

UNIVERSIDADE DE LISBOA  
FACULDADE DE CIÊNCIAS  
DEPARTAMENTO DE BIOLOGIA ANIMAL



**MACROBENTHIC COMMUNITIES OF TRÓIA SAND BEACHES (SETÚBAL,  
PORTUGAL): SAMPLE SIZE AND COMMUNITY STRUCTURE VS. ENVIRONMENTAL  
FACTORS**

MARIA LUÍS ADRIÃO DO VALE

MESTRADO EM BIOLOGIA E GESTÃO DE RECURSOS MARINHOS



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Dissertação submetida para obtenção do  
grau de Mestre no curso de Mestrado em  
Biologia e Gestão de Recursos Marinhos

Dissertação orientada por:

Francisco A. de L. Andrade – Professor do Departamento de Biologia Animal da FCUL

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## RESUMO

Desde os primórdios da sociedade humana, que os ambientes costeiros protegidos constituem locais de atracção para o homem. À medida que estas sociedades se foram desenvolvendo, a exploração destes ambientes costeiros provocou pressões cada vez maiores sobre os ecossistemas. A península de Tróia corresponde a um destes ambientes costeiros. Devido às suas características singulares, Tróia foi desde sempre alvo de impactes de origem antrópica, datando do século I e II os vestígios mais antigos que se conhecem na península - as ruínas romanas, que foram consideradas monumento nacional em 1910. Em 1998, a secção Norte da península de Tróia (a Norte do empreendimento da SOLTRÓIA) e os respectivos direitos de desenvolvimento, foram adquiridos pelo grupo Sonae com o intuito de desenvolver um novo empreendimento turístico – *TroiareSORT*. No âmbito deste empreendimento está previsto um casino, um hotel 5 estrelas, diversos apart-hotéis e apartamentos turísticos, um campo de golfe, uma marina, um novo cais dos “ferries” e diversas áreas de serviços. Este projecto resultou de um processo interactivo de integração das condicionantes ambientais presentes na península de Tróia, identificadas no “Estudo Ambiental Estratégico” produzido pelo Instituto do Mar (IMAR) (Andrade et al. 1998). Entre o início de 1999 e 2005, os projectos referentes à marina e novo cais dos “ferries”, foram sujeitos a um processo de avaliação de impacte ambiental realizado pelo IMAR (Andrade & Melo 2003). Este estudo contém a caracterização da situação de referência, a avaliação da sensibilidade do território e dos efeitos globais do empreendimento e, um conjunto de recomendações e medidas de minimização, incluindo um programa de monitorização e um sistema de

gestão ambiental (SGA). O IMAR manteve a sua relação com o proponente, sendo neste momento a entidade que executa o plano de monitorização da secção Norte da península de Tróia. Este, tem como objectivo acompanhar e avaliar os potenciais impactes, decorrentes não só da implantação do empreendimento, mas também da sua fase de exploração. O programa teve início em fase de pré-construção, em 2004, e engloba campanhas periódicas de amostragem, num horizonte temporal de pleno funcionamento de empreendimento utilizando para isso, determinados indicadores (*ex.* ambientes intertidais, dinâmica costeira, avifauna, mamíferos, etc.). A parceria entre o IMAR e a Sonae, permitiu ainda o desenvolvimento de diversos trabalhos de índole académico (teses de licenciatura e mestrados), que forneceram informação mais detalhada acerca da península, com vista a melhorar a gestão do *TroiareSORT*. Neste contexto, e inserido no programa de monitorização ambiental que decorre, o presente trabalho é mais uma contribuição para o conhecimento detalhado do local, mais especificamente no que se refere às comunidades macrobentónicas intertidais na península de Tróia. Com o presente trabalho pretendeu-se dar resposta a uma questão fulcral que surgiu durante a execução do programa de monitorização ambiental. Dos dados recolhidos até à data, concluiu-se que o número de amostras colhidas (replicados) para o estudo da macrofauna bentónica poderia ter influência nos resultados finais obtidos. Pretendeu-se assim, determinar qual o número de replicados ideal tendo em consideração o ratio esforço/rendimento de amostragem. Com este trabalho pretendeu-se ainda determinar os padrões (distribuição e estrutura) das comunidades bentónicas em função dos parâmetros ambientais. Assim, o presente trabalho tem como principais objectivos:

1) determinar qual o número de replicados ideal tendo em consideração o ratio esforço/rendimento de amostragem. No presente trabalho, através dos dados recolhidos no estuário do Sado - 12 replicados por nível em 3 transectos (108 amostras), foi possível estudar qual o número de amostras mínimas necessárias para cada local (número de replicados);

2) determinar os padrões (distribuição e estrutura) das comunidades bentónicas em função dos parâmetros ambientais que variam ao longo da península de forma a não só melhorar e rentabilizar o plano de monitorização em curso, como também fornecer informação que possa ser incorporada na actual gestão do *TroiareSORT*. No presente trabalho, através de dados recolhidos no estuário do Sado – 2 replicados x 3 nível x 9 transectos x 4 vezes ao ano x 2 anos (432 amostras individuais), foi possível relacionar a distribuição e estrutura das comunidades macrobentónicas com os factores ambientais.

A análise do ambiente morfo-sedimentar da área de estudo, foi parte integrante de ambos os objectivos enunciados e os resultados demonstraram um gradiente ambiental da margem marinha (ambiente marinho exposto) para a margem estuarina (ambiente estuarino protegido). A macrofauna bentónica demonstrou um gradiente crescente no número de indivíduos, riqueza específica e diversidade do supralitoral (SL) para o infralitoral (IL) e da margem marinha (exposto) para a margem estuarina (protegido).

De modo a atingir o primeiro objectivo deste trabalho, em cada nível e transecto, diversas abordagens foram testadas de forma a determinar o número de replicados ideal:

1) Análise Combinatória; 2) Análise Combinatória Aleatória; 3) Análise Probabilística;

4) Mao Tau; 5) ICE; 6) Lei de Zipf e Teste de Cattell's Scree. As primeiras cinco abordagens falharam, uma vez que a informação tendia a aumentar sempre com o número de replicados. No geral, as diferentes abordagens utilizadas sugerem que o número de replicados ideal se encontra entre o 5º (0.0825 m<sup>2</sup>) e o 12º (0.198 m<sup>2</sup>) replicado, sendo este último valor na maioria dos casos estudados inferior ao número de replicados ideal, o que é impraticável no âmbito de um programa de monitorização, uma vez que existem limitações no que se refere ao tempo e recursos disponíveis. No entanto, estes resultados são aplicáveis somente para a área de estudo. Este tipo de estudo deveria ser a primeira abordagem em qualquer monitorização biológica, uma vez que, a perda de habitats costeiros cria a necessidade de se obter análises comparativas e previsões de confiança relativamente à riqueza específica e biodiversidade existentes, de forma a melhorar a sua gestão ambiental.

O segundo objectivo deste trabalho foi atingido através de uma Análise Canónica de Correspondências (CCA). A estrutura das comunidades intertidais de substratos móveis estudadas demonstraram uma clara dominância dos padrões espaciais sobre os temporais. Através desta análise 4 comunidades foram definidas: 1) uma comunidade dominada por *Angulus tenuis*, na margem marinha da península; 2) uma comunidade dominada por *Euclymene* sp.A e *Apseudes latreillei*, na área de transição entre o estuário e o mar, relacionada com a presença de *Zostera* spp.; 3) uma comunidade dominada por *Glycera* sp. e *Scoloplos arminger*, na área de transição entre o estuário e o mar; 4) uma comunidade dominada por *Notomastus latericeus*, *Nassarius reticulatus* e *Cyathura carinata*, na margem estuarina. Os factores ambientais que mais

contribuíram para a análise multivariada foram o declive de praia e os teores em matéria orgânica e carbonato de cálcio, os quais aparentam ter um importante papel na definição da estrutura das comunidades macrobentónicas, uma vez que os *taxa* aparentam ter preferências relativamente a estes factores. Através da CCA foi possível determinar os padrões (distribuição e estrutura) das comunidades bentónicas em função dos parâmetros ambientais, melhorando e rentabilizando o plano de monitorização em curso, como também fornecendo informação que passou a ser incorporada na actual gestão do *TroiareSORT*. No entanto, devemos salientar a necessidade de estudos com uma amplitude temporal mais elevada assim como a inclusão de outros factores ambientais que influenciam a distribuição e estrutura das comunidades macrobentónicas.

**PALAVRAS CHAVE:** macrofauna bentónica, ambiente estuarino, amostra mínima, esforço de amostragem, factores ambientais, Análise Canónica de Correspondências, estuário do Sado.



## **ABSTRACT**

Coastal environments have always constituted places of attraction for mankind for a number of reasons. Tróia peninsula corresponds to one of those coastal environments, which was acquired in 1998 by a private group – Sonae, with the intention to develop a new tourist project – *Troiaresort*. In this project it is foreseen a casino, a fivestar hotel, hotel apartments, tourist apartments, a marina, golf course, a new ferry harbor and several service areas. In the scope of the ongoing environmental monitoring program, the present study had two objectives:

1) determine the minimum sample size (number of replicates) having in consideration the ratio effort/information of the sampling;

2) determine the patterns (distribution and structure) of the benthic communities in relation to the environmental parameters which vary along the peninsula.

The morpho-sedimentary environment analysis was part of both objectives of this work, and results revealed an environmental gradient, from the marine margin (exposed marine environment) to the estuarine margin (sheltered estuarine environment). Benthic macrofauna analysis showed a gradient of increasing number of individuals, species richness and diversity from upper intertidal (SL) to lower intertidal (IL) and from the marine margin (Exposed) to the estuarine margin (Sheltered).

To achieve the first objective of this work, in each level and transect, several approaches were tested to determine the minimum sample size. In general, the different approaches

used, suggest minimum sample sizes between 5 (0.0825 m<sup>2</sup>) and 12 (0.198 m<sup>2</sup>) replicates, with this value still below a minimum sampling size in most of the cases.

The second objective of this work was achieved through a Canonical Correspondence Analysis (CCA). The structure of the sand beach intertidal communities studied showed the definition of four communities and a clear dominance of the spatial patterns over the seasonal ones. The most important environmental factors were beach slope, organic matter and calcium carbonate contents, which appear to have an important role in defining the structure of macrobenthic communities.

**KEYWORDS:** benthic macrofauna, estuarine habitat, minimum sample size, sampling effort, environmental factors, CCA, Sado estuary.

## **INDEX**

Acknowledgements.....	i
Resumo.....	iii
Abstract.....	ix

## **CHAPTER 1**

General Introduction.....	1
Literature Cited.....	4

## **CHAPTER 2**

MACROBENTHIC COMMUNITIES OF TRÓIA SAND BEACHES (SETÚBAL, PORTUGAL): SAMPLE SIZE.....	6
Abstract.....	6
Introduction.....	8
Study area.....	11
Material and Methods.....	12
Sampling design.....	12
Morpho-sedimentary environment.....	14
Benthic macrofauna.....	14
Data analysis.....	14
Morpho-sedimentary environment.....	14
Benthic macrofauna.....	14

Combinatory analysis.....	15
Random Combinatory analysis.....	15
Probabilistic analysis.....	15
Mao Tau.....	15
ICE.....	16
Zipf law and Cattell's Scree test.....	16
Results and Discussion.....	18
General characterization of the morpho-sedimentary environment.....	18
General characteristics of benthic macrofauna.....	19
Minimum sample size.....	20
Conclusion.....	31
Literature Cited.....	34

### **CHAPTER 3**

MACROBENTHIC COMMUNITIES OF TRÓIA SAND BEACHES (SETÚBAL, PORTUGAL): COMMUNITY STRUCTURE VS. ENVIRONMENTAL FACTORS.....	49
Abstract.....	49
Introduction.....	51
Study area.....	53
Material and Methods.....	54
Sampling design.....	54
Morpho-sedimentary environment.....	56
Benthic macrofauna.....	56

Data analysis.....	56
Morpho-sedimentary environment.....	57
Benthic macrofauna.....	57
Results and Discussion.....	59
General characterization of the morpho-sedimentary environment.....	59
General characteristics of benthic macrofauna.....	61
Environmental Vs biological variables .....	65
Conclusion.....	73
Literature Cited.....	75

#### **CHAPTER 4**

Conclusions.....	84
------------------	----



# CHAPTER 1

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## GENERAL INTRODUCTION

Coastal environments have always constituted places of attraction for mankind for a number of reasons, and consequently, the development of human societies has increased the exploration and pressure on coastal ecosystems (European Commission 2001). Tróia peninsula corresponds to one of those coastal environments, due to its singular characteristics. Tróia has always been target of anthropogenic impacts, the first evidences dating from the 1<sup>st</sup> and 2<sup>nd</sup> century – period of the Roman occupation on the peninsula. More recently, in the 70's, the North portion of the Tróia peninsula was target of one ambitious tourist project by a private group – Torralta, which foresaw the implantation of a tourist complex with 67 200 beds in a total area of 320 ha. However, this project did not reach its totality, since it was verified the bankruptcy situation of the Torralta. In 1998 the North portion of the Tróia peninsula and the respective rights of development were acquired by a private group – Sonae, with the intention to develop a new tourist project – *TroiareSORT*. In the scope of this project it is foreseen a casino, a fivestar hotel, hotel apartments, tourist apartments, a marina, golf course, a new ferry harbor and several service areas. This project resulted of an interactive process of integration of the environmental conditionings present in the Tróia peninsula, identified in the Strategical Environmental Study produced by the Institute of Marine Research (IMAR) (Andrade et al. 1998).

Between 1999 and 2005, the projects concerning the marina and the new ferry harbor were subject to a process of environmental impact assessment carried out by IMAR (Andrade & Melo 2003). This study contains the characterization of the reference

situation, the evaluation of the sensitivity of the territory, the global effects of the project and, a set of recommendations and mitigation measures, including a monitoring program and an environmental management system. The monitoring program that is being executed by IMAR, has the objective of following and evaluating the potential impacts that may be consequence, not only of the implementation but also of the exploration of the projects. The monitoring program started in 2004 (pre-construction), and consists of periodic surveys for collection of data regarding a number of pre-determined environmental indicators (*ex.* intertidal environments, coastal morphodynamic, mammals, etc.), in a temporal horizon of full functioning of the project. The partnership between IMAR and Sonae also allowed the development of several graduate work (PhD and MSc thesis), that managed to supply more detailed information concerning the peninsula, and thus, helped improve the management of the *Troiaresort* (*ex.* Carapuço, 1999; Ferreira, 2001; Carapuço, 2005; Silveira, 2006; Lourenço, 2007). In this context and in the scope of the ongoing environmental monitoring program, the present work represents a contribution for the detailed knowledge of the site, specifically in what concerns the intertidal macrobenthic communities of the Tróia peninsula. The first objective deals with a very important issue that is related with the execution of the environmental monitoring program. From the data collected, we concluded that the number of sampling units (replicates) could influence the final results for the study of the macrobenthic communities. Thus, we intend to determine the minimum sample size (number of replicates) having in consideration the ratio effort/information of the sampling.

The second objective of this work is to determine the patterns (distribution and structure) of the benthic communities in relation to the environmental parameters which vary along the peninsula.

The aim of the present work is to deliver important input that can improve the monitoring program that is ongoing, as well as to supply information that can be incorporated in the current environmental management system of the *TroiareSORT*.

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## **CHAPTER 2**

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## **MACROBENTHIC COMMUNITIES OF TRÓIA SAND BEACHES (SETÚBAL, PORTUGAL): SAMPLE SIZE**

Vale M, Andrade F

### **ABSTRACT**

One of the most generally accepted and intensively studied issues on ecological communities is the relationship between area and species richness. Many sandy beach ecologists have used species accumulation curves in order to evaluate the precision of sampling protocols and to estimate the minimum sample area required to supply practical estimates of species richness on sandy beaches. In this study, a data set of 12 replicates for each level of 3 transects (108 samples) of the Sado estuary allowed to study the optimal sample size (number of replicates). Morpho-sedimentary environment analysis revealed an environmental gradient, from the marine margin (exposed marine environment) to the estuarine margin (sheltered estuarine environment). Benthic macrofauna analysis showed a gradient of increasing number of individuals, species richness and diversity from upper intertidal (SL) to lower intertidal (IL) and from the marine margin (Exposed) to the estuarine margin (Sheltered). In each level and transect several approaches were tested to determine the minimum sample size: 1) Combinatory analysis; 2) Random Combinatory analysis; 3) Probabilistic analysis; 4) Mao Tau; 5) ICE; 6) Zipf law and Cattell's Scree test. The first five approaches failed, since information tends to always increase with the number of replicates. In general, the

different approaches used, suggest minimum sample sizes between 5 (0.0825 m<sup>2</sup>) and 12 (0.198 m<sup>2</sup>) replicates, with this value still below a minimum sampling size in most of the cases, which is unfeasible in a monitoring program, since there are limitations in terms of time and available resources.

**KEYWORDS:** benthic macrofauna, estuarine habitat, minimum sample size, sampling effort, Sado estuary.

## INTRODUCTION

One of the most generally accepted and intensively studied issues on ecological communities is the relationship between area and species richness (e.g. Arrhenius 1921, Gleason 1922, Palmer 1990, Colwell & Coddington 1994, Jaramillo et al. 1995, Ney-Nifle & Mangel 1999, Gotelli & Colwell 2001, Connor & McCoy 2001, Williamson et al. 2001, Schoeman et al. 2003, Ugland et al. 2003, McLachlan & Dorvlo 2007), i.e. the number of species with increasing sampled area (Ugland et al. 2003, McLachlan & Dorvlo 2007). This increase has been documented for a considerable range of habitats and taxa (McLachlan & Dorvlo 2007). Species-area curves are suitable tools for examining the rate at which species are added to the estimate for species richness with an increase in sampling effort (Schoeman et al. 2003). Many sandy beach ecologists have used species accumulation curves in order to evaluate the precision of sampling protocols and to estimate the minimum sample area required to supply practical estimates of species richness on sandy beaches (Hartnoll 1983 in McLachlan & Dorvlo 2007, Jaramillo et al. 1995, Brazeiro 2001, Schoeman et al. 2001). These curves are asymptotic and represent the increasing proportion of total species richness collected within a habitat as sampling effort increases (McLachlan & Dorvlo 2007). According to James & Fairweather (1996 in Schoeman et al. 2003) the size, shape and distribution of the sampling units in the habitat is very important to the efficiency of the sampling design. According to Schoeman et al. (2003) the sampling units are usually arranged in shore transects across the intertidal, each corresponding to an area between 0.009 and 0.25 m<sup>2</sup>, covering total areas between 0.03 and 1m<sup>2</sup>. After determining the size, shape

and distribution of sampling, one of the factors that remains is the number of sampling units (replicates) to be sampled at each specific level (Schoeman et al. 2003).

One of the common ways to contain costs in monitoring programs is to reduce the number of such replicates, which can compromise the representativity, the statistical reliability and obstruct the detection of important environmental changes (Fairweather 1991 and Streever 1998 in Halse et al. 2002, Ysebaert & Herman 2002). Despite the importance of the number of sampling units (replicates) in the design of sampling programs, for beach ecology, few are the studies that have attempted to evaluate the consequences on the parameter estimates used for the interpretation of biological patterns and the processes that they infer (Schoeman et al. 2003). The number of sampling units (replicates) is a universal problem in benthic invertebrate studies, the majority of them resorting to estimates of the mean densities based on the collection of 3 or less replicates (Downing & Downing 1992 and Resh & McElravy 1993 in Veijola et al. 1996). On the other hand, collecting larger samples, with more replicates is very time consuming and implies large resources, being most of the times limited by the availability of people and time. In the majority of the benthic invertebrate studies consulted, irrelevant of the geographic location, sampling method and sample size, the number of sampling units (replicates) was defined without any kind of previous quantitative study (**Table 1.**). Therefore, there is a need to arrive at a practicable statistical commitment. The number of sampling units (replicates) collected must be a commitment between the best possible representation of the community and the time and effort spent in collecting and processing the samples (Gray 1981).

**Table 1.** Summary of the bibliography reviewed, with the number of sampling units (replicates) collected by the different authors (- denotes dimension not specified in the work).

Replicates number	Individual dimension (m <sup>2</sup> )	Author
1	0.529	Chester et al. 1983
	—	Dexter 1988
	0.1	Rodrigues 1992
	0.04	Behbehani & Croker 1982 in Costa 1988
2	—	Albertelli et al. 2001
	0.025	Slepukhina et al. 1996
	0.06	Sardá et al. 1999
	0.1	Carvalho et al. 2001
	0.082	Edgar & Barrett 2002
3	0.0165	Ferreira 2001
	—	Halse et al. 2002
	0.06 - 0.07	Boesch 1973
	0.1	Rodrigues & Quintino 1990
	0.06	Weston 1990
	0.07	Crema et al. 1991
	0.047	Morrisey et al. 1992
	0.1	Rosenberg et al. 1992
	0.0184	Alden et al. 1997
	0.095	Amaral & Costa 1999
	0.05	Boaventura et al. 1999
	0.065	Chimenz Gusso et al. 2001
4	0.004	Garmendia et al. 2003
	0.223	Guerra-García et al. 2003
	0.083	Manté et al. 2003
	—	Martins et al. 2005
	0.05	Jones et al. 1986
1 to 4	0.0184	Dauer et al. 1987
	0.05	Jones 1987
	0.027	Rainer 1981 in Costa 1988
	—	Boyd & Rees 2003
5	0.01	Dexter 1990
	0.1	Pearson 1975
	0.0274	Leber 1982
	0.083	López-Jamar & Mejuto 1986
	—	Gamito 1989
	—	Underwood 1991
	0.0824	Edgar et al. 1994
	0.1	Zmarzly et al. 1994
	0.1	Grémare et al. 1998
	0.008	Paiva 2001
6	0.01	Cunha & Ravara 2003
	0.1	Lampadariou et al. 2005
10	0.1	Martínez & Adarraga 2003
	0.0133	Baden 1990
	0.02	Guerreiro 1994
8 to 12	0.25	Ozolin'sh 2002
	0.05	Beisel et al. 1998
14	0.011	Mucha & Costa 1999
16	0.023	Essink & Beukema 1986 in Costa 1988
	0.1	Somerfield & Gage 2000
18	0.011	Costa 1988
	0.011	Costa et al. 1990
24	—	Cao et al. 1998 in Rumohr et al. 2001
32	0.1	Gentil & Dauvin 1988 in Rumohr et al. 2001
15 to 40	0.1	Holme 1953 in Rumohr et al. 2001

The main objective of the present work was to study the optimal sample size (number of replicates) for the sand beaches intertidal communities in Tróia (Portugal), in order to improve the ongoing monitoring program for the environmental management of the area. The effort/information ratio was used to assess the minimum sampling effort needed to ensure a good characterization of the communities present, in order to ensure that patterns detected in field samples reflect real ecological processes rather than methodological effects (Schoeman et al. 2003)

## STUDY AREA

The Tróia peninsula is a sand spit in the west coast of Portugal, oriented Southeast-Northwest, limited in the north by the mouth of the Sado estuary. The study area (**Figure 1.**) corresponds to the distal part of the peninsula, between the open coast and the transition between the marine and estuarine margins, which exhibits a high lateral variation in the hydrodynamic characteristics and thus, the main driving forces acting on the beach (Andrade et al. 1998, Ferreira 2001, Silveira 2006). The distal part of the peninsula corresponds to a low-energy environment, sheltered and fetch-restricted, affected by local and nonlocal generation waves and presents a high spatial variability of the morphodynamic pattern (Silveira 2006). The Southwest (marine) margin is affected by low intensity waves, due to the presence of the ebb tide delta of the Sado River (Silveira 2006). The Northwest margin of the peninsula (mouth of the estuary) is predominantly subject to tidal circulation as the main driving force, and to waves generated by the local wind regime, corresponding to the most dynamic area with

significant morphological and sedimentary changes comparable to high energy environments (Silveira 2006). Sediment variability correspond essentially to differences in the proportions of sand-sized shell debris, which preferably concentrate in the coarser tail of the dimensional spectrum (Silveira 2006).

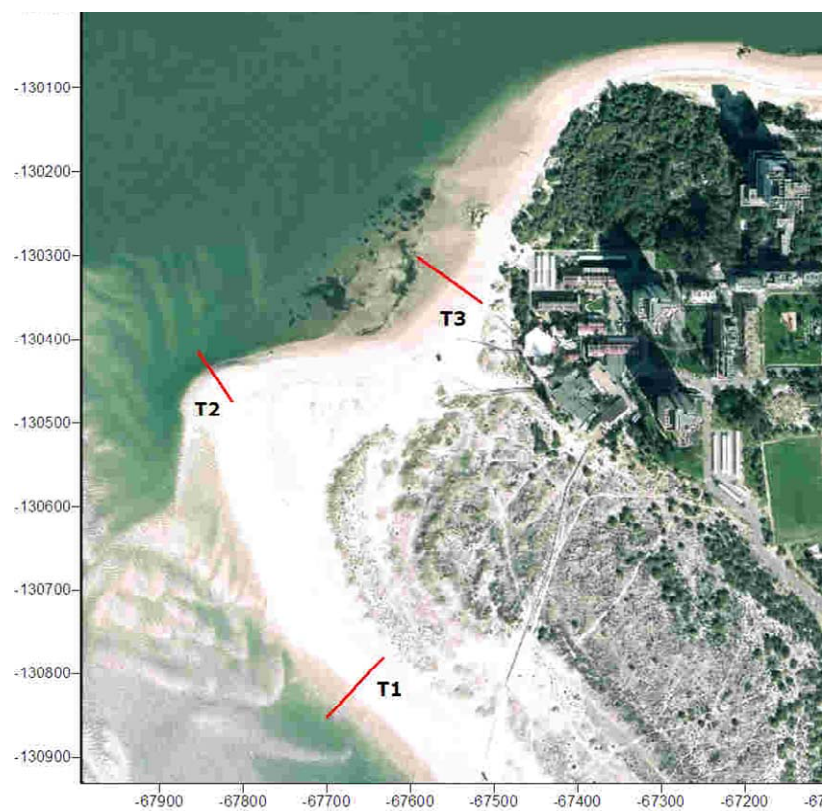


**Figure 1.** Location of Tróia on the Portuguese coast (©2006 Google Maps).

## MATERIALS AND METHODS

**Sampling design.** Since the main problem with studying numerous sites is that the sampling involved is time consuming, and so this kind of studies must be performed on a limited scale (Palmer 1990), the study area was divided in 3 sectors, according to the exposure to the forcing agents – tides, waves and winds. Each sector, each characterized by a transect, covering the intertidal area of the beach – Exposed (T1), Transition (T2) and Sheltered (T3). The marine margin transect (T1) is mostly influenced by the ocean waves and thus, will be referred to as the exposed one; The sea-estuary transect (T2) is

located in a very heterogeneous area in the outer estuarine margin, which is mostly influenced by the ebb currents of the estuary. This transect will be referred to as the transition transect; The sea-estuary transect (T3) is located on a small bay in the outer estuarine margin, with a *Zostera* spp. meadow, and thus, will be referred to as the sheltered one (**Figure 2.**).



**Figure 2.** Location of the 3 transects sampled (UTM coordinates, Datum 73 Zone 29N).

Each of the 3 transects was sampled between the 25<sup>th</sup> and 28<sup>th</sup> of September 2003, during the low-water of the largest spring tide of the month.

Morpho-sedimentary environment: Surface sediment samples – upper 1 cm (c. 200 g in each sample), were collected on the beach face. Beach profiles were surveyed using a TCR307 *Leica Geosystems* total station.

Benthic macrofauna: Macrofauna was sampled at three levels on the beach – lower intertidal (IL), mid intertidal (ML) and upper intertidal (SL), with a 14.5 cm Ø corer (0.0165 m<sup>2</sup>), taking the upper 20 cm of sediment, with twelve replicates (total area 0.198 m<sup>2</sup>) collected at each site and level. A total of 108 individual samples were collected (12 replicates x 3 levels x 3 transects). Each individual sample was separately washed through a 1 mm mesh sieve and the biological material and sediment retained on the sieve were labelled and stored in individual bags until the sorting and collection of the organisms *in situ* and *in vivo*. Collected organisms were preserved in a 70 % alcohol solution. Laboratory taxonomic determinations were carried out later, to the lowest possible taxonomic level.

**Data analysis.** Morpho-sedimentary environment: Sediment data - grain size analysis was carried out by dry sieving, using half-phi intervals from  $-2 \phi$  to  $4 \phi$ . The grain size parameters - mean grain size (**Mz**) and sorting (**s1**) - were determined through Folk's graphic method (Folk & Ward 1957). Calcium carbonate contents (**CaCO<sub>3</sub>**) was determined by means of hydrochloric acid test (e.g. Freitas 1995, Cruces 2001). Morphological data - the beach face slope, corresponding to the steepest section of the intertidal area, was calculated using simple trigonometry and expressed in  $\tan\beta$ . Benthic macrofauna: For each replicate, total number of individuals and densities

(inds/m<sup>2</sup>) were computed per *taxon*, together with species richness and Shannon-Weaver diversity (1948) and evenness indexes, using *Microsoft Excel* vs. 2003.

In order to determine the minimum sample size, for each transect and level, several approaches were tested:

(1) **Combinatory analysis** - in which replicates were combined in an ascending number, irrelevant of an order ( ${}_{12}C_1, {}_{12}C_2, {}_{12}C_3, \dots, {}_{12}C_{12}$ ). Since no simple process to perform the successive combinations between the 12 replicates is available, only the total calculations for  ${}_{12}C_1, {}_{12}C_{11}$  and  ${}_{12}C_{12}$  and the partial calculations for  ${}_{12}C_2$  (replicate 1 with all the others) and  ${}_{12}C_3$  (replicates 1 and 2 with all the others) were performed, for the transect and level with the highest species richness and diversity values – T3IL. Species richness and diversity were used as information values;

(2) **Random combinatory analysis** – in which 12 combinations for each number of replicates (1 to 12) were randomly selected for each transect and level. In this analysis we only considered sites with more than 6 *taxa*. For each number of replicates, the mean value of species richness and diversity were used as the corresponding information values;

(3) **Probabilistic analysis** – based on the occurrence probability of each *taxa* in the whole replicate universe, for each transect and level. Species richness and diversity were used as the corresponding information values;

(4) **Mao Tau** – This method is based on the rarefaction curves through successive replicates (Gotelli & Colwell 2001) and consisted of the creation of cumulative species richness curves using the formulas of Colwell et al. (2004). The rarefaction curves are used to compare *taxon* richness at comparable levels of sampling effort, thus the number

of *taxa* was plotted as a function of the accumulated number of individuals, not the accumulated number of replicates, since datasets may differ systematically in the mean number of individuals per replicate. Therefore it is assumed that *taxon* richness is the question, not *taxon* density (Gotelli & Colwell 2001). In this analysis, we chose the random option without reposition of replicates (each replicate was selected only once). Analysis of those curves allowed us to identify the replicate number after which no more species were being added. This analysis was performed using the *EstimateS* software (vs. 7.5.) (Colwell 2005);

(5) **ICE** – This approach uses an estimator of the species richness based on the incidence of each species in the replicates group (Chao & Yang 1993, Lee & Chao 1994, Chazdon et al. 1998). The method is based on the statistical concept of *sample coverage*, which is defined as the sum of the parametric relative abundance probabilities of the observed classes (Turing & Good 1953 in Chao & Lee 1992). This approach was used because it tends to give better species richness estimates when compared to the previous ones, when the number of replicates is low, and also because it is less sensitive to sampling density and species *patchiness* (Chazdon et al. 1998);

(6) **Zipf law and Cattell's Scree test** – According to Zipf's law (1949), the relation between rank and size is constant since, in most instances, the rank-size curves are very nearly segments of rectangular hyperbolas, that is, curves whose equations are of the form  $XY = \text{constant}$ , or as expressed in logarithmic coordinates -  $\log X + \log Y = \text{constant}$ . So, when such rank-size curves are plotted in a log/log system, they are very nearly straight lines with slopes close to -1. A rank-size curve is essentially the integral of the size-frequency curve (Kruskal & Tanur 1978). In this work, we assumed that in

the rank/size relation, size is the absolute frequency of the *taxa*. So, this law gives special relevance to the relation between the absolute frequency of the *taxa* (total number of individuals) and rank position occupied by the *taxa* (e.g. 1<sup>st</sup> - *Euclymene* cf. *santanderensis* - 443 inds; 2<sup>nd</sup> *Euclymene* sp. -134 inds; 3<sup>rd</sup> *Euclymene* cf. *collaris* - 88 inds), absolute frequency being inversely proportional to the rank, thus following a hyperbolic distribution (Kruskal & Tanur 1978, Mandelbrot 1982). Zipf's law assumes that, as the sampling effort rises, the information will also raise. The intersection of the absolute frequency/rank hyperbole with any given frequency level will give the corresponding best relation between effort and information. The hyperbole cut frequency was defined as 2 individuals per *taxon*. A logarithm transformation was applied both to absolute frequency and rank values. The Cattell's Scree test (1966) was also used, which allowed the identification of the discontinuity (factor - *taxon*) through the observation of the graphic of the *log* absolute frequency/*log* order relation, by finding the inflection point of the function. The projection of this point in the XX axis indicates the number of factors to extract (Lattin et al. 2003). Using this method, the factors (*taxa*) that correspond to the inflexion points in the graph, were reported to the probabilistic table of occurrences (from the probabilistic analysis), yielding the correspondent replicate number and therefore, the best effort/information ratio.

## RESULTS AND DISCUSSION

**General characterization of the morpho-sedimentary environment:** Table 2. shows the results of the data analysis for the morpho-sedimentary parameters. These results reflect the difference in exposure to the local forcing factors along the study area.

**Table 2.** Morpho-sedimentary parameters for the study area.

	Exposed (T1)	Transition (T2)	Sheltered (T3)
<b>MZ</b>	Medium sand	Medium sand	Coarse sand
<b>s1</b>	Moderately well sorted	Moderately well sorted	Very well sorted
<b>CaCO<sub>3</sub> (%)</b>	12.38	9.73	15.26

Sediment analysis showed a gradient of increasing mean grain size from the marine margin (Exposed) to the outer estuarine margin (Sheltered). The exposed sector (T1) is composed almost entirely of sand, due to the re-working induced by the waves, while the sheltered sector (T3) suffers the occasional incorporation of coarser grains in the sediment. Usually, surface sediments are coarser on estuarine beaches, with a similar source of sand and pebbles, than on ocean or ocean-facing beaches (Nordstrom 1977 in Nordstrom 1992). The highest carbonate contents of T3 correspond mainly to shell fragments. This could be explained by the low hydrodynamic conditions which allow the deposition of shell fragments and also by the presence of the nearby *Zostera* spp. meadow. T2 was characterized by a steep beach slope (11.5°) and a well marked berm crest, while T1 and T3 were both characterized by gentle beach slopes (between 5.5° and 6°). This suggests that T1 and T3 are less acted upon by the wave action, in T1 due to the presence of the Sado ebb delta – Cambalhão and, in T3, due to the presence of the

low-tide terrace with *Zostera* spp., which, in both cases, prevents local waves from reaching the beach face at low-water.

**General characteristics of benthic macrofauna:** Table 3. shows the results of the data analysis for the benthic macrofauna. For T3SL no organisms were found. A total of 112 macroinvertebrate *taxa* (1179 individuals) were identified in the survey area. Annelida (42 *taxa* – 81.2 % of individuals), Crustacea (42 *taxa* – 9.1 % of individuals) and Mollusca (23 *taxa* – 9.7 % of individuals) were the most abundant groups. Results show that vertical distribution of the macrofauna is in conformity with the zonation pattern proposed by Dahl (1952): (1) the supralittoral zone (SL) of air breathers is typically inhabited by talitrid amphipods and oniscid isopods; (2) the midlittoral zone (ML) is typically inhabited by true intertidal *taxa*, such as cirolanid isopods and spionid polychaetes; (3) the sublittoral zone (IL) presents a wide variety of *taxa*, such as bivalves, mysids, amphipods and polychaetes.

**Table 3.** Overall values for density, species richness, diversity and evenness on the study area (IL - lower intertidal; ML - mid intertidal; SL – upper intertidal).

	Exposed (T1)			Transition (T2)			Sheltered (T3)	
	IL	ML	SL	IL	ML	SL	IL	ML
Density (inds/m <sup>2</sup> )	277.8	10.1	35.4	80.8	10.1	15.2	5404	121.2
Species Richness ( <i>taxa</i> )	20	2	2	11	2	3	82	16
Diversity Shannon-Weaver (bit)	3.6	1	0.9	3.3	1	1.6	3.6	3.8
Evenness	0.83	1	0.86	0.96	1	1	0.57	0.95

Results show a gradient of increasing density, species richness and diversity from the upper (SL) to the lower beach levels (IL) and from the marine margin (Exposed) to the outer estuarine margin (Sheltered), consistent with the results of Ferreira (2001) in the

same study area, and Junoy & Viéitez (1990) in the Ría de Foz (Galicia - Spain). According to Junoy & Viéitez (1990) and Edgar & Barret (2002), level is an important factor in the distribution of intertidal *taxa*.

It is also known that organic enriched sheltered areas contain higher numbers of individuals than exposed areas, with sheltered areas promoting the enrichment of macrofauna (Dexter 1988, Junoy & Viéitez 1990, McLachlan & Jaramillo 1995). Density, species richness and diversity tend to increase as exposure to wave action decreases (Dexter 1992). This pattern is supported by our results, with T3 located in a sheltered bay in the vicinity of a *Zostera* spp. meadow that suggest an important homogeneity of the environmental conditions (Pérès & Picard 1964, Riggs & Fralick 1975 in Andrade 1986), and with a substantially more diverse fauna than unvegetated areas (Edgar et al. 1994, Edgar & Barrett 2002); T1 and T2, on the other hand, are located in a very dynamic and unstable sector of the study area, in part because of the proximity to the main ebb tide channel of the estuary.

**Minimum sample size: (1) Combinatory analysis** – This analysis, applied to T3IL, showed that the mean species richness increased with the number of combined replicates (sampling effort). However the mean diversity for 3 replicates reached the value of 3.6 bit, the same value for the total combinations with 11 and 12 replicates. So we can assume that the mean diversity stabilized for 3 replicates (**Table 4.**).

**Table 4.** Species richness and diversity from combinatory analysis for site T3IL.

Combinations	Species Richness	Diversity
${}_{12}C_1$	19,6	3,0
${}_{12}C_2$ -	31,3	3,4
${}_{12}C_3$ -	45,2	3,6
${}_{12}C_{11}$	78,2	3,6
${}_{12}C_{12}$	82	3,6

This analysis suggests that after the 3<sup>rd</sup> replicate (0.0495 m<sup>2</sup>), there is no significant gain of specific information. However, not all possible combinations were analyzed, so this approach doesn't provide a high confidence level. Although this analysis could be considered the "ideal" approach to determine the minimum sample size, it wasn't possible to carry it in full, because of the extremely high number of possible combinations, which made this method not viable.

(2) **Random combinatory analysis** – This analysis was applied to T1IL, T2IL, T3IL and T3ML. For the 12 random combinations of 1 to 12 replicates, species richness tended to increase with the number of replicates (sampling effort). However, diversity tended to stabilize for 3 replicates at T1IL and for 2 replicates at T3IL. It followed an increasing trend at T2IL and T3ML (**Figure 3**).

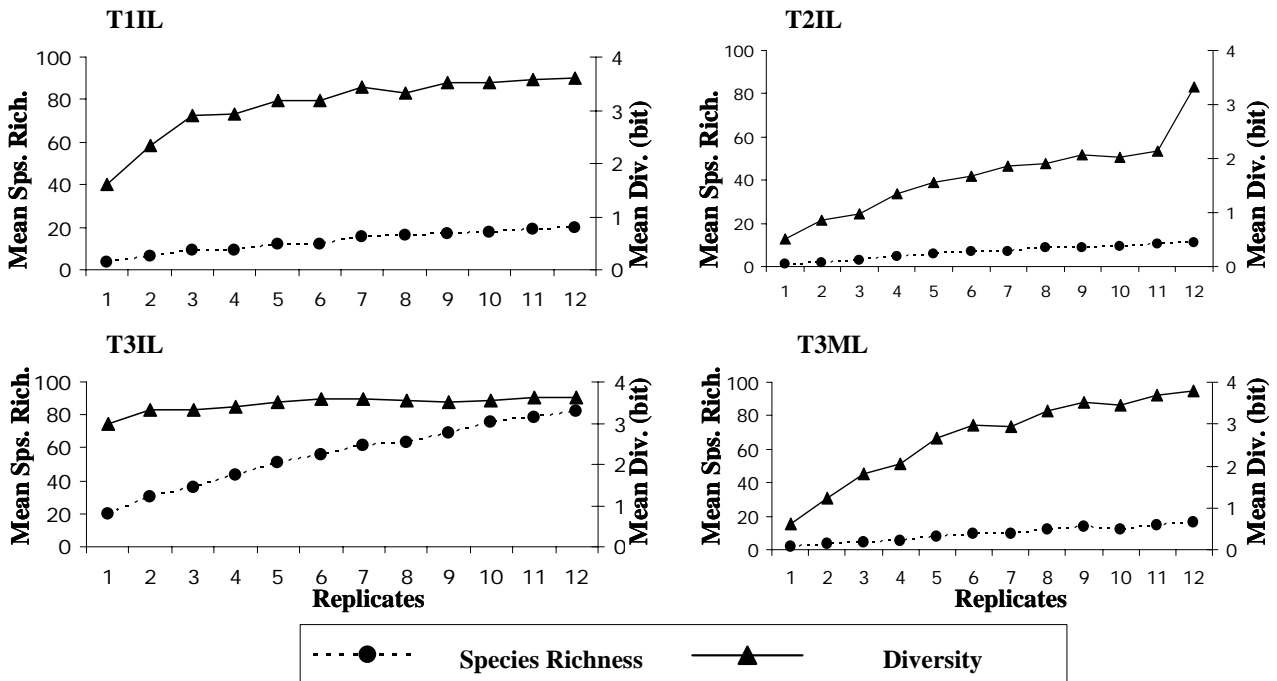


Figure 3. Species richness and diversity for random combinatory analysis at sites T1IL, T2IL, T3IL and T3ML.

This analysis did not produce any specific information on the minimum sample size. However, it provided indications that, in the majority of the transects and levels, the optimum replicates number was approached, since all the effort/information curves show a stabilization trend. Yet, the analysis suggests that the 12 replicates collected weren't enough to attain the minimum sample size, making this approach inconclusive.

(3) **Probabilistic analysis** – In this analysis, both indexes increased with the number of replicates (sampling effort). For the IL level, this analysis highlighted T3, because this site presented the overall highest values of species richness and diversity. Therefore, the corresponding communities are more diversified / structured. For the ML level, low values of richness and diversity were always found, although slightly higher at site T3ML. For the SL level, values of both indexes were very low and similar for the 3

transects. Therefore, we can assume that the minimum sample size for T3 corresponds to a much higher number of sampling units, since this transect presented the richest and more diverse communities (**Table 5**). According to Jaramillo et al. (1995) beaches harbouring the highest number of species need to be sampled more extensively comparing to beaches having lower species richness.

**Table 5.** Species richness and diversity for the probabilistic analysis (IL - lower intertidal; ML - mid intertidal; SL – upper intertidal).

			Number of replicates											
			1	2	3	4	5	6	7	8	9	10	11	12
IL	T1	Species Richness	0	0	0	0	0	2	2	2	4	5	7	20
		Diversity						0.98	0.98	0.98	1.99	2.31	2.78	4.29
	T2	Species Richness	0	0	0	0	0	0	0	0	0	0	3	11
		Diversity											1.56	3.41
	T3	Species Richness	5	6	6	7	10	11	14	20	21	27	37	82
		Diversity	1.46	1.82	1.82	1.96	2.26	2.48	2.72	3.22	3.29	3.71	4.12	5.46
ML	T1	Species Richness	0	0	0	0	0	0	0	0	0	0	0	2
		Diversity												1
	T2	Species Richness	0	0	0	0	0	0	0	0	0	0	0	2
		Diversity												1
	T3	Species Richness	0	0	0	0	0	0	0	0	0	0	2	16
		Diversity											0.97	3.83
SL	T1	Species Richness	0	0	0	0	0	0	0	1	1	1	2	2
		Diversity								0	0	0	1	1
	T2	Species Richness	0	0	0	0	0	0	0	0	0	0	0	3
		Diversity												1.58

This analysis only provided information on the minimum sample size for T1SL (11 replicates - 0.1815 m<sup>2</sup>). However, we should take in consideration that only 2 *taxa* were collected from this site. In all other transects and levels, no potential stabilization was evident. This result was expectable, since with this method, each *taxon* is only considered as present for the number of replicates that ensures, with a 100% probability that it will be found. So, this is a method that will tend to overestimate the minimum sample size.

(4) **Mao Tau** –For T1ML, T2ML, T3ML and T2SL the slope of the curves is constant, suggesting that each additional replicate is also adding new species, without any indication of a possible curve stabilization. The same is reinforced by the corresponding steady increase in standard deviation. For T1IL, T2IL and T3IL the curve slope decreases slightly, suggesting that despite each new replicate adding a number of new species, some stabilization may be occurring. For T1SL a decreasing slope curve, suggests that the addition of new species decreases until it becomes null for the 11<sup>th</sup> replicate, with no extra information being added then (**Figure 4.**).

This analysis only provided information on a minimum sample size for T1SL (11 replicates – 0.1815 m<sup>2</sup>), again probably, because of the low number of *taxa* (7 *taxa*) found in this location. For the remaining transects and levels, only for site T3IL does the cumulative species richness curve suggest a potential stabilization for the totality of the replicates (12). According to these results, the sampling effort of 12 replicates (0.198 m<sup>2</sup>) wasn't enough to attain a minimum sample size in the intertidal beach environments in Tróia. Using the same method, Santos & Simon (1980), in Tampa Bay (Florida, USA), showed that for 9 replicates (9 x 0.456 = 4.104 m<sup>2</sup>) the cumulative species richness showed no increment, up to a total of 16 replicates (7.2 m<sup>2</sup>). However, it should be considered that the size of 1 replicate (0.456 m<sup>2</sup>) in the work developed by Santos & Simon corresponds approximately to 27 times the size of 1 replicate on the present study (0.0165 m<sup>2</sup>).

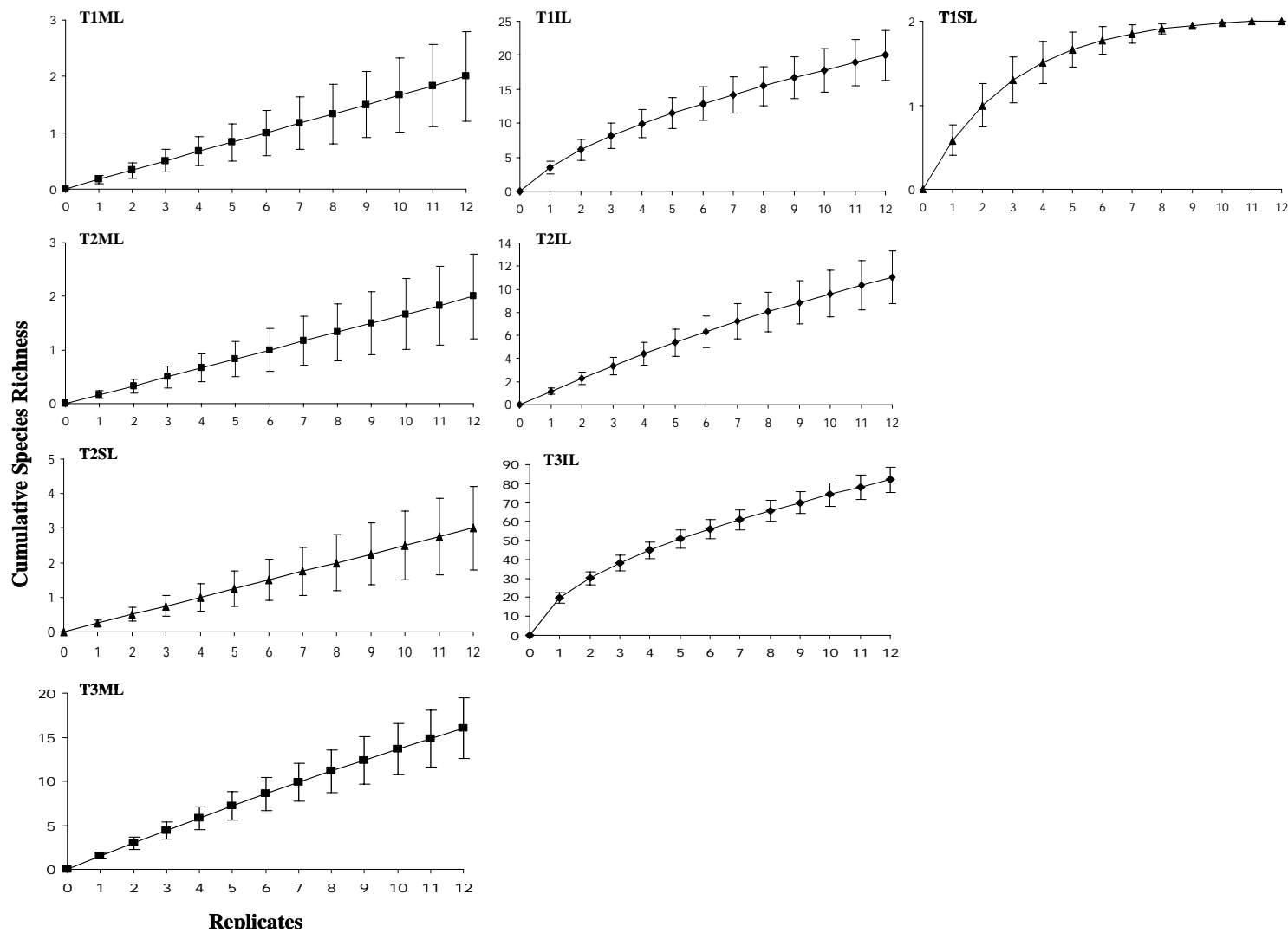


Figure 4. Mao Tau analysis for the sampling sites (IL - lower intertidal; ML - mid intertidal; SL – upper intertidal).

5) **ICE** - Results of this analysis, show that for T1SL, T2IL and T3ML, cumulative species richness reaches its maximum for 6, 9 and 11 replicates respectively, suggesting that added sampling effort does not add more information. For T1ML, T2ML and T2SL the curve slope remains constant, showing that each new replicate is still adding new species. However, there is a decrease in standard deviation, suggesting an eventual stabilization. For T1IL, the curve slope decreases, showing that the addition of new species with each new replicate is decreasing, this is reinforced by the reduction of the standard deviation. So we can state that cumulative species richness seems to be close to the stabilization. For T3IL, cumulative species richness presents an abrupt peak for the 1<sup>st</sup> replicate, decreasing after this, never reaching the asymptote. Such fact can be explained by *patchiness*, since during this analysis the 1<sup>st</sup> replicate probably presented a higher number of species when compared to the remaining. After the 3<sup>rd</sup> replicate, the curve slope shows again a positive trend, however, with a small addition of new species with each new replicate. The same information is strengthened by the standard deviation, so we can state that the curve seems to be close to the stabilization (**Figure 5**). The order in which samples are added to the species cumulative curve affects the shape of the curve, inducing variations that may result from sampling error and heterogeneity among the species in the samples (Colwell & Coddington 1994). According to Ugland et al. (2003), initially, many species are found as larger areas are sampled and a cumulative plot of number of species *vs.* area sampled rises steeply at first and then more slowly as the increasingly rare species are added. The ICE analysis only produced information on the minimum sample size for T1SL (6 replicates – 0.099 m<sup>2</sup>), T2IL (9 replicates – 0.1485 m<sup>2</sup>) and T3ML (11 replicates – 0.1815 m<sup>2</sup>).

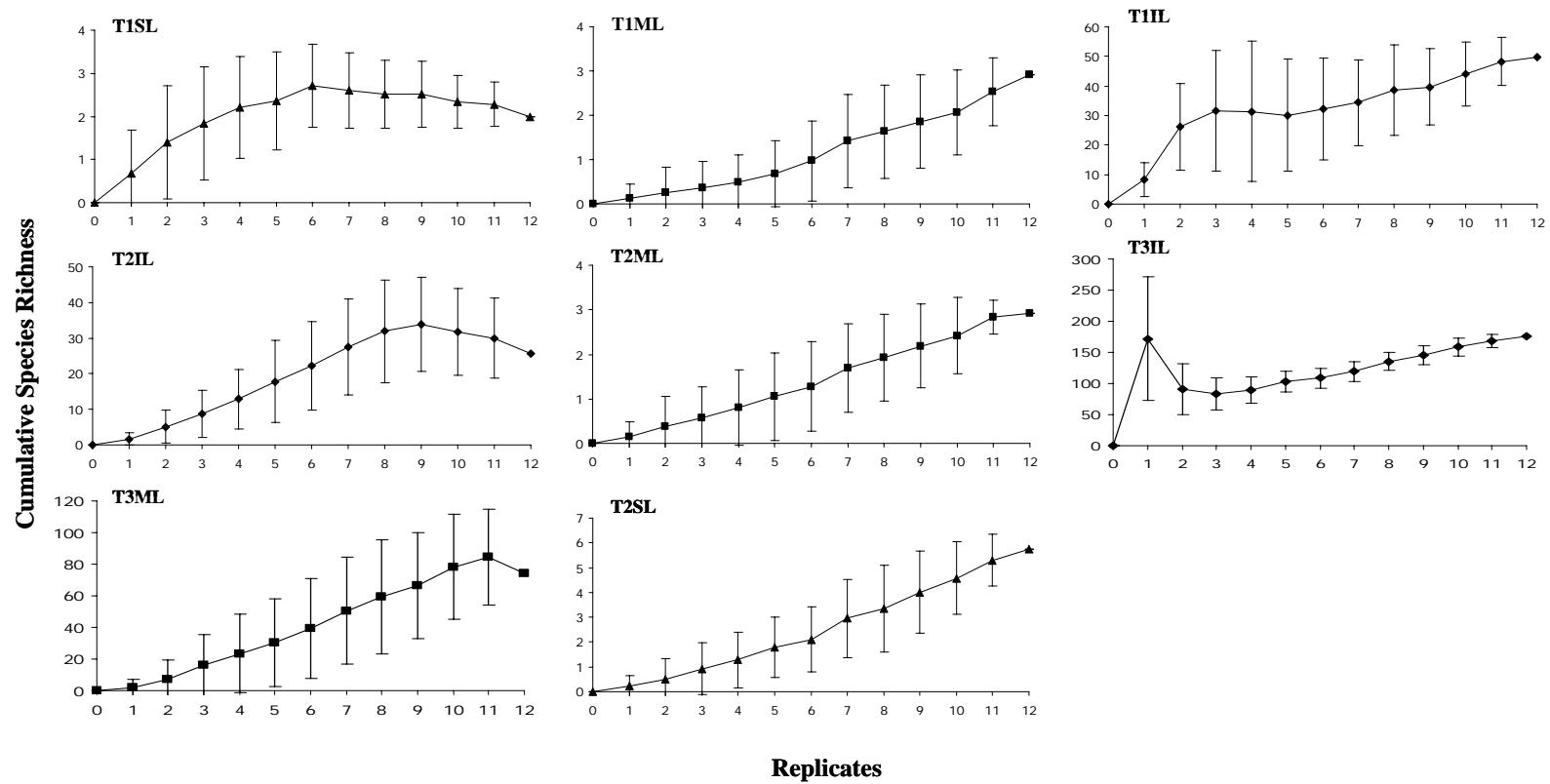


Figure 5. ICE analysis for the sampling sites (IL - lower intertidal; ML - mid intertidal; SL – upper intertidal).

In the remaining transects and levels, the ideal replicate number is probably not much above 12, since the slope of the curves indicates a possible stabilization. In this method, species richness estimation, especially when the number of samples is reduced, is highly dependent of the *patchiness* in the data (Chazdon et al. 1998). Thus, the 12 collected replicates (0.198 m<sup>2</sup>) weren't enough to determine the minimum sample size in all transects and levels analyzed, which could be justified by the high *patchiness* of the sampled communities. According to Ugland et al. (2003), this approach doesn't come close to an asymptote and the predicted total number of species is much lower than the one predicted by other methods.

(6) **Zipf law and Cattell's Scree test** – As described before, the hyperbole cut level was defined at 2 individuals per *taxon*, so T1ML, T2ML and T2 SL were not submitted to this method. T1SL was submitted to this approach, but it was not possible to detect the inflexion point in the graph, which made this approach inconclusive in the detection of the best replicate number to this location. **Table 6.** presents the extracted factors (*taxa*) that were reported to the probabilistic table of occurrences yielding the correspondent replicate number, with the largest sample size of 12 replicates for T3ML and T2 IL, and the lowest of 5 replicates for T3IL, giving us the best ratio between effort and information.

**Table 6.** Synthesis of the results from the application of Zipf law and Cattell's Scree test (IL - lower intertidal; ML - mid intertidal) (*taxon* – species corresponding to the inflexion point).

Level	Transect	Inflexion point		Extracted factors ( <i>Taxon</i> )	Replicate number
		Rank (Log)	Absolute frequency (Log)		
ML	3	0.5	0.3	<i>Elasmopus rapax</i> cf.	12 <sup>th</sup>
IL	1	0.5	0.8	<i>Nephtys caeca</i>	9 <sup>th</sup>
	2	0.3	0.3	Amphipoda	12 <sup>th</sup>
	3	0.8	1.3	Polychaeta sp.A	5 <sup>th</sup>

This approach only provided information on the minimum sample size for 4 locations. For the lower beach (IL), the results can be justified by the presence of high density, species richness and diversity occurring in the more sheltered area (T3), while the more exposed areas (T1 and T2) present lower corresponding values. The largest minimum sample size corresponds to 12 replicates for T2 (0.198 m<sup>2</sup>), the location with the lowest values of density, species richness and diversity, probably because this is the more hydrodynamic and unstable location in the study area. This can be justified by the proximity of the ebb tide channel of the estuary, which also does not allow for the formation of submerged bars that elsewhere reduce the energy of waves acting on the beach, with higher exposure, steeper beach profile and coarser sediment size having profound effects in the survival conditions of the organisms in the sediment (Raffaelli & Hawkins 1996). The smallest minimum sample size corresponds to 5 replicates for T3 (0.0825 m<sup>2</sup>), which probably relates to the fact that this site presents the highest values of density, species richness and diversity of all the study area, due to the proximity of the *Zostera* spp. meadow, that suggest an important homogeneity of the environmental conditions (Pérès & Picard 1964, Riggs & Fralick 1975 in Andrade 1986). These results agree with Jaramillo et al. (1995), since they have shown that beaches of different

morphodynamic types present differences in macrofaunal community attributes including species richness, abundance and biomass. According to McLachlan et al. (1993 in Jaramillo et al. 1995), species richness increases linearly from reflective to dissipative conditions, with changes in beach type resulting in predictable changes in macrofaunal species richness.

According to Jaramillo et al. (1995), beaches presenting the highest number of species need to be sampled more extensively to collect most of the species, with the estimated value for sampling effort increasing from reflective to dissipative morphodynamic states (Schoeman et al. 2003). Pires (1983), also in the Sado estuary (in an innermost part of the estuary), but with Elliott's (1971) formula ( $n = t^2 s^2 / D x^2$ ), defined a minimum sample size for the lower beach level of 0.099 m<sup>2</sup>. Our results using the Zipf law approach suggest a minimum sample size between 5 (0.0825 m<sup>2</sup>) and 12 (0.198 m<sup>2</sup>) replicates, according to the local characteristics. Jaramillo et al. (1995) in order to define the minimum total area to be sampled, collected 3 replicates (0.1 m<sup>2</sup> each) in 15 different levels in the beach (total area: 4.5 m<sup>2</sup>) and constructed species area curves for sample areas up to 5 m<sup>2</sup>. With this method, they concluded that a minimum of 4 m<sup>2</sup> needs to be sampled in order to detect at least 95 % of the species present on an exposed sandy beach of a variety of morphodynamic types. However, Schoeman et al. (2003) from his study in three beaches (Maitlands, Sundays River and Kings beaches) of the Eastern Cape, South Africa stated that with a sampled area of 4 m<sup>2</sup>, more than 30 % of the species present can be missed, suggesting the need for an increased sampling effort. To ensure sampling more than 90 % of the species on any given transect, sampling

effort must exceed 12 m<sup>2</sup>, since rare species will always be missed at low levels of sampling effort (Schoeman et al. 2003).

## CONCLUSION

Our results show that the first five approaches, failed in the definition of a minimum sample size, since information tends to always increase with the number of replicates, for the total of 12 replicates collected.

In general, the different approaches used suggest minimum sample sizes between 5 (0.0825 m<sup>2</sup>) and 12 (0.198 m<sup>2</sup>) replicates, with this value still below a minimum sampling size in most of the cases, which is unfeasible in a monitoring program, since there are limitations in terms of time and available resources (**Table 7**).

However these results are applicable only for the study area and therefore, further studies will be needed for different locations and environmental settings. According to Hartnoll (1983 in McLachlan & Dorvlo 2007), Jaramillo et al. (1995), Brazeiro (2001) and Schoeman et al. (2001), large areas (of at least 4 m<sup>2</sup>) need to be sampled to estimate macrofauna species richness in intertidal transects on sandy beaches. With most published works corresponding to rough underestimates (McLachlan & Dorvlo 2007), as our bibliographic research confirmed (cf. **Table 1**) which has profound implications to our understanding of the ecology and biodiversity of sandy beach macrofauna (Jaramillo et al. 1995, Ugland et al. 2003).

**Table.7.** Synthesis of the results from the six approaches used (▲ denotes no stabilization up to the 12 replicates level;\* denotes some tendency to stabilization for 12 replicates; - denotes method not applied; n – minimum sample size in replicates).

Approaches			Combinatory analysis	Random combinatory analysis	Probabilistic analysis	Mao Tau	ICE	Zipf law and Cattell's Scree test
T1	IL	Sp.Rich.	-	▲	▲	▲	*▲	9
		Div.	-	*▲	▲	-	-	-
	ML	Sp.Rich.	-	-	▲	▲	*▲	-
		Div.	-	-	▲	-	-	-
	SL	Sp.Rich.	-	-	11	11	6	-
		Div.	-	-	11	-	-	-
T2	IL	Sp.Rich.	-	▲	▲	▲	9	12
		Div.	-	▲	▲	-	-	-
	ML	Sp.Rich.	-	-	▲	▲	*▲	-
		Div.	-	-	▲	-	-	-
	SL	Sp.Rich.	-	-	▲	▲	*▲	-
		Div.	-	-	▲	-	-	-
T3	IL	Sp.Rich.	▲	▲	▲	*▲	*▲	5
		Div.	3	*▲	▲	-	-	-
	ML	Sp.Rich.	-	▲	▲	▲	11	12
		Div.	-	▲	▲	-	-	-

Since the objective of any sampling survey is to supply a baseline relative to which changes in the composition of the macrobenthic communities can be detected, the only option is to increase the sampling effort above the levels traditionally attempted (Schoeman et al. 2003). According to Schoeman et al. (2003), the only way in which these levels could be increased is through a greater investment in personnel or via mechanical innovation, which would require greater levels of funding for beach research. However, the lack of this kind of resources was what made the beach researchers adopt a relatively conservative approach to the sampling design, since they tend to develop methods that suit their resources (time and money) and then stick with them irrelevant of the sampled habitat (Schoeman et al. 2003).

Therefore, this kind of study should be the first approach to any biological monitoring, since the loss of coastal habitats creates the need for comparative analyses and reliable

predictions of species richness and biodiversity in order to improve environmental management.

During the development of the present work, we came across some limitations, not only because of the bibliography scarcity referring to this subject, but also because of the restrictions inherent to the methods used to reach the objective. Results showed that some of the approaches did allow for the definition of a sample size ranging from 5 to 12 replicates. However, in practice, such samples cannot be achieved due both to the available resources and the time available for field work – spring tides low water. So, due to these limitations and in order to improve the ongoing monitoring program for the environmental management of the Tróia peninsula (Portugal), we decided to increase the number of sampling units from 2 to 3 replicates, as a compromise to increase information gathered with the available resources.

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# CHAPTER 3

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## MACROBENTHIC COMMUNITIES OF TRÓIA SAND BEACHES (SETÚBAL, PORTUGAL): COMMUNITY STRUCTURE VS. ENVIRONMENTAL FACTORS

Vale M, Andrade F

### ABSTRACT

Several studies have dealt with the spatial and temporal variations specifically on benthic macrofauna and their relationships with abiotic environmental factors. In this study, a data set (432 individual samples) of the Sado estuary allowed to relate macrobenthic species distribution and community structure to environmental factors. Morpho-sedimentary data analysis revealed an environmental gradient, from the marine margin (exposed marine environment) to the estuarine margin (sheltered estuarine environment). Benthic macrofauna analysis showed a gradient of increasing number of individuals, species richness and diversity from upper intertidal (SL) to lower intertidal (IL) and from the marine margin (Exposed) to the estuarine margin (Sheltered). Canonical Correspondence Analysis (CCA) showed the dominant patterns in the community structure to be explained by the environmental variables. The structure of the sand beach intertidal communities studied showed a clear dominance of the spatial patterns over the seasonal ones. Four communities were defined – (1) a community dominated by *Angulus tenuis*, on the marine margin of the peninsula; (2) a community dominated by *Euclymene* sp.A and *Apseudes latreillei*, on the sea-estuary transition area

and related to the presence of a *Zostera* spp. meadow; (3) a community dominated by *Glycera* sp. and *Scoloplos arminger*, on the sea-estuary transition area; (4) a community dominated by *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata*, on the estuarine margin. The most important environmental factors were beach slope, organic matter and calcium carbonate contents, which appear to have an important role in defining the structure of macrobenthic communities, since *taxa* appear to have preferences.

**KEYWORDS:** benthic macrofauna, estuarine habitat, environmental factors, CCA, Sado estuary.

## INTRODUCTION

It is recognized that soft-bottom benthic organisms do not present a spatial pattern of random distribution, and their communities occur in a structured way (e.g. Brown & McLachlan 1990) which makes the study of temporal and spatial variations of benthic communities essential for the determination of their dynamics and structure.

Many studies have dealt with the spatial and temporal variability of biological communities (e.g. Andrew & Mapstone 1987, Ibanez & Dauvin 1988, Wiens 1989, Barry & Dayton 1991, Schneider 1994, Horne & Schneider 1995, Ysebaert 2000, Ysebaert & Herman 2002). Some of these results suggest that communities do not show a significant inter-annual variability (Ibanez & Dauvin 1988 and Ysebaert & Herman 2002), even when intra-annual variability is high (Ysebaert 2000). In temperate seas, differences tend to be maximum, between the end of summer/beginning of autumn and the end of winter/beginning of spring (Dauvin 1984 in Ibanez & Dauvin 1988), due to the large seasonal fluctuations in macrobenthic soft-sediments populations (Ysebaert 2000). Benthic communities tend to show a high spatial-temporal stability and inertia, which enables them to reflect the prevailing local conditions (Costa et al. 1990). Thus, they behave as true memorizers, accumulators and in some cases as amplifiers of the environmental conditions (Hily et al. 1986). Therefore, they are excellent indicators in marine pollution studies, being used in a large number of monitoring programs (Bilyard 1987, Rosenberg & Resh 1993, Quintino 1996 in Carvalho et al. 2001, Cabral & Murta 2004). Generally, these communities are conditioned by environmental factors that do not affect all the individuals of the community in the same way (e.g. Brown &

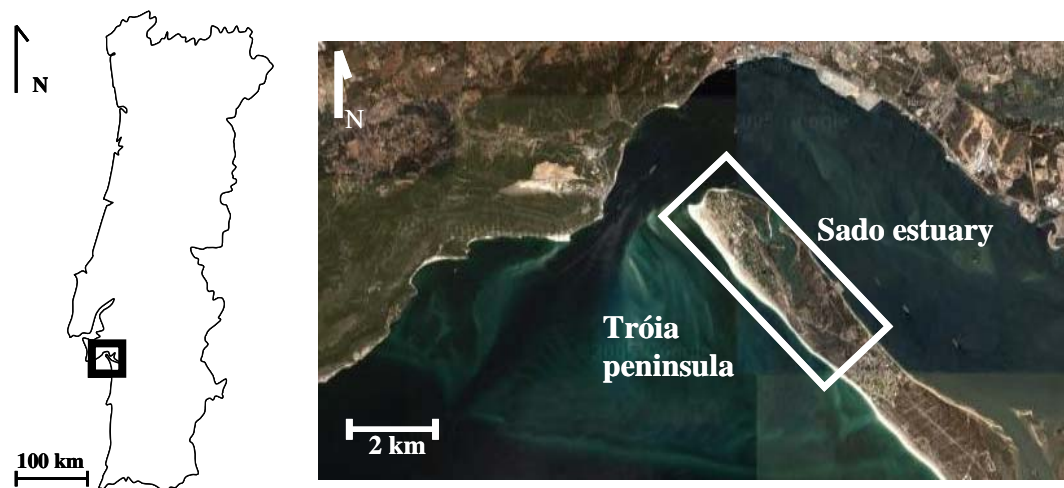
McLachlan 1990). Several studies have dealt with the spatial and temporal variations specifically on benthic macrofauna and their relationships with abiotic environmental factors (e.g. Brown & McLachlan 1990, Picard 1965 in Costa et al. 1990, McLachlan 1983 in Dexter 1990, Junoy & Viéitez 1990, McLachlan 1990 in McArdle & McLachlan 1992, Edgar & Barrett 2002, Ysebaert & Herman 2002, Ysebaert et al. 2003, Defeo & McLachlan 2005, Silva et al. 2006). Some of these studies evidenced strong relations between the structure of the benthic communities and the sediment (e.g. Brown & McLachlan 1990, Picard 1965 in Costa et al. 1990, Junoy & Viéitez 1990, Warwick et al. 1991, McLachlan 1990 in McArdle & McLachlan 1992, Ysebaert & Herman 2002, Ysebaert et al. 2003, Silva et al. 2006) and also with the exposure to the wave action (Warwick & Uncles 1980, Brown & McLachlan 1990, McLachlan 1983 in Dexter 1990, McLachlan 1990 in McArdle & McLachlan 1992), this last factor being considered determinant to the density and diversity of the intertidal organisms according to Dexter (1988). A study on the spatial distribution of macrobenthic communities was developed by Ferreira (2001) in the Troia peninsula (Portugal). Through a PCA analysis of the biotic (*taxa*) and abiotic (environmental parameters) variables, Ferreira (2001) was able to determine the spatial distribution pattern of the macrobenthic communities along the peninsula, and concluded that the distal part of the peninsula is characterized by typical marine and estuarine (high salinity) communities, with the communities of the higher levels in the beach, homogeneous throughout the study area and characteristic of temperate sandy beaches under marine influence. In the lower level of the beach, a heterogeneous environment was found, with three different communities along the peninsula: 1) a community dominated by *Angulus tenuis* – *Nephtys* sp.,

predominantly in the marine margin; 2) a community dominated by *Euclymene* sp. A., in the sea-estuary transition area; 3) a community dominated by *Spiochaepertus costarum*, in the estuarine margin. Building on the above mentioned work (Ferreira 2001), the main objective of the present study was to analyse the distribution and structure of the macrobenthic communities in an estuarine to marine intertidal soft-bottom environment and their relationships with environmental factors, in order to further understand the distribution and variability of the macrobenthic communities in the Tróia peninsula (Portugal).

## STUDY AREA

The Tróia peninsula is a sand spit in the west coast of Portugal, oriented Southeast-Northwest, limited in the north by the mouth of the Sado estuary (**Figure 1.**). The study area corresponds to the distal part of the peninsula, between the open coast and the estuary, which exhibits a variation in the hydrodynamic characteristics and thus, the environmental parameters of the beach (Andrade et al. 1998, Ferreira 2001). The distal part of the peninsula corresponds to a low-energy environment, sheltered and fetch-restricted, affected only by local and nonlocal generation waves and presents a high spatial variability of the morphodynamic pattern (Silveira 2006). The Southwest (**marine**) margin is affected by low intensity waves, due to the ebb tide delta of the Sado River (Silveira 2006). The Northwest margin of the peninsula (**mouth of the estuary**) is predominantly subject to tidal circulation as the main driving force, and to waves generated by the local wind regime, corresponding to the most dynamic area with

significant morphological and sedimentary changes comparable to high energy environments (Silveira 2006). The Northeast (**estuarine**) margin is only affected by local generation incident weak waves and the corresponding beach is stable (Silveira 2006). Sedimentary variations of the beach correspond essentially to differences in the proportions of sand-sized shell debris, which preferably concentrate in the coarser tail of the dimensional spectrum (Silveira 2006).

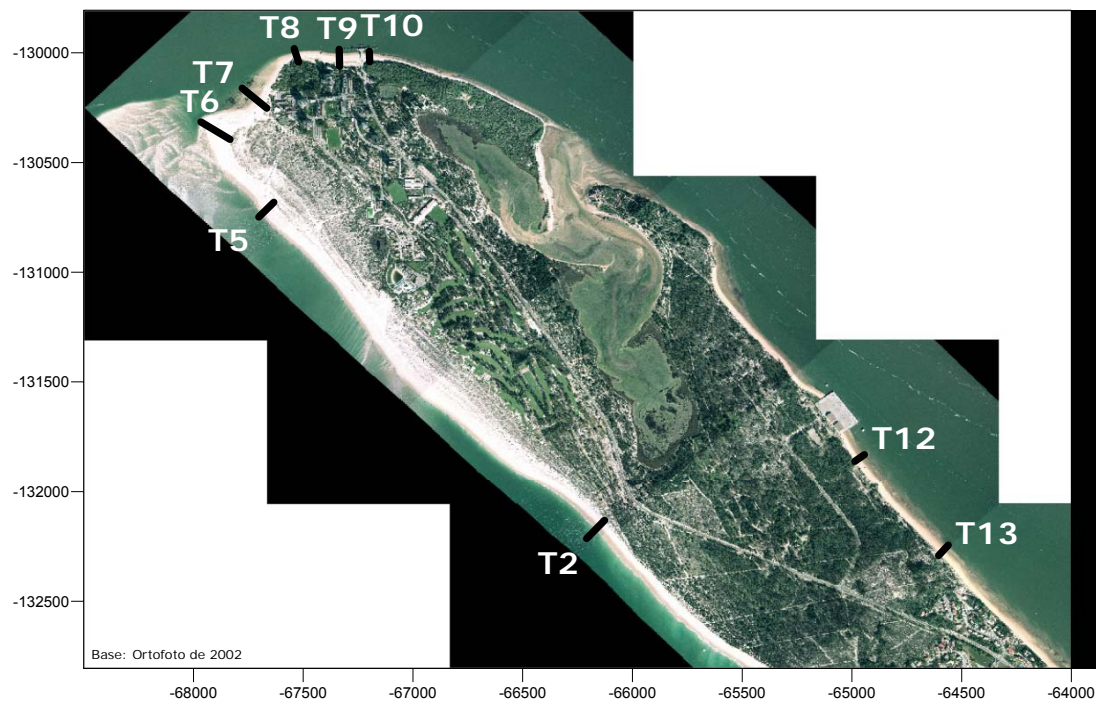


**Figure 1.** Location of Tróia on the Portuguese coast (©2006 Google Maps).

## MATERIALS AND METHODS

**Sampling design.** A part of the 13 transects in the work developed by Andrade et al. (1998) were used for the current study, maintaining the nomenclature. According to the exposure to the forcing agents – tides, waves and wind, the study area was divided into 3 sectors characterized by 9 intertidal transects – Marine margin (transects T02 and T05), Transition (transects T06, T07, T08, T09 and T10) and Estuarine margin (transects T12 and T13). The marine margin transects (T02 and T05) are mostly

influenced by the ocean waves and thus, will be referred to as the exposed ones; The sea-estuary transects (T06, T07, T08, T09 and T10) are located along a very heterogeneous area in the transition between the estuarine and the marine margins, which is mostly influenced by the ebb currents of the estuary (transect – T07 - is located on a small bay with a *Zostera* spp. meadow). These transects will be referred to as the transition transects; Estuarine margin transects (T12 and T13) are mostly influenced by the estuarine action and will be referred to as sheltered (**Figure 2.**).



**Figure 2.** Location of the 9 transects sampled (UTM coordinates, Datum 73 Zone 29N).

Each of the 9 transects was sampled during 2004 and 2005 with a seasonal periodicity (winter, spring, summer, autumn), during the low-water of the largest spring tide of the corresponding month.

Morpho-sedimentary environment: Surface sediment samples – upper 10 cm (c. 200 g in each sample), were collected on the beach face, at the same location where sediment compaction was also measured. Compaction was evaluated by measuring the depth of the impact of a lead sphere (100 g and 2.5 cm Ø) in the sediment dropped from a constant height of 100 cm. Beach profiles were surveyed using both a TCR307 *Leica Geosystems* total station and a profiler according to the method described by Andrade & Ferreira (2006).

Benthic macrofauna: Macrofauna was sampled at three levels on the beach – lower intertidal (IL), mid intertidal (ML) and upper intertidal (SL), with a 14.5 cm Ø corer (0.0165 m<sup>2</sup>), from the upper 20 cm of sediment, with two replicates (total area 0.033 m<sup>2</sup>) collected at each level. A total of 432 individual samples were collected (2 replicates x 3 levels x 9 transects x 4 times a year x 2 years). Each individual sample was separately washed through a 1 mm mesh sieve and the biological material and sediment retained on the sieve were labelled and stored in individual bags until the sorting and collection of the organisms *in situ* and *in vivo*. Collected organisms were preserved in a 70 % alcohol solution. Laboratory taxonomic determinations were carried out later, to the lowest possible taxonomic level.

**Data analysis.** Since the macrobenthic soft-sediments communities tend not to display significant inter-annual variability but rather, high intra-annual variability (Ibanez & Dauvin 1988, Ysebaert 2000, Ysebaert & Herman 2002), and since we only covered a two years sampling period, we decided to combine the data from 2004 and 2005 working with the average seasonal values.

Morpho-sedimentary environment: Sediment data - grain size analysis was carried out by dry sieving, using 1 phi intervals from  $-1 \phi$  to  $4 \phi$ . The grain size parameters - mean grain size (**Mz**), sorting (**s1**), skewness (**SKI**) - were determined through Folk's graphic method (Folk & Ward 1957). Sediment fraction with a diameter higher than  $1\phi$  (0.5 mm) (**Coarse**) was also estimated. Calcium carbonate contents (**CaCO<sub>3</sub>**) was determined by means of hydrochloric acid test (e.g. Freitas 1995, Cruces 2001). Organic matter contents (**OrgMat**) was determined by the Loss on Ignition (LOI) method at 450-500 °C (e.g. Byers et al. 1978, Kristensen & Andersen 1987); Sediment compaction - the depth of the impact of the sphere in the sediment was expressed in 0.5 cm classes; Morphological data - the beach face slope, corresponding to the steepest section of the intertidal area, was calculated using simple trigonometry and expressed in  $\tan\beta$ .

Benthic macrofauna: For each sample, absolute and relative densities (inds/m<sup>2</sup>) of each *taxon* were computed, together with species richness and Shannon-Weaver diversity (1948) and evenness indexes using *Microsoft Excel* vs. 2003. In order to relate the biologic variables with the environmental variables, a multivariate ordination technique – Canonical Correspondence Analysis (CCA) - was applied. CCA is a nonlinear eigenvector ordination technique related to CA (Correspondence Analysis), but which constrains the axes to be linear combinations of the measured environmental variables (Ter Braak 1986, Grothues & Cowen 1999). This technique analyzes the spatial-temporal variation in the *taxa* abundance data set (Ter Braak & Smilauer 1998). *Canoco for Windows (version 4.5)* (Ter Braak & Smilauer 2002), was used for this analysis. We specifically focused in the inter-species distances, without any type of preliminary data transformation. From the three levels sampled on the beach – lower intertidal (IL), mid

intertidal (ML) and upper intertidal (SL), we decided that for the ordination analysis we would only use the lower intertidal (IL), because the relationships between environmental and biological variables are poorly defined in the highest intertidal levels (Edgar & Barrett 2002), and also because this is the level that showed the higher richness and diversity. To remove any undue effects of rare *taxa* on the ordination analysis, *taxa* occurring in less than 30 % of the total 36 averaged samples (9 transects x 4 times a year) i. e. occurrence < 11, were excluded (e.g. Ysebaert et al. 2003). The *taxon* determined as the subtidal species *Ervilia castanea* (Rodriguez Babio & Bonnin 1987, Quintino et al. 1989, Morton 1990, Moreno 1998), was also excluded from this analysis, since its high densities would potentially mask the overall distribution patterns of the benthic macrofauna. So, in this analysis, in order to detect/determine the patterns of seasonality and spatial (transects) distribution of the communities, we considered the *taxa* at the lowest taxonomic level reached, and eight environmental variables: mean grain size (MZ), sorting (s1), skewness (SKI), sediment fraction with a diameter higher than  $1\phi$  (0.5 mm) (Coarse), calcium carbonate contents ( $\text{CaCO}_3$ ), organic matter contents (OrgMat), compaction degree (Compct) and beach slope (Slope).

## RESULTS AND DISCUSSION

**General characterization of the morpho-sedimentary environment:** **Table 1.** shows the average values – 2004/2005 – for the morpho-sedimentary data. Analysis of the morpho-sedimentary data revealed an environmental gradient, from the marine margin (exposed marine environment) to the estuarine margin (sheltered estuarine environment), as previously described by Ferreira (2001). Grain size distribution showed a high variability in time and space, which seems to be random, with no spatial-temporal pattern. However, sediments from the innermost sheltered transect - T13 (**Figure. 2**), are consistently coarser than all of the rest, which is consistent with estuarine beaches (**Table. 1**). Sorting did not present significant variations in space and time, with the sediments of all transects moderately well sorted, except for the estuarine/sheltered transect - T12 (**Figure. 2**) that presented a well sorted sediment distribution in the winter, with moderately well sorted sediments throughout the rest of the year. In general, sediment distributions showed that the estuarine/sheltered transect - T12 has a predominance of coarser sediments, while the marine/exposed transect - T05 shows a predominance of finer ones (**Figure. 2**). All the other transects showed a near-symmetrical sediment distribution (**Table. 1**). The textural distribution is in agreement with the sedimentary environment. The exposed marine margin is composed almost entirely of sand, due to the re-working induced by the waves, while the estuarine margin is characterized by the incorporation of coarser grains in the sediment. Usually surface sediments are coarser on estuarine beaches than on ocean or ocean-facing beaches within estuaries that have a similar source of sand and pebbles (Nordstrom 1977 in

Nordstrom 1992). The sediment fraction with a diameter higher than  $1\phi$  (0.5 mm) varied both in time and space. In general, it was always higher than 15 %, reaching almost 80 % of the total sample (T07 and T13 – summer; T06 - autumn) (**Figure. 2**) (**Table. 1**). The transect with higher calcium carbonate and organic matter contents was consistently T07 (transition transect) (**Figure. 2**) (**Table. 1**). This could be explained by the low hydrodynamic conditions which allow the deposition of shell fragments and also by the nearby presence of the *Zostera* spp. meadow. On the other hand, the lowest values of calcium carbonate were observed in the innermost/sheltered transects - T12 and T13, which is consistent with estuarine beaches (**Figure. 2**) (**Table. 1**). Also, the coarser fraction present in the sheltered transects is a result of the input of sediments brought by the river. These results reflect the difference in exposure to the local forcing factors along the study site. According to Wolf (1973 in Silva et al. 2006), fine sediments present a reduced interstitial space thus decreasing their permeability. Results of the compaction degree are in agreement with Wolf's statement since, in general, transects exposed to the wave action, with fine sands, were the ones that presented the higher compaction degree (T02, T05 and T06), while the innermost/sheltered transects (T12 and T13), with coarser sediments, presented the lowest compaction degree (**Figure. 2**) (**Table. 1**). The highest beach slopes were measured in transects located at the sea-estuary transition area - T06 and T10, while the innermost/sheltered transect - T13 showed the lowest beach slope (**Figure. 2**) (**Table. 1**).

**Table 1.** Morpho-sedimentary parameters for the study area (average values 2004/2005) (**MZ** - mean grain size (MS – medium sand / CS – coarse sand); **s1** – sorting (MWS – moderately well sorted / WS – well sorted); **SKI** – skewness (CSK – coarse skewed / FSK – fine skewed / NS – near symmetrical / VCSK – very coarse skewed); **Coarse** - sediment fraction with a diameter higher than 1 $\phi$  (0.5 mm); **CaCO<sub>3</sub>** - calcium carbonate contents; **OrgMat** - organic matter contents; **Compct** - compaction degree; **Slope** – beach slope).

Morpho-sediment parameters	Seasons	Exposed			Transition				Sheltered	
		T02	T05	T06	T07	T08	T09	T10	T12	T13
MZ ( $\phi$ )	Winter	MS	MS	MS	MS	MS	MS	MS	MS	CS
	Spring	MS	CS	MS	MS	CS	MS	MS	MS	CS
	Summer	MS	CS	MS	CS	CS	MS	MS	CS	CS
	Autumn	MS	CS	CS	MS	MS	MS	MS	MS	CS
s1 ( $\phi$ )	Winter	MWS	MWS	MWS	MWS	MWS	MWS	MWS	WS	MWS
	Spring	MWS	MWS	MWS	MWS	MWS	MWS	MWS	MWS	MWS
	Summer	MWS	MWS	MWS	MWS	MWS	MWS	MWS	MWS	MWS
	Autumn	MWS	MWS	MWS	MWS	MWS	MWS	MWS	MWS	MWS
SKI ( $\phi$ )	Winter	CSK	FSK	NS	NS	NS	NS	CSK	CSK	NS
	Spring	NS	FSK	NS	NS	FSK	NS	NS	VCSK	NS
	Summer	NS	FSK	VCSK	FSK	NS	NS	NS	CSK	NS
	Autumn	CSK	NS	NS	CSK	NS	NS	NS	VCSK	NS
Coarse (%)	Winter	24.76	52.44	30.33	37.72	42.66	37.00	23.01	17.99	58.25
	Spring	43.93	69.93	41.91	36.72	66.37	35.20	39.06	30.62	58.58
	Summer	41.35	71.05	17.52	76.49	58.99	40.77	42.02	48.54	78.84
	Autumn	34.14	58.99	79.36	35.21	33.22	38.69	41.02	35.98	69.30
CaCO <sub>3</sub> (%)	Winter	12.62	13.87	9.10	20.45	9.90	10.46	12.64	0.58	1.48
	Spring	17.75	11.38	19.21	33.82	11.03	10.58	13.58	1.11	1.14
	Summer	15.74	11.84	11.24	25.25	15.02	10.79	17.36	1.70	4.29
	Autumn	10.36	8.64	24.55	41.24	10.21	21.88	14.32	1.13	1.36
OrgMat (%)	Winter	0.44	0.54	0.28	0.62	0.35	0.39	0.38	0.16	0.24
	Spring	0.54	0.40	0.61	0.92	0.40	0.37	0.48	0.16	0.22
	Summer	0.53	0.44	0.66	0.83	0.64	0.46	0.46	0.43	0.22
	Autumn	0.49	0.41	0.76	0.94	0.41	0.61	0.43	0.24	0.22
Compct (°)	Winter	11.00	11.00	10.00	8.50	9.50	9.50	9.00	7.50	8.00
	Spring	9.50	10.50	10.50	9.50	9.00	10.00	9.50	7.50	9.50
	Summer	11.00	9.50	11.00	7.50	10.00	9.00	9.50	10.00	8.00
	Autumn	11.00	10.50	10.00	11.00	11.00	11.00	11.00	9.50	8.50
Slope (°)	Winter	5.00	5.00	8.00	5.00	5.00	5.50	7.50	4.50	4.00
	Spring	6.00	5.00	8.00	5.00	5.00	5.50	8.00	5.00	4.00
	Summer	6.00	5.00	8.00	5.00	5.00	6.00	8.00	5.50	4.50
	Autumn	5.50	5.00	7.50	5.00	5.50	7.00	7.50	4.50	4.00

**General characteristics of benthic macrofauna:** A total of 219 macroinvertebrate *taxa* (5612 individuals) were identified in the survey area during the years of 2004 and 2005 (for the 432 individual samples). In 2004 and 2005 a total of 157 *taxa* (2331 individuals) and 139 *taxa* (3281 individuals) were determined, respectively. Results

show that vertical distribution of the macrofauna is in conformity with the zonation pattern proposed by Dahl (1952): (1) the supralittoral zone (SL) of air breathers is typically inhabited by talitrid amphipods and oniscid isopods; (2) the midshore zone (ML) is typically inhabited by true intertidal *taxa*, such as cirrolanid isopods and spionid polychaetes; (3) the sublittoral zone (IL) presents a wide variety of *taxa*, such as bivalves, mysids, amphipods and polychaetes.

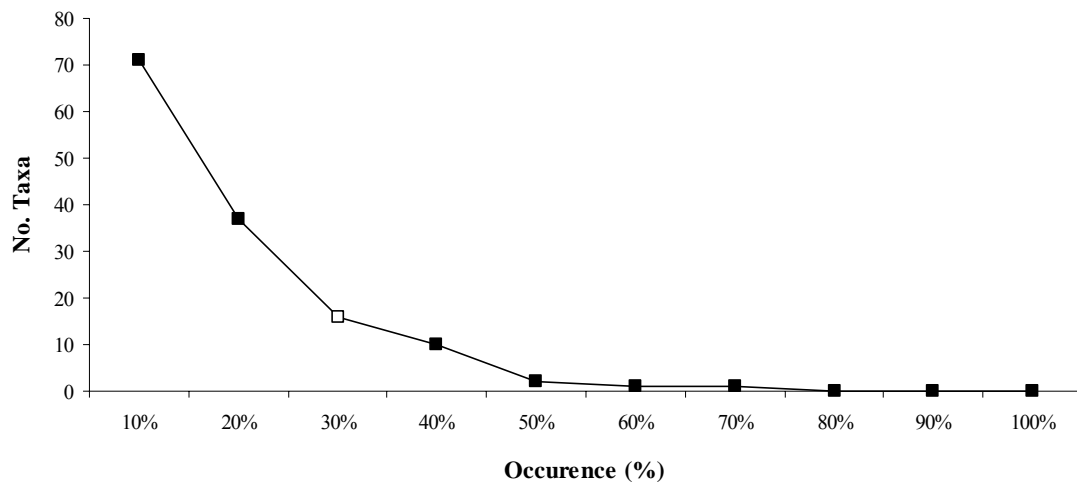
Results show a gradient of increasing number of individuals, species richness and diversity from SL to IL and from the marine margin (Exposed) to the estuarine margin (Sheltered), consistent with the results of Ferreira (2001) in the same study area, and Junoy & Viéitez (1990) in the Ría de Foz (Galicia - Spain) (**Table 2.**). According to Junoy & Viéitez (1990) and Edgar & Barret (2002) the tidal level is an important factor in the distribution of intertidal *taxa*. The idea that organic enriched sheltered areas contain higher number of individuals than exposed areas is also supported by our results, with such sheltered areas promoting the enrichment of macrofauna (Dexter 1988, Junoy & Viéitez 1990, McLachlan & Jaramillo 1995) since density, species richness and diversity tend to increase as exposure to wave action decreases (Dexter 1992) (**Table 2.**). In general, the highest numbers of individuals, species richness and diversity occur in the summer, while the lowest values occur in the autumn, in agreement, e. g. with the results of Dexter (1990) (**Table 2.**). On the other hand, irrelevant of level and season, transects T07, T12 and T13 showed the highest number of individuals, species richness and diversity, while T06 presented the lowest values (**Table 2.**). This can be justified by the location of the transects: (1) T12 and T13 are located in the estuarine margin of the peninsula, the less hydrodynamic and most

sheltered and stable region of the study area; (2) T07 is located in a sheltered bay in the vicinity of a *Zostera* spp. meadow that suggest an important homogeneity of the environmental conditions (Pérès & Picard 1964, Riggs & Fralick 1975 in Andrade 1986), and with a substantially more diverse fauna than unvegetated areas (Edgar et al. 1994, Edgar & Barrett 2002) ; (3) T06 is located in the most dynamic and unstable sector of the study area, in part because of the proximity to the main ebb tide channel of the estuary. In some seasons and levels, T05 presents the highest number of individuals and density, which can be justified by the occurrence of the subtidal species *Ervilia castanea* (Rodríguez Babio & Bonnin 1987, Morton 1990, Moreno 1998) on the intertidal area (**Figure. 2**) (**Table 2.**).

**Table 2.** The overall average values - 2004/2005, for total number of individuals, species richness, diversity and evenness on the study area (IL - lower intertidal; ML - mid intertidal; SL – upper intertidal).

Biological indices	Seasons	Exposed									Transition										Sheltered							
		T02			T05			T06			T07			T08			T09			T10			T12			T13		
		IL	ML	SL	IL	ML	SL	IL	ML	SL	IL	ML	SL	IL	ML	SL	IL	ML	SL	IL	ML	SL	IL	ML	SL	IL	ML	SL
No.Individuals	Winter	10	5	19	23	14	1	1	1	1	192	13	2	15	4	1	15	10	2	13	3	21	73	12	55	30	7	21
	Spring	28	9	14	19	3	3	6	2	1	157	13	1	21	8	3	13	6	2	18	13	19	89	5	80	15	34	63
	Summer	90	5	13	339	3	1	5	1	1	141	18	0	124	6	1	31	13	1	17	8	10	94	51	21	44	8	66
	Autumn	12	7	5	19	1	1	2	6	2	163	9	0	10	10	2	17	6	1	17	4	13	83	27	4	68	8	15
Species Richness	Winter	6	3	2	9	2	1	1	1	1	26	6	2	7	3	1	9	4	1	9	3	4	21	5	5	12	6	3
	Spring	9	3	2	7	3	2	3	2	1	22	6	1	10	4	2	8	2	2	14	3	4	24	3	4	8	5	4
	Summer	11	4	3	21	3	1	3	1	1	21	6	0	12	3	1	13	5	1	11	2	2	26	14	4	21	4	3
	Autumn	2	1	2	7	1	1	2	1	1	21	5	0	9	4	1	11	3	1	7	2	2	18	7	1	13	4	3
Diversity Shannon-Weaver (bit)	Winter	0.04	0.08	0.22	0.08	0.17	0.02	0.00	0.01	0.01	0.32	0.18	0.04	0.05	0.07	0.02	0.05	0.14	0.05	0.05	0.05	0.18	0.17	0.17	0.38	0.09	0.11	0.19
	Spring	0.08	0.12	0.13	0.06	0.05	0.05	0.03	0.03	0.01	0.29	0.15	0.02	0.07	0.12	0.05	0.05	0.10	0.04	0.06	0.14	0.22	0.19	0.08	0.41	0.05	0.26	0.29
	Summer	0.19	0.08	0.15	0.34	0.05	0.02	0.02	0.02	0.01	0.27	0.21	0.00	0.21	0.10	0.03	0.09	0.17	0.02	0.06	0.11	0.11	0.21	0.41	0.22	0.12	0.13	0.27
	Autumn	0.04	0.08	0.09	0.06	0.02	0.01	0.01	0.00	0.00	0.30	0.14	0.00	0.04	0.15	0.02	0.06	0.09	0.02	0.06	0.07	0.14	0.19	0.29	0.06	0.15	0.13	0.18
Evenness	Winter	0.01	0.02	0.04	0.01	0.03	0.00	0.00	0.00	0.00	0.06	0.03	0.01	0.01	0.01	0.00	0.01	0.03	0.01	0.01	0.01	0.03	0.03	0.03	0.07	0.02	0.02	0.04
	Spring	0.02	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.00	0.06	0.03	0.00	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.03	0.04	0.04	0.02	0.08	0.01	0.05	0.06
	Summer	0.04	0.02	0.03	0.07	0.01	0.00	0.00	0.00	0.00	0.05	0.04	0.00	0.04	0.02	0.01	0.02	0.03	0.00	0.01	0.02	0.02	0.04	0.08	0.04	0.02	0.02	0.05
	Autumn	0.01	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.06	0.03	0.00	0.01	0.03	0.00	0.01	0.02	0.00	0.01	0.01	0.03	0.04	0.06	0.01	0.03	0.02	0.03

**Environmental Vs biological variables:** As mentioned before, only the 16 *taxa* with an occurrence higher than 30 % were considered in the Canonical Correspondence Analysis (CCA) (**Figure.3**), with *Ervilia castanea* also being excluded from the analysis.



**Figure 3.** Number of *taxa* considered in the CCA (□)

The first two axes of the CCA explain 29.2 % (respectively, 22 % and 7.2 %) of the cumulative percentage variance of response variables (*taxa*) and 70 % (respectively, 52.8 % and 17.2 %) of the cumulative percentage variance of species-environment relation. Through this analysis we can state that the environmental variables selected explain almost the entire variability associated with the composition of the communities, since the correlation between *taxa* and environmental data is very high for the first two axes (respectively, 0.90 and 0.77) (**Table 3.**).

**Table 3.** Results of the Canonical Correspondence Analysis (CCA).

Axes	1	2	3	4
Eigenvalues	0.488	0.159	0.127	0.059
Species-environment correlations	0.904	0.774	0.715	0.53
Cumulative percentage variance of species data	22.0	29.2	34.9	37.5
Cumulative percentage variance of species environment relation	52.8	70.0	83.8	90.1

**Figure 4.** shows the diagram of the CCA performed to analyze the distribution of the 16 *taxa* retained, in relation to the environmental variables, showing the dominant patterns in the community structure that can be explained by the environmental variables. Through this analysis a strong correlation was evidenced between organic matter (OrgMat) and calcium carbonate contents (CaCO<sub>3</sub>), and also between compaction degree (Compct) and skewness (SKI), with the corresponding vectors pairs forming very small angles. The majority of the samples from the same transects were grouped, showing a clear spatial pattern. This pattern is so strong that it masks the seasonal effect, with a clear dominance of the spatial pattern over the seasonal pattern. The 1<sup>st</sup> axis is most strongly correlated with organic matter contents and beach slope, which show similar gradients. The second axis is mainly correlated with calcium carbonate contents, which also shows a strong correlation with the 1<sup>st</sup> axis.

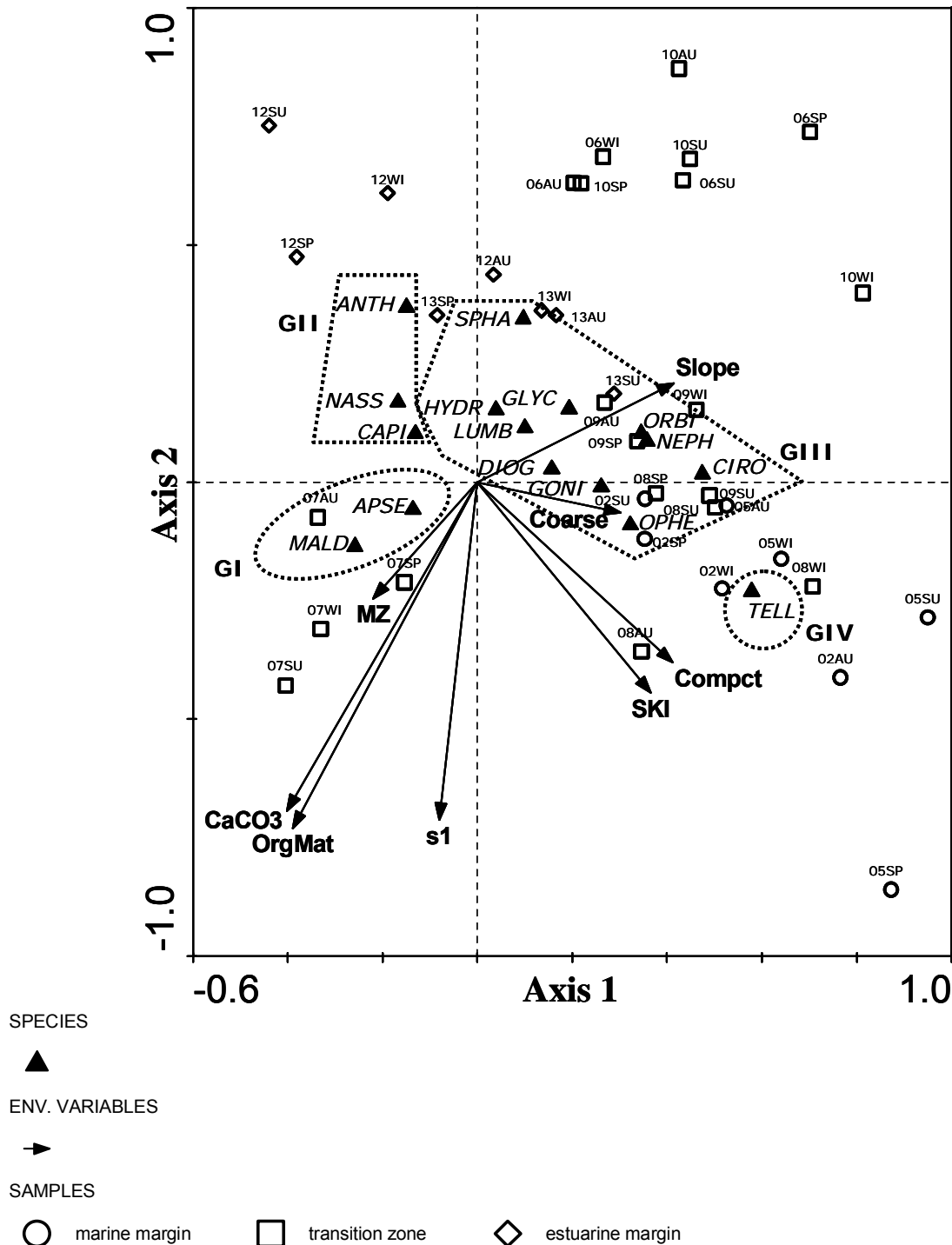
The distribution along the 1<sup>st</sup> axis shows a gradient relative to the exposure degree – from less exposed/sheltered (positive sector), to more exposed (negative sector). The environmental variable **beach slope**, gives an indication of the degree of exposure and the variable **organic matter contents**, gives an indication of sheltered environments. Species which were mainly observed in exposed/energetic environments, with low organic matter contents, were found along the positive sector (e.g. *Angulus tenuis*, *Eurydice naylori*, *Nephtys* sp., *Scoloplos armingeri* and *Ophelia laubieri*), whereas

species characteristic of sheltered environments, with high organic matter contents, were distributed along the negative sector (e.g. *Euclymene* sp.A, *Nassarius reticulatus*, *Cyathura carinata*, *Apseudes latreillei* and *Notomastus latericeus*). As stated by Raffaelli & Hawkins 1996, the most obvious change that occurs along the exposed-sheltered gradient is the gradual *taxa* addition, with the simultaneous, loss of other *taxa* not tolerant to less turbulent environments. Samples from exposed/energetic environments, with high beach slope and low organic matter contents, were distributed along the positive sector (e.g. T02, T05 and T06), whereas samples from sheltered environments, with low beach slope and high organic matter contents, were distributed along the negative sector (e.g. T07 and T12). The distribution along the 2<sup>nd</sup> axis shows a gradient relatively to the **calcium carbonate contents**, with the highest shell fragments contents in the negative sector (T02, T05 and T07), and the lowest shell fragments contents in the positive sector (T12 and T13). This shell fragments contents gradient can be justified by the presence of empty valves of *Ervilia castanea*, since, as previously related, this subtidal species can present a very high density occurrence in the intertidal zone of the study area. Ferreira (2001) refers that in the same study area, the highest densities of *Ervilia castanea* were found in T05 and T07. According to Morton (1990) and Ferreira (2001), the empty valves are found in such high numbers that sometimes, the beach is dyed in pink. CCA results show that the biological community is mainly aggregated in 4 different *taxa* groups: Group I – *Euclymene* sp.A and *Apseudes latreillei* (GI); Group II – *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata* (GII); Group III – *Sphaeroma serratum*, *Glycera* sp., *Hydrobia ulvae*, *Lumbrineris* sp., *Diogenes pugilator*, *Scoloplos arminger*, *Nephtys* sp., *Eurydice naylori*, *Goniada* sp.

and *Ophelia laubieri* (GIII); Group IV – *Angulus tenuis* (GIV), which appear to be indifferent to any seasonal association. Edgar & Barrett (2002), in their study of the Tasmanian estuaries, concluded that the variability associated with temporal fluctuations (monthly, seasonal and lower intra-annual scales) is generally less than the spatial variation, which is in agreement with the present results. The spatial heterogeneity of macrobenthos along the estuarine gradient can be described in relation to salinity and sediment composition (e.g. Ysebaert et al. 2003). Our results showed that variations on the morphology and sediment composition are very important to the structure of the biological communities, since sediment properties determine environmental conditions of the benthic habitat (Junoy & Viéitez 1990). *Euclymene* sp.A and *Aapseudes latreillei* (GI) showed a preference for fine sands, with high contents of calcium carbonate and organic matter and lower beach slopes, while *Sphaeroma serratum*, *Glycera* sp., *Hydrobia ulvae*, *Lumbrineris* sp., *Diogenes pugilator*, *Scoloplos arminger*, *Nephtys* sp., *Eurydice naylori*, *Goniada* sp. and *Ophelia laubieri* (GIII), seem to prefer coarser sands, with low calcium carbonate and organic matter contents and higher beach slopes. *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata* (GII) tended to be mostly related to less compact, coarser sediments, and *Angulus tenuis* (GIV) seems to prefer high beach slopes and more compact, fine sands. These results agree with the ones from Ferreira (2001), in that the lowest level in the intertidal area of Tróia is a very heterogeneous environment with 3 different but well defined communities (GI, GII and GIV). The 4<sup>th</sup> community is not well defined (GIII) (**Figure 5.**). On the marine margin of the peninsula, the most hydrodynamic area with medium and fine sands and low organic matter contents, a community numerically

dominated by *Angulus tenuis* (GIV) was found. On the transition zone, a very heterogeneous area with a high biological diversity, 2 communities were found: a first community (GI) in a stable area (transect 07) associated with a *Zostera* spp. meadow and high carbonate contents, numerically dominated by *Euclymene* sp.A and *Apseudes latreillei*; a second community (GIII), numerically dominated by *Glycera* sp. and *Scoloplos arminger*, that also includes the true intertidal specie *Eurydice naylori* found predominantly in semi-exposed or sheltered sand beaches (Jones & Pierpoint 1997). The polychaete *Scoloplos arminger* is characteristic of sandy sediments in polyhaline zones (Ysebaert & Herman 2002, Ysebaert et al. 2003). On the estuarine margin, mostly sheltered, with coarser sediments, moderate to high organic matter contents and low calcium carbonate contents, a community numerically dominated by *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata* (GII) was found. The polychaete *Notomastus latericeus* is an opportunistic *taxon* common on soft bottoms and tolerant to excess organic matter (Pearson & Rosenberg 1978, Hily et al. 1986, Ibanez & Dauvin 1988, Grall & Glémarec 1997, Borja et al. 2000), while the isopod *Cyathura carinata* who is also tolerant to organic enrichment shows preferences for mesohaline zones (Pearson & Rosenberg 1978, Hily et al. 1986, Grall & Glémarec 1997, Borja et al. 2000, Ysebaert & Herman 2002, Ysebaert et al. 2003). Ferreira (2001) defined 3 main communities for the same study area: (1) Marine margin – *Angulus tenuis* and *Nephtys* sp. community; (2) Transition zone – *Euclymene* sp.A community associated with transect T07; (3) Estuarine margin – *Spiochaetopterus costarum* community. Comparing the results of the present study with the study developed by Ferreira (2001), we can see that the communities present along the marine

margin and in the transition zone (associated with transect T07) seem to have remained constant. However, the estuarine margin community seems to have suffered changes, being presently dominated by *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata*, instead of the *Spiochaetopterus costarum*, *Notomastus latericeus*, *Pista cristata* and *Euclymene* sp.A.



**Figure 4.** CCA ordination diagram for the analysis of the 16 retained *taxa* and environmental variables (**HYDR** – *Hydrobia ulvae*; **NASS** – *Nassarius reticulatus*; **TELL** – *Angulus tenuis*; **CAPI** – *Notomastus latericeus*; **GLYC** – *Glycera* sp.; **GONI** – *Goniada* sp.; **LUMB** – *Lumbrineris* sp.; **MALD** – *Euclymene* sp.A.; **NEPH** – *Nephtys* sp.; **OPHE** – *Ophelia laubieri*; **ORBI** – *Scoloplos arminger*; **APSE** – *Aapseudes latreillei*; **ANTH** – *Cyathura carinata*; **CIRO** – *Eurydice naylori*; **SPHA** – *Sphaeroma serratum*; **DIOG** – *Diogenes pugilator*) (**MZ** - mean grain size; **s1** – sorting; **SKI** – skewness; **Coarse** - sediment fraction with a diameter higher than 1φ (0.5 mm); **CaCO<sub>3</sub>** - calcium carbonate contents; **OrgMat** - organic matter contents; **Compct** - compaction degree; **Slope** - beach slope).



**Figure 5.** Taxa groups distribution along the peninsula (**GI** - *Euclymene* sp.A and *Aapseudes latreillei*; **GII** - *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata*; **GIII** - *Sphaeroma serratum*, *Glycera* sp., *Hydrobia ulvae*, *Lumbrineris* sp., *Diogenes pugilator*, *Scoloplos arminger*, *Nephtys* sp., *Eurydice naylori*, *Goniada* sp. and *Ophelia laubieri*; **GIV** - *Angulus tenuis*).

## CONCLUSIONS

The structure of the sand beach intertidal communities studied showed a clear dominance of the spatial patterns over the seasonal ones. The CCA showed the dominant patterns in the community structure to be explained by the environmental variables. Significant correlations between abundance, grain-size and slope, but not with wave action were found by McLachlan et al. (1981 in Jones & Pierpoint 1997), suggesting that the fauna is not limited as much by wave action directly but rather by steep slopes and coarse sands. However, studies on the distribution and abundance of sand beach fauna, have shown that exposure to wave action exert the greatest influence either indirectly or directly (Jones & Pierpoint 1997). Junoy & Viéitez (1990) stated that sediment characteristics and tidal height are the most important factors in the distribution and abundance of the intertidal communities. However, in the present study, the environmental variables with the highest contributions to the multivariate analysis were beach slope, organic matter and calcium carbonate contents, that appear to have an important role in defining the structure of macrobenthic communities, since *taxa* appear to have preferences. As other authors have shown (e.g. Warwick & Uncles 1980, Brown & McLachlan 1990, Picard 1965 in Costa et al. 1990, McLachlan 1983 in Dexter 1990, Junoy & Viéitez 1990, Warwick et al. 1991, McLachlan 1990 in McArdle & McLachlan 1992, Ysebaert & Herman 2002, Ysebaert et al. 2003, Silva et al. 2006), wave energy, beach slope and sediment grain size have a high influence in the conditions of survival, and therefore, they influence the distribution of the macrobenthic organisms dwelling in the sediments (Raffaelli & Hawkins 1996). Compaction degree and beach slope results

suggest that the marine margin of the peninsula behaves as a reflective beach while the estuarine margin behaves as a dissipative beach, in agreement with the results obtain by Ferreira (2001).

In the present study, 4 communities were defined – (1) a community dominated by *Angulus tenuis*, on the marine margin of the peninsula; (2) a community dominated by *Euclymene* sp.A and *Apseudes latreillei*, on the sea-estuary transition area and related to the presence of a *Zostera* spp. meadow; (3) a community dominated by *Glycera* sp. and *Scoloplos armingi*, on the sea-estuary transition area; (4) a community dominated by *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata*, on the estuarine margin.

The distribution and structure of macrobenthic communities was also assessed, in its relation with the environmental factors, which can help to improve the environmental monitoring and management of the Tróia peninsula beaches.

Nevertheless, the need for longer time scale studies (with longer data series), should be emphasised, as well as the inclusion of other factors influencing the distribution and structure of the macrobenthic communities.

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# CHAPTER 4

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## CONCLUSIONS

The present study contributes to the detailed knowledge of the intertidal macrobenthic communities of the Tróia peninsula. The results of the determination of the minimum sample size (number of replicates) study, demonstrate that in general the different approaches used, suggest minimum sample sizes between 5 (0.0825 m<sup>2</sup>) and 12 (0.198 m<sup>2</sup>) replicates, with this value still below a minimum sampling size in most of the cases, which is unfeasible in a monitoring program, since there are limitations in terms of time and available resources. Due to some limitations (bibliography scarcity referring to this subject and also restrictions inherent to the methods used) and in order to improve the ongoing monitoring program for the environmental management of the Tróia peninsula, we decided to increase the number of sampling units from 2 to 3 replicates, as a compromise to increase the amount of information gathered with the available resources. However, these results are applicable only for the study area and therefore, further studies will be needed for different locations and environmental settings. The better option to detect changes in the composition of the macrobenthic communities is to increase the sampling effort above the levels traditionally attempted. However, this can only be achieved through a greater investment in personnel or via mechanical innovation, which would require greater levels of funding for beach research (Schoeman et al. 2003). Therefore, the determination of the minimum sample size study should be the first approach to any biological monitoring, since the loss of coastal habitats creates the need for comparative analyses and reliable predictions of species richness and biodiversity in order to improve environmental management.

The results of the determination of the patterns (distribution and structure) of the benthic communities in relation to the environmental parameters, demonstrate the dominant patterns in the community structure to be explained by the environmental variables. A clear dominance of the spatial patterns over the seasonal ones was evidenced. The environmental variables with the highest contributions to the multivariate analysis were beach slope, organic matter and calcium carbonate contents, which appear to have an important role in defining the structure of macrobenthic communities, since *taxa* appear to have preferences. Through this study, 4 communities were defined – (1) a community dominated by *Angulus tenuis*, on the marine margin of the peninsula; (2) a community dominated by *Euclymene* sp.A and *Apseudes latreillei*, on the sea-estuary transition area and related to the presence of a *Zostera* spp. meadow; (3) a community dominated by *Glycera* sp. and *Scoloplos arminger*, on the sea-estuary transition area; (4) a community dominated by *Notomastus latericeus*, *Nassarius reticulatus* and *Cyathura carinata*, on the estuarine margin.

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