing an economic threat to several marine associated industries because of attach to ship hulls, creating drag and increasing the fuel costs. Also in nuclear power plants it can be found as fouling organism so the removal process has to be carried out. Like other invasive species when it is removed of their natural habitat can produce food webs alterations and several impact on the ecosystem.

REMARKS

A. amphitrite belongs to a complex of species with very similar morphological characteristics. It is mostly confused with *A. improvisus*, *A.eburneus*, *A. reticulatus*, *A. subalbidus* and *A. variegatus.*

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SYSTEMATICS

***Perforatus perforatus* (Bruguière, 1789)**

(Figure.16 & 17)

COMMON NAMES

An acorn barnacle (UK); craka (PT).

DESCRIPTION

It is one of the larger barnacles of European coasts; it is 30 mm in diameter and 30 mm tall. The shell wall consists of 6 purplish plates that are often vertically ridged. The regular conical shape and the small opening are two easy features to identify this specie. Also the pink or purple colouration of the shell plates and the beaked terga are. The scutum shows moderate growth ridges but is without longitudinal striations. The tergo-scutal flaps are usually reflexed, but sometimes more or less erect.

DISTRIBUTION & HABITAT

P. perforatus (Bruguière, 1789) is a southern species, occurring in the Mediterranean and along the eastern Atlantic seaboard from south-west Wales to West Africa, but has not been found in Ireland. The species are common in south-west England and along the coast of Brittany and Cherbourg on the French coast. The species is regularly found on floating objects washed ashore in the southern North Sea. It usually occurs in the lower half of the littoral zone and may extend into the sublittoral. It is found in variable density on a wide range of hard substrata along wave-beaten shores and in ria-type estuaries.

Figure.16 – One specimen of *Perforatus perforatus* (Bruguière, 1789) photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

P. perforatus is a filter-feeding species. Egg masses are present from June to September, and the larva are released from the end of June to the end of August, finally settlement takes place mainly in August and September.

Figure.17 – Typical *P. perforatus* operculum photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

REMARKS

Another isopod crustacean, *Naesa bidentata*, normally lives in rock crevices and under seaweed and stones but with the spread of *P. perforatus*, it has adopted the empty shells of the barnacle as its home.

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SYSTEMATICS

Family Austrobalanidae Newman & Ross, 1976

Austrominius modestus **(Darwin, 1854)**

(Figure.18 & 19)

COMMON NAMES

Australasian barnacle, New Zealand barnacle (UK); Craca Australiana (PT).

DESCRIPTION

A. modestus (Darwin, 1854) has only 4 symmetrical calcareous wall plates surrounding the body. The plates are thin and often carry rounded ridges giving the shell a sinuously octoradiate outline. The basic is membranous. In young and uneroded specimens, each scutum carries a slaty grey line. Sublittoral specimens may in occasions resemble *Balanus crenatus* Bruguière, 1789 among which they grow and it is necessary to clean the shells thoroughly of epizoic growths to check identity. The tergo-scutal flaps of live specimens are held flat, basically white, with brown marks at the pylorus and two blackish bands in the rostral half.

DISTRIBUTION & HABITAT

A. modestus is native to Australia and New Zealand. In European waters (e.g. France, Germany, United Kingdom, Ireland, Italy, Japan, Netherland, Spain and Portugal) and in South Africa it is considered an invasive species. It is found at all levels of the shore but is more common in the mid-shore and may extend to shallow sublittoral, preferring sheltered areas. It grows very fast and tolerates lower salinity, turbidity and higher temperatures than most native barnacles. *A. modestus* attaches to a wide variety of substrata including rocks, stones, shells, other crustaceans and artificial structures including ships.

Figure.18 – One specimen of *Austrominius modestus* (Darwin, 1854) photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

Benthic and epibiotuc species that colonizes a wide variety of substrata including rocks, stones, shells, other crustaceans and artificial structures including ships from the intertidal and subtidal zones. It grows very fast and tolerates turbidity, lower salinity and higher temperatures than most native barnacles. *A. modestus* is a filter-feeding species, feeding mainly on zooplankton, but can also filter phytoplankton. Like in almost all barnacles, it is hermaphroditic, requiring cross-fertilization. Specimens reach sexual maturity at about 8 weeks after settling, and a diameter of 6-7 mm. Breeding begins when the water temperature exceeds 6º C. An average sized animal produces about 1800-4000 eggs and can produce up to 12 broods per year.

Figure.19 – Typical *A. modestus* operculum photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

IMPACTES

A. modestus competes for space with native barnacles found along the coasts of Europe. Because it may reproduce throughout the year it has a high reproductive potential spreading widely and in some places it is the dominating barnacle species. It is frequently found fouling ships' hulls, which not only causes further spread of the species, but also is a nuisance to the shipping industry. As an epibiont on commercial shellfish such as mussels and oysters, it is assumed to compete with these organisms for food.

REMARKS

A. modestus has been reported as early as 1945 from the coast of the United Kingdom. It is believed to have arrived on hulls of navy vessels during World War II.

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SYSTEMATICS

Family Chtamalidae Darwin, 1854

***Chthamalus montagui* Southward, 1976**

(Figure.20 & 21)

COMMON NAMES

Montagu's stellate barnacle (UK).

DESCRIPTION

C. montagui Southward, 1976 is a small barnacle normally between 1 and 2 cm. It has 6 calcareous (solid but not porose) wall plates surrounding the body and it has basis membranous. Usually it has less than 10 mm rostro-carina diameter. The rostrum is narrow, being overlapped by the rostro-lateral plates, which gives a distinctive character that is useful to separate this genus from *Semibalanus* and *Balanus* genus. Normally flattened, height less than 5 mm, but when crowded may become columnar up to 10 mm high. This specie can be separated from *C. Stellatus* (Poli, 1791) by the kite-shaped operculum and the smaller terga. In water, with the operculum open, the tergo-scutal flaps show a dull blue colour, sometimes almost white and the spot at the micropylar opening is brownish rather than bright orange and shorter than in *C. stellatus.*

DISTRIBUTION & HABITAT

C. montagui is an European common barnacle on rocky shores in South West England, Ireland and Southern Europe. The main distribution is from Ireland to Mediterranean areas. It is abundant in Spain, Portugal and the Atlantic coast of Morocco to Senegal.

But it is absent from the Atlantic islands of Madeira and Azores, but it is present in Canaries. Usually, it is record in the high to mid eulitoral zone on expose to moderately expose rocky shores. Its vertical distribution overlaps with that of *C. stellatus* and *S. Balanoides* (Linnaeus,1767).

Figure.20 – Three specimens of *Chthamalus montagui* Southward, 1976 photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

Chthamalus montagui is able to breed in its first year. The duration of larval stage is 11-30 days. Normally they preferred cross-fertilization, but *Chthamalus* has been shown to self-fertilize when it isolated. *C. montagui* generally feed on small plankton. They can consume diatoms, but were found not to grow under regime dominated by diatoms. Normal feeding involves a cirral beat however, in high wave exposure they tend to hold their cirri out stiffy against the water current for a long period of time, retracting when food is captured. *C. montagui* breeds from April to late September and settlement takes place from late July to December.

Figure.21 – Typical *C. montagui* operculum photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

REMARKS

Empty barnacle's cases provide homes for small periwinkles, small bivalves and the isopod as *Campecopea hirsuta* (Montagu, 1804).

In order to protect themselves from changes in temperature/desiccation and a lowering of salinity, intertidal barnacles are usually able to close their aperture tightly.

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SYSTEMATICS

Family Archaeobalanidae Newman & Ross, 1976

***Hesperibalanus fallax* (Broch, 1927)**

(Figure.22 & 23)

COMMON NAMES

Balane feinte (F); Craka (PT).

DESCRIPTION

H. fallax (Broch, 1927) is a small barnacle which is comprised of 6 shell plates (parietes) and can reach 12 mm in diameter. This specie has solid thick walls which are smooth or slightly ribbed. The wall plates can be ribbed, but not as strongly as in *Balanus crenatus*, but it is without pores. The shell is tubo-conic, verging towards globose, with relatively large diamond-shaped opercular opening that is rounded at the rostral and carina ends. The carina tends to grow higher than the rostrum. The shell colour is very variable, but the majority has the carina and lateral plates pink and the rostrum white. Although some specimens are almost entirely white with pink tinges and other can all be strong red coloured.

DISTRIBUTION & HABITAT

Hesperibalanus fallax is considered to be non-native within the British Isles as well as northern Europe believed to have relatively recently expanded its range northward from Africa. British records are currently restricted to the south-west coast, from Portland Harbour to S. Wales, is almost the same than *P. perforatus*. They are also found in Netherlands and Belgium. There are no records from the north side of the Mediterraanean, but it has been found on algae and cnidarians from Algeria and Morocco through the Gulf of guinea down the African coast to Angola. The depth range is from the upper sublittoral to at least 60 m. They can occur singly or in clusters on the upper valves of the queen scallop, *Aequipecten opercularis* (Linnaeus, 1758), in the shells of *Buccinum* inhabited by the hermit crab *Pagarus benhardus* (Linnaeus, 1758), on the carapace of the spider crab, *Maja squinado* (Herbst, 1788), on hydroids, and on the sea-fan *Eunicella verrucosa* (Pallas, 1766). Additional records are man-made plastic object, including fishing nets, lobster pots and supermarket bags.

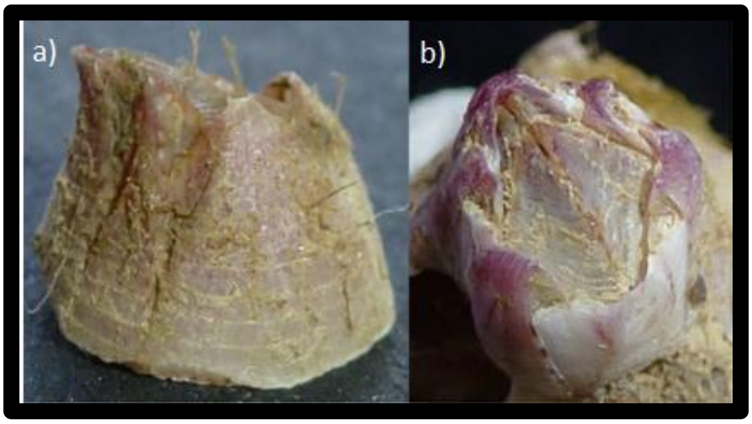

Figure.22 – Specimens of *Hesperibalanus fallax* (Broch, 1927) photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

BIOLOGY & ECOLOGY

H. fallax is a filter-feeding species. This specie breeds in summer. Usually they incube embryos in May but the major settlement of spat are from September to November.

Figure.23 – Typical *Hesperibalanus fallax* operculum photographed in laboratory facilities (Image by Carlos M. L. Afonso & Sofia Tristancho, Algarve, Portugal, 2015).

REMARKS

H. fallax does not occur on rocks or stones, in contrast to *Balanus crenatus* and *Amphibalanus amphitrite* which it may be confused. Also this specie can be distinguished by the Opercular membrane yellow with brown or black banding.

REFRENCES

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ANNEX II: The average percentage of abundance for barnacle and sea squirts

Barnacles

Table 1 The average percentage of abundance for the total data of barnacle species found in the grid showing the differences between all the factors: local, type of port, season, port location and geographic location.

	Amphibalanus amphitrite	*Austrominius modestus*	*Chthamalus montegui*	*Perforatus perforatus*	Amphibalanus sp.
Faro	2,64	0,30	89,07	0,37	7,62
Lagos	40,85	2,32	34,78	0,62	21,43
Olhao	5,34	1,17	42,94	0,10	50,46
Quar-Vil	12,86	1,12	78,61	0,08	7,33
recreational	20,13	1,48	53,82	0,18	24,39
fishing	4,35	0,60	81,76	0,29	13,00
spring	11,88	1,04	63,51	0,28	23,27
winter	8,11	0,80	77,16	0,32	13,61
summer	9,35	0,86	76,17	0,03	13,60
sheltered	3,47	0,57	74,79	0,29	20,88
unsheltered	19,04	1,39	68,92	0,20	10,45
west	3,47	0,57	74,79	0,29	20,88
east	40,85	2,32	34,78	0,62	21,43
center	12,86	1,12	78,61	0,08	7,33

<u>Sea squirts</u>

Table 2 The average percentage of abundance for the total data of sea squirts species found in the grid showing the differences between all the factors: local, type of port, port location and season.

	Ascidiella aspersa	*Ciona intestinalis*	*Corella eumyota*	*Microcosmus squamiger*
Faro	0,33	2,55	0,08	59,08
Lagos	3,53	7,69	0,00	54,49
Olhao	0,67	3,11	0,00	35,67
Qua-Vil	1,83	4,41	0,00	25,62
fishing	1,18	3,12	0,00	45,04
recreational	1,19	4,47	0,06	38,04
sheltered	0,47	2,79	0,05	49,13
unsheltered	2,18	5,09	0,00	31,57
spring	0,26	1,28	0,05	48,31
summer	2,26	6,60	0,00	34,27

	Phallusia mammilata	*Ascidia* **cf.** *mentula*	*Styela canopus*	*Styela plicata*
Faro	0,00	0,00	1,07	18,73
Lagos	0,00	0,32	0,32	32,69
Olhao	3,33	0,00	0,00	57,11
Qua-Vil	2,16	0,00	0,00	64,31
fishing	2,35	0,05	0,00	42,53
recreational	0,60	0,00	0,83	46,87
sheltered	1,42	0,00	0,61	35,05
unsheltered	1,72	0,07	0,07	57,79
spring	1,85	0,00	0,67	47,18
summer	1,19	0,06	0,06	41,46

ANNEX III: Frequency of occurrence of species comparing all the factors one by one.

Barnacles

Table 3 Frequency of abundance of the barnacles species obtain for each factor: season, type of port, port location, geographic location and local.

	A. amphitrite	*A. modestus*	*C. montegui*	*P. perforatus*	*Amphibalanus* sp.
spring	78,57	48,57	52,86	20,00	81,43
winter	63,24	52,94	72,06	4,41	79,41
summer	82,50	37,50	75,00	5,00	67,50
recreational	88,64	51,14	50,00	10,23	76,14
fishing	58,89	44,44	78,89	11,11	77,78
sheltered	61,22	36,73	50,00	7,14	82,65
unsheltered	90,00	62,50	83,75	15,00	58,75
east	62,24	35,71	50,00	7,14	82,65
west	100,00	76,67	86,67	26,67	100,00
central	86,00	46,00	82,00	8,00	54,00
Faro	56,00	26,00	50,00	6,00	72,00
Lagos	100,00	76,67	86,67	26,67	100,00
Olhao	66,67	45,83	50,00	8,33	93,75
Quart-Vil	82,00	54,00	54,00	8,00	54,00

Sea squirts

Table 4 Frequency of abundance of the tunicates species obtain for each factor: local, type of port, port location and season.

	A. aspersa	*C. intestinalis*	*C. eumyota*	*M. squamiger*	*M. occidentalis*	*P. mammilata*	*Ascidia* cf. *Mentula*	*S. canopus*	*S. plicata*
Faro	10,00	50,00	5,00	100,00	65,00	0,00	0,00	15,00	90,00
Lagos	27,27	36,36	0,00	72,73	18,18	0,00	9,09	9,09	72,73
Olhao	20,00	30,00	0,00	95,00	5,00	50,00	0,00	0,00	100,00
Qua-Vil	50,00	60,00	0,00	90,00	25,00	35,00	0,00	0,00	100,00
fishing	22,50	37,50	0,00	92,50	27,50	30,00	2,50	0,00	90,00
recreational	32,26	54,84	3,23	90,32	29,03	16,13	0,00	12,90	96,77
sheltered	15,00	40,00	2,50	97,50	32,50	27,50	0,00	7,50	95,00
unsheltered	41,94	51,61	0,00	83,87	22,58	22,58	3,23	3,23	90,32
spring	5,71	28,57	2,86	91,43	8,57	37,14	0,00	8,57	85,71
summer	47,22	61,11	0,00	91,67	47,22	11,11	2,78	2,78	100,00

ANNEX IV: Diversity index

Barnacles

Sample	S	N	d	J'	H'(loge)
S1	3	14	0,7578	0,6908	0,7589
S2	2	19	0,3396	0,2975	0,2062
S3	3	60	0,4885	0,4257	0,4677
S4	2	32	0,2885	0,8113	0,5623
S5	1	9	0	****	0
S6	2	57	0,2473	0,9621	0,6669
S7	2	61	0,2433	0,9998	0,693
S8	2	42	0,2675	0,9934	0,6886
S9	2	73	0,2331	0,9966	0,6908
S10	2	53	0,2519	0,9874	0,6844
S11	3	38	0,5498	0,5352	0,5879
S12	3	40	0,5422	0,7566	0,8312
S13	3	70	0,4708	0,5817	0,639
S14	3	75	0,4632	0,6571	0,7219
S15	3	24	0,6293	0,5607	0,616
S16	3	36	0,5581	0,7912	0,8692

Sample	S	N	d	J'	H'(loge)
S70	3	76	0,4618	0,522	0,5734
S71	2	1160	0,1417	0,2486	0,1723
S72	2	897	0,1471	0,5463	0,3787
S73	1	72	0	****	0
S74	1	45	0	****	0
S75	1	52	0	****	0
S76	1	74	0	****	0
S77	3	28	0,6002	0,2793	0,3068
S78	1	24	0	****	0
S79	4	85	0,6753	0,2912	0,4037
S80	2	72	0,2338	0,1056	7,32E-02
S81	3	94	0,4402	0,3162	0,3474
S82	4	102	0,6487	0,3136	0,4347
S83	3	81	0,4551	0,4426	0,4863
S84	5	143	0,806	0,4983	0,8019
S85	2	36	0,2791	0,5033	0,3488

S17	2	41	0,2693	0,965	0,6689
S18	2	69	0,2362	0,7554	0,5236
S19	2	120	0,2089	0,8113	0,5623
S20	2	50	0,2556	0,7602	0,5269
S21	1	618	0	****	0
S22	3	44	0,5285	0,7991	0,8779
S23	2	574	0,1574	0,1746	0,121
S24	1	449	0	****	0
S25	1	220	0	****	0
S26	1	473	0	****	0
S27	1	445	0	****	0
S28	3	417	0,3315	8,39E-02	9,21E-02
S29	1	355	0	****	0
S30	1	587	0	****	0
S31	3	1116	0,285	5,69E-02	6,25E-02
S32	3	464	0,3257	0,1878	0,2063
S33	2	229	0,184	0,2186	0,1515
S34	2	816	0,1492	7,12E-02	4,94E-02
S35	2	309	0,1744	5,64E-02	3,91E-02
S36	3	1795	0,2669	0,1838	0,202

S86	3	36	0,5581	0,43	0,4724
S87	2	45	0,2627	0,5033	0,3488
S88	3	72	0,4677	0,3137	0,3446
S89	3	87	0,4478	0,2264	0,2487
S90	3	164	0,3922	0,5722	0,6286
S91	3	143	0,403	0,3961	0,4352
S92	3	116	0,4207	0,5369	0,5899
S93	3	106	0,4289	0,6113	0,6716
S94	2	114	0,2111	0,2975	0,2062
S95	2	66	0,2387	0,4395	0,3046
S96	3	44	0,5285	0,4185	0,4597
S97	2	82	0,2269	0,4992	0,346
S98	2	25	0,3107	0,4022	0,2788
S99	4	41	0,8078	0,8191	1,135
S100	3	23	0,6379	0,6098	0,6699
S101	5	85	0,9004	0,8357	1,345
S102	4	103	0,6473	0,6311	0,875
S103	5	136	0,8142	0,6419	1,033
S104	4	51	0,763	0,5358	0,7428
S105	2	23	0,3189	0,4262	0,2954

S37	3	881	0,2949	5,51E-02	6,06E-02
S38	3	106	0,4289	0,881	0,9678
S39	1	788	0	****	0
S40	2	258	0,1801	0,583	0,4041
S41	4	209	0,5616	0,734	1,017
S42	4	90	0,6667	0,6778	0,9396
S43	4	129	0,6173	0,7576	1,05
S44	4	108	0,6407	0,4741	0,6573
S45	3	92	0,4423	0,5975	0,6564
S46	3	98	0,4362	0,6963	0,765
S47	3	70	0,4708	0,7939	0,8721
S48	5	109	0,8526	0,5962	0,9596
S49	4	110	0,6382	0,6091	0,8444
S50	3	51	0,5087	0,5896	0,6478
S51	3	74	0,4647	0,9442	1,037
S52	4	116	0,6311	0,8279	1,148
S53	4	147	0,6012	0,7971	1,105
S54	5	84	0,9028	0,7096	1,142
S55	5	56	0,9937	0,7279	1,171
S56	4	167	0,5862	0,4953	0,6867

S106	4	19	1,019	0,6864	0,9515
S107	1	32	0	****	0
S108	2	50	0,2556	0,9248	0,641
S109	3	77	0,4604	0,4014	0,441
S110	3	77	0,4604	0,4842	0,5319
S111	3	120	0,4178	0,8637	0,9489
S112	3	206	0,3754	0,7151	0,7856
S113	3	201	0,3771	0,3985	0,4378
S114	3	48	0,5166	0,7049	0,7744
S115	3	82	0,4539	0,6267	0,6885
S116	3	39	0,5459	0,7748	0,8512
S117	2	22	0,3235	0,7732	0,536
S118	3	47	0,5195	0,5369	0,5898
S119	4	92	0,6635	0,7708	1,069
S120	4	122	0,6245	0,6175	0,856
S121	3	107	0,428	0,6629	0,7283
S122	3	81	0,4551	0,5999	0,659
S123	2	69	0,2362	0,9031	0,626
S124	3	493	0,3226	0,1355	0,1488
S125	3	463	0,3259	0,1047	0,115

S57	3	204	0,3761	0,7116	0,7817
S58	4	89	0,6684	0,73	1,012
S59	4	92	0,6635	0,6814	0,9446
S60	4	65	0,7187	0,7791	1,08
S61	2	121	0,2085	0,7805	0,541
S62	3	121	0,417	0,5684	0,6245
S63	3	528	0,319	0,5653	0,621
S64	4	312	0,5224	0,6183	0,8572
S65	2	70	0,2354	0,422	0,2925
S66	4	117	0,63	0,6524	0,9044
S67	4	54	0,7521	0,8802	1,22
S68	4	97	0,6558	0,6579	0,9121
S69	3	110	0,4255	0,5638	0,6194

S126	1	460	0	****	0
S127	1	247	0	****	0
S128	1	327	0	****	0
S129	4	112	0,6358	0,7648	1,06
S130	3	46	0,5224	0,4802	0,5276
S131	3	73	0,4662	0,5559	0,6107
S132	4	55	0,7486	0,8076	1,12
S133	2	24	0,3147	0,995	0,6897
S134	3	319	0,3469	0,1831	0,2012
S135	3	149	0,3997	0,2426	0,2665
S136	3	520	0,3198	0,1516	0,1666
S137	2	1072	0,1433	8,86E-02	6,14E-02
S138	3	585	0,3139	0,1443	0,1585

ANNEX V: Percentage of species contribution to the dissimilarity of the samples (SIMPER).

Barnacles

a) Type of port

Groups Recreational Port & Fishing Port
Average dissimilarity = 57,75

	Group Recreational Port	Group Fishing Port				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Chthamalus montegui	3,98	10,02	28,56	1,19	49,46	49,46
Amphibalanus sp.	4,44	4,55	13,25	1,25	22,94	72,40
Amphibalanus amphitrite	4,36	2,19	11,36	1,46	19,66	92,07

b) Port location

Groups sheltered & unsheltered
Average dissimilarity = 58,14

	Group sheltered	Group unsheltered				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Chthamalus montegui	6,89	7,21	26,72	1,25	45,96	45,96
Amphibalanus sp.	5,58	3,17	14,03	1,31	24,12	70,09
Amphibalanus amphitrite	1,87	4,96	12,60	1,50	21,66	91,75

c) Port characteristics

Groups west & center
Average dissimilarity = 61,21

	Group west	Group center				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum. %**
Chthamalus montegui	6,89	8,57	29,19	1,22	47,69	47,69
Amphibalanus sp.	5,58	2,53	15,69	1,32	25,64	73,33
Amphibalanus amphitrite	1,87	4,36	11,87	1,38	19,39	92,72

d) locals

Groups Faro & Lagos
Average dissimilarity = 55,57

	Group Faro	Group Lagos				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Chthamalus montegui	10,34	4,95	28,89	1,72	51,98	51,98
Amphibalanus amphitrite	1,85	5,96	12,94	1,80	23,28	75,27
Amphibalanus sp.	3,69	4,22	8,35	1,46	15,03	90,30

Groups Faro & Olhao
Average dissimilarity = 59,57

	Group Faro	Group Olhao				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Chthamalus montegui	10,34	3,30	30,58	1,12	51,33	51,33
Amphibalanus sp.	3,69	7,55	16,49	1,65	27,68	79,01
Amphibalanus amphitrite	1,85	1,89	8,25	1,11	13,86	92,87

Groups Lagos & Olhao
Average dissimilarity = 50,40

	Group Lagos	Group Olhao				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Chthamalus montegui	4,95	3,30	16,10	1,39	31,94	31,94
Amphibalanus amphitrite	5,96	1,89	14,72	1,73	29,20	61,14
Amphibalanus sp.	4,22	7,55	14,26	1,79	28,29	89,43
Austrominius modestus	1,23	0,78	3,79	1,25	7,52	96,95

Groups Faro & Quarteira-Vilamoura
Average dissimilarity = 60,30

	Group Faro	Group Quarteira-Vilamoura				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Chthamalus montegui	10,34	8,57	33,11	1,35	54,91	54,91
Amphibalanus sp.	3,69	2,53	11,71	1,23	19,42	74,32
Amphibalanus amphitrite	1,85	4,36	11,11	1,42	18,42	92,75

Groups Olhao & Quarteira-Vilamoura
Average dissimilarity = 62,15

	Group Olhao	Group Quarteira-Vilamoura				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Chthamalus montegui	3,30	8,57	25,10	1,12	40,39	40,39
Amphibalanus sp.	7,55	2,53	19,85	1,57	31,94	72,32
Amphibalanus amphitrite	1,89	4,36	12,66	1,36	20,36	92,69

Sea squirts

a) *season*

Groups Spring & Summer
Average dissimilarity = 41,90

Species	Group Spring **Av.Abund**	Group Summer **Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	4,56	4,07	11,66	1,26	27,83	27,83
Microcosmus squamiger	4,78	3,66	11,23	1,09	26,80	54,63
Molgula occidentalis	0,13	1,57	6,54	0,80	15,61	70,24
Ciona intestinalis	0,43	1,22	5,30	1,02	12,65	82,89
Ascidiella aspersa	0,09	0,68	3,05	0,87	7,28	90,17

b) *port location*

Groups sheltered & unsheltered
Average dissimilarity = 41,03

Species	Group sheltered **Av.Abund**	Group unsheltered **Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	3,95	4,77	11,98	1,28	29,20	29,20
Microcosmus squamiger	4,80	3,45	11,24	1,07	27,39	56,59
Molgula occidentalis	1,23	0,38	5,80	0,73	14,14	70,73
Ciona intestinalis	0,66	1,06	5,14	1,03	12,54	83,26
Ascidiella aspersa	0,19	0,64	2,93	0,85	7,15	90,41

c) <u>local</u>

Groups Faro & Lagos
Average dissimilarity = 48,25

Species	Group Faro **Av.Abund**	Group Lagos **Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Microcosmus squamiger	5,87	3,29	15,38	1,08	31,87	31,87
Molgula occidentalis	2,41	0,22	11,30	1,08	23,41	55,28
Styela plicata	2,96	2,39	11,00	1,28	22,81	78,09
Ciona intestinalis	0,75	0,84	5,36	1,05	11,11	89,20
Ascidiella aspersa	0,14	0,50	2,54	0,65	5,26	94,46

Groups Faro & Olhao
Average dissimilarity = 42,02

Species	Group Faro **Av.Abund**	Group Olhao **Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Microcosmus squamiger	5,87	3,73	11,18	1,16	26,60	26,60
Styela plicata	2,96	4,94	10,42	1,37	24,80	51,40
Molgula occidentalis	2,41	0,05	9,97	1,05	23,72	75,12
Ciona intestinalis	0,75	0,56	4,10	0,87	9,75	84,87
Phallusia mammilata	0,00	0,83	3,34	0,92	7,95	92,82

Groups Lagos & Olhao
Average dissimilarity = 45,17

Species	Group Lagos **Av.Abund**	Group Olhao **Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	2,39	4,94	16,72	1,41	37,02	37,02
Microcosmus squamiger	3,29	3,73	12,80	1,13	28,34	65,36

Ciona intestinalis	0,84	0,56	5,70	0,85	12,62	77,98
Phallusia mammilata	0,00	0,83	4,22	0,92	9,33	87,31
Ascidiella aspersa	0,50	0,24	3,25	0,75	7,19	94,50

Groups Faro & Quarteira-Vilamoura
Average dissimilarity = 45,21

	Group Faro	Group Quarteira-Vilamoura				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	2,96	6,08	13,96	1,59	30,87	30,87
Microcosmus squamiger	5,87	3,54	11,13	1,21	24,63	55,50
Molgula occidentalis	2,41	0,47	9,05	1,13	20,02	75,52
Ciona intestinalis	0,75	1,18	4,62	1,18	10,22	85,75
Ascidiella aspersa	0,14	0,72	2,80	0,97	6,20	91,94

Groups Lagos & Quarteira-Vilamoura
Average dissimilarity = 49,33

	Group Lagos	Group Quarteira-Vilamoura				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	2,39	6,08	20,59	1,50	41,73	41,73
Microcosmus squamiger	3,29	3,54	11,86	1,16	24,04	65,77
Ciona intestinalis	0,84	1,18	6,25	1,18	12,67	78,45
Ascidiella aspersa	0,50	0,72	4,12	1,05	8,36	86,81
Phallusia mammilata	0,00	0,60	2,75	0,62	5,57	92,37

Groups Olhao & Quarteira-Vilamoura
Average dissimilarity = 30,59

	Group Olhao	Group Quarteira-Vilamoura				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Microcosmus squamiger	3,73	3,54	8,20	1,14	26,80	26,80
Styela plicata	4,94	6,08	7,93	1,23	25,93	52,73
Ciona intestinalis	0,56	1,18	5,24	1,08	17,13	69,86
Phallusia mammilata	0,83	0,60	4,10	1,05	13,40	83,26
Ascidiella aspersa	0,24	0,72	3,11	1,01	10,15	93,42

d) *port abundance*

Groups 2 & 1
Average dissimilarity = 49,72

	Group 2	Group 1				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	4,77	2,39	18,36	1,30	36,92	36,92
Microcosmus squamiger	4,43	3,29	15,70	1,16	31,58	68,50
Molgula occidentalis	1,14	0,22	5,99	0,68	12,04	80,54
Ciona intestinalis	0,65	0,84	5,37	0,99	10,80	91,34

Groups 3 & 1
Average dissimilarity = 46,49

	Group 3	Group 1				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	4,41	2,39	13,93	1,41	29,98	29,98
Microcosmus squamiger	4,64	3,29	11,54	1,07	24,83	54,80

Ciona intestinalis	1,19	0,84	6,45	1,12	13,87	68,67
Molgula occidentalis	1,19	0,22	5,83	0,71	12,54	81,21
Ascidiella aspersa	0,53	0,50	3,64	0,96	7,84	89,04
Phallusia mammilata	0,63	0,00	2,98	0,71	6,42	95,46

Groups 1 & 4

Average dissimilarity = 46,61

	Group 1	Group 4				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Styela plicata	2,39	5,21	18,10	1,37	38,84	38,84
Microcosmus squamiger	3,29	3,50	14,06	1,15	30,17	69,01
Phallusia mammilata	0,00	0,98	4,97	0,94	10,67	79,68
Ciona intestinalis	0,84	0,10	4,55	0,75	9,75	89,43
Ascidiella aspersa	0,50	0,00	2,52	0,56	5,41	94,84

ANNEX VI: Percentage of species contribution to the dissimilarity of the samples (SIMPER) in the case study.

a) *surface*

Groups cement plates & closed plastic wallet
Average dissimilarity = 82,81

	Group cement plates	Group closed plastic wallet				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	5,97	1,00	40,05	1,63	48,37	48,37
Molgula occidentalis	0,08	1,37	9,03	0,75	10,91	59,27
Styela plicata	0,28	1,01	8,36	0,87	10,10	69,37
Botryllus schlosseri	0,08	0,39	4,44	0,62	5,37	74,74
Ciona intestinalis	0,00	0,60	4,40	0,79	5,31	80,04
Molgula sp	0,17	0,52	3,99	0,68	4,82	84,86
Ascidiacea	0,14	0,11	2,84	0,38	3,43	88,29
Amphibalanus cf. *eburneus*	0,39	0,07	2,81	0,61	3,39	91,67

Groups cement plates & PVC tubes
Average dissimilarity = 59,59

	Group cement plates	Group PVC tubes				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	5,97	2,58	38,81	1,67	65,12	65,12
Amphibalanus cf. *eburneus*	0,39	0,19	3,82	0,73	6,41	71,53
Perforatus perforatus	0,24	0,10	3,24	0,42	5,43	76,96
Botryllus schlosseri	0,08	0,14	2,72	0,40	4,56	81,52
Styela plicata	0,28	0,05	2,57	0,53	4,31	85,83
Ascidiacea	0,14	0,05	2,52	0,34	4,24	90,07

Groups closed plastic wallet & PVC tubes
Average dissimilarity = 81,69

Species	Group closed plastic wallet **Av.Abund**	Group PVC tubes **Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	1,00	2,58	28,06	1,19	34,34	34,34
Styela plicata	1,01	0,05	12,79	0,84	15,66	50,00
Molgula occidentalis	1,37	0,00	11,64	0,74	14,25	64,25
Botryllus schlosseri	0,39	0,14	8,28	0,64	10,14	74,40
Ciona intestinalis	0,60	0,00	6,61	0,74	8,09	82,48
Molgula sp	0,52	0,00	4,06	0,66	4,97	87,45
Ascidiacea	0,11	0,05	3,02	0,32	3,69	91,15

b) *<u>times of visits</u>*

Groups 1 & 2
Average dissimilarity = 75,76

Species	Group 1 **Av.Abund**	Group 2 **Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	0,80	2,76	34,99	1,29	46,19	46,19
Botryllus schlosseri	0,58	0,29	12,10	0,82	15,97	62,16
Ascidiacea	0,53	0,00	8,84	0,65	11,67	73,84
Styela plicata	0,00	0,47	8,12	0,65	10,71	84,55
Perforatus perforatus	0,22	0,07	4,16	0,40	5,49	90,04

Groups 1 & 3
Average dissimilarity = 81,73

	Group 1	Group 3				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	0,80	3,76	36,50	1,46	44,66	44,66
Styela plicata	0,00	0,68	10,13	0,60	12,40	57,05
Botryllus schlosseri	0,58	0,19	9,60	0,74	11,75	68,80
Ascidiacea	0,53	0,07	7,70	0,67	9,43	78,23
Perforatus perforatus	0,22	0,36	6,10	0,64	7,46	85,69
Ciona intestinalis	0,00	0,16	3,27	0,34	4,01	89,70
Amphibalanus cf. *eburneus*	0,00	0,33	3,25	0,57	3,97	93,67

Groups 1 & 4
Average dissimilarity = 82,39

	Group 1	Group 4				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	0,80	3,10	33,04	1,31	40,11	40,11
Molgula occidentalis	0,00	1,44	14,73	0,82	17,87	57,98
Botryllus schlosseri	0,58	0,00	8,21	0,70	9,96	67,94
Ascidiacea	0,53	0,00	6,76	0,64	8,21	76,15
Molgula sp	0,00	0,53	5,02	0,70	6,09	82,24
Ciona intestinalis	0,00	0,32	3,60	0,54	4,37	86,62
Amphibalanus cf. *eburneus*	0,00	0,34	3,50	0,61	4,25	90,87

Groups 2 & 4
Average dissimilarity = 67,27

	Group 2	Group 4				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	2,76	3,10	30,12	1,34	44,77	44,77
Molgula occidentalis	0,00	1,44	13,11	0,80	19,49	64,26
Styela plicata	0,47	0,07	5,56	0,65	8,26	72,52
Molgula sp	0,00	0,53	4,52	0,70	6,72	79,24
Botryllus schlosseri	0,29	0,00	4,02	0,52	5,97	85,21
Ciona intestinalis	0,07	0,32	3,48	0,58	5,18	90,39

Groups 3 & 4
Average dissimilarity = 66,32

	Group 3	Group 4				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	3,76	3,10	28,59	1,38	43,12	43,12
Molgula occidentalis	0,00	1,44	11,54	0,78	17,39	60,51
Styela plicata	0,68	0,07	7,01	0,61	10,57	71,08
Amphibalanus cf. *eburneus*	0,33	0,34	4,19	0,79	6,32	77,39
Molgula sp	0,00	0,53	4,02	0,68	6,06	83,45
Ciona intestinalis	0,16	0,32	3,88	0,58	5,86	89,31
Perforatus perforatus	0,36	0,07	3,22	0,65	4,85	94,16

Groups 1 & control
Average dissimilarity = 84,88

	Group 1	Group control				
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Diss/SD**	**Contrib%**	**Cum.%**
Amphibalanus amphitrite	0,80	5,94	43,15	1,58	50,83	50,83
Styela plicata	0,00	0,93	7,24	0,86	8,53	59,37
Molgula occidentalis	0,00	0,88	6,62	0,56	7,80	67,17
Botryllus schlosseri	0,58	0,00	5,82	0,71	6,86	74,03
Ascidiacea	0,53	0,00	4,94	0,65	5,83	79,86
Molgula sp	0,00	0,63	4,72	0,63	5,56	85,42
Amphibalanus cf. *eburneus*	0,00	0,42	3,12	0,63	3,67	89,09
Ciona intestinalis	0,00	0,40	3,09	0,57	3,64	92,73

ANNEX VII: Results from case study

Device after recollection

Control