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SHOREBIRDS AS INDICATORS OF ECOLOGICAL STATUS AND SEDIMENT QUALITY IN THE RIA FORMOSA LAGOON

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IN WATER AND COASTAL MANAGEMENT

SERGEI DANCHENKO

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NOME / NAME: Sergei Danchenko

DEPARTAMENTO / DEPARTMENT: Faculdade de Ciências e Tecnologia

ORIENTADOR / SUPERVISOR:

1) Prof. Dr Alice Newton, Department of Chemistry and Biochemistry, Faculty of Science and Technology, University of Algarve, Portugal.
2) Prof. Dr Jaime Albino Ramos, IMAR – Instituto do Mar da Universidade de Coimbra.

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JURI:

Presidente: Doutor Tomasz Boski, Professor Catedrático da Faculdade de Ciências e Technologia da Universidade do Algarve.

Vogais: Doutor Stephen Michael Mudge, Senior Lecturer, Bangor School of Ocean Science, University of Wales, United Kingdom;

Doutor John David Icely, Investigador Principal do CIMA – Centro de Investigação Marinha e Ambiental da Universidade do Algarve;

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RESUMO

A lagoa da Ria Formosa é um local de importância ambiental internacional, incluido na rede Natura 2000, na Convenção Ramsar e na Directiva – Quadro da Água (DQA). Esta tese é centrada no estudo das comunidades de aves costeiras que se alimentam nos terrenos de interridais em relação com as propriedades dos sedimentos e às comunidades de meiobentos e macrobentos. O lugares do estudo seriam presumivelmente diferentes em estatuto ecológico, ambos localizados perto dos locais de intercalibração da DQA com estatuto ecológico definido. Os lugares não diferem significativamente na granulametria dos sedimentos, mas diferem no seu conteúdo orgânico total e no redox potencial dos sedimentos. As comunidades de aves consistem nas mesmas espécies mas são diferentes na densidade relativa entre locais. No foi encontrada relação entre a densidade das comunidades de aves e a bentos. As outras características do habitat, canais de maré, a mistura dos sedimentos operada pela pesca de bivalves e a distância aos locais de descanso, e a presença de locais complementares de alimentação influenciam as comunidades de aves.

Palavras-chaves: Aves, limicolas, macrobenthos, meiobenthos, estado ecologico, Ria Formosa.

ABSTRACT

The Ria Formosa lagoon is a site of international environmental importance, included into Natura 2000 Network, Ramsar Convention and European Water Framework Directive (WFD). This thesis focuses on the study of shorebird assemblages feeding on intertidal flats in relation with physical properties of the sediments, meiobenthos and macrobenthos communities. The study sites were presumably different in ecological status, both located close to the WFD intercalibration sites with defined ecological status. The study sites did not differ significantly by sediment granulometry, but differed in total organic content and redox potential of the sediment. Bird communities consisted of the same species but differed in relative densities between sites. No relation was found between bird densities and their benthic prey. The other habitat characteristics, tidal channels, disturbance of sediment by shellfishery and distance to roost sites, presence of complementary feeding sites influenced bird communities.

Keywords: Shorebirds, waders, macrobenthos, meiobenthos, ecological status, Ria Formosa.

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LIST OF ABBREVIATIONS

BQE – Biological Quality Element

EQS – Ecological Quality Status

EU – European Union

IndVal – Indicator Values analysis

MI – Maturity index

GPS – Global Positioning System

PCA – Principal Component Analysis

SD – Standard Deviation

SE – Standard Error

TOC – Total Organic Content

WFD – Water Framework Directive

WWTP – Waste Water Treatment Plant

1. INTRODUCTION

1.1. Wetlands and shorebirds conservation

Wetlands and coasts are of essential importance for support of population of many bird species and also biological diversity of other taxa. For this reason, Ramsar Convention on Wetlands (1971) was one of the first international environmental agreements to be developed and one of its main focuses is protection and conservation of bird populations. In the European Union the Directive 79/409/EEC on the protection of wild birds (Birds Directive), Water Framework Directive (2000/60/EC), Directive on the Conservation of Natural Habitats (92/43/EEC) form a legislation framework arranged to organize the nature conservation measures at all levels, especially on the level of decision-making. Management of many coastal territories declared a Ramsar site and Natura 2000 sites requires knowledge about bird communities and their response to various human activities. The knowledge of bird communities' response to human impact should be a basis for implementing the EU Directive on Birds and Habitat Directive at the level of decision making. As per Council Directive 92/43/EEC (Habitats Directive), 1992, "*conservation status of a natural habitat means the sum of the influences acting on a natural habitat and its typical species that may affect its long-term natural distribution, structure and functions as well as the long-term survival of its typical species*".

Wetlands and coasts are among the ecosystems under the highest anthropogenic pressure due to their economical goods and services. The impact comes in forms of physical alteration (coastal engineering, sediment dredging, and construction), pollution by urban sewage, agricultural runoff and industrial waste, overexploitation of the biological resources (fish, shellfish), changes at the watersheds which impact hydrological regime (Barnes, 1980).

Shorebirds are one of the most threatened animal groups, dependant heavily on estuarine areas and showing clear decline during last decades, mainly because of habitat loss and side-effects of human activity impact (Stroud et al., 2006; Wader Study Group, 2003). Council

Directive 79/409/EEC (“Bird Directive”) states: ”*A large number of species of wild birds naturally occurring in the European territory of the Member States are declining in number, very rapidly in some cases; whereas this decline represents a serious threat to the conservation of the natural environment, particularly because of the biological balances threatened thereby…*” The future of this group depends on availability of suitable quality habitat to support sustainable bird populations. Knowledge about the importance of physical habitat characteristics to the site selection of birds is a prerequisite for understanding their habitat ecology and distribution, as well as managing their habitats (Boere et al., 2006; Granadeiro et al., 2007; Lourenço et al., 2005). Shorebirds may represent itself flagship species for conservation of the wetlands and coastal areas, as they can relatively easy attract attention of the broad public (BirdLife Int., 2004) to problems of conservation.

1.2. Birds as environmental indicators

Indicator species in general are considered to be able to provide a cost- and time-efficient mean to assess the impacts of environmental disturbances on an ecosystem (Carignan & Villard, 2002). Birds are considered suitable group to be used as ecological indicators (Koskimies, 1989) in monitoring of ecological integrity and ecosystem change.

Some characteristics of birds as ecological indicators are reported as follows:

- Birds are valid indicators of biodiversity, species richness and endemism patterns (Gregory et al., 2003);
- Birds have wide geographical distribution in almost all kinds of habitats worldwide;
- Birds respond to environmental change over large and small spatial scales (Temple & Wiens, 1989);
- Bird populations’ species composition, abundance and reproductive success are influenced by the habitats characteristics (Mazerolle and Villard (1999) and references cited therein);

- Birds are on top of the trophic chain in ecosystems and so can provide integrated information on the ecosystem status (Temple & Wiens, 1989) and are indicative of more general situation among other taxa;
- Changes in bird populations tend to integrate a set of ecological factors, including biotical and physical (Gregory et al., 2009; BirdLife Int., 2004);
- Birds are mobile and responsive to environmental changes (BirdLife Int., 2004);
- Birds can be effectively censused on different spatial scales (Carignan & Villard, 2002);
- Populations can be relatively easily assessed, detected and identified by physical appearance, vocalization and traits, sometimes without requirement of high expertise of observer (Hutto, 1998);
- Scientific knowledge available on birds' biology and ecology make them one of the best studied groups of indicator organisms (BirdLife Int., 2004);
- Many bird species are specialized in their requirements and have narrow distributions or ecological niches, potentially being sensitive indicator of local ecosystem change (Cramp and Simmons, 1983;);
- Some bird species are of economical importance, linking natural and socio-economic components (BirdLife Int., 2004), or can serve as "flagship" species to rise public attention to the conservation problems.

Ecological indicators based on birds were used in study and monitoring of various ecosystems. Birds were used as indicators to assess forest ecological integrity (Canterbury et al., 2005; Florenzano, 2004) using population trends of multiple species. Bird assemblages are reported to respond to landscape patch characteristics in forest and agricultural landscapes (Mazerolli & Villard, 1999). Songbird assemblages were used for assessment of the conditions of forested watersheds (Bryce et al., 2005). Some groups of birds (woodpeckers) were shown to be reliably indicative of avian diversity of forests (Drever et al., 2008). Aquatic birds have been used as indicators of trophic change of the estuarine food-webs, and were found to respond to nutrient impact and density of plankton and fish, as well as habitat alteration (Fernandez et al., 2005). Seabirds were suggested to be used as indicators of the status of the marine environment, and as indicators of the food supply of vertebrate predators (Parsons et al., 2008).

Bird population trends of 124 European species were shown to respond to recent climatic change in the way of divergent trends (populations respond positively or negatively) and biological indicator of climatic change impacts was developed (Gregory et al., 2009). Geographical range of species and population size were parameters used as indicators of climatic change. In some countries, indicators based on birds were included in the national monitoring schemes, e.g. UK wild bird indicator (Gregory et al., 2005). Parameters used for assessment of bird indicators were species occurrence, abundance (Gregory et al., 2003), population size, population trends, geographical range (Gregory et al., 2009), reproductive success, nest abundance, predation (Mazerolle & Villard, 1999).

There were identified constraints that arise form the use of indicator species as integrative measure for ecosystem status (Hutto, 1998): 1) There is not possible to monitor all species, and choice of several indicator species requires excessive time and effort; 2) The narrow group of chosen species usually does not represent the conditions required by all others; 3) The costs required to monitor rare species which are usually used as indicators are high, and 4) The research attention given to the indicator species group is diverted from other species, environmental requirements of which could be different. Therefore, it was suggested to use wide species groups, including birds as indicators. The principal problem regarding using birds' population change data as bio-indicators is that the exact cause of detected change is usually unclear, and is a function of many complicating factors, including natural population cycles. There is also a difficulty in associating the observed effect with acting factors, as well as which direction of population change corresponds to adverse change in the environment (Temple & Wiens, 1989).

Shorebirds' ecological peculiarities suggest this group as suitable ecological indicator. According to International Wader Study Group (2003), "*Knowledge of the status of wader populations can provide important information on the wider environment, including those caused by climate change as well as from habitat loss, and degradation of habitat quality as well as those caused by climate change*."

Shorebirds are closely connected to their habitat and linked to environmental changes, abundant and can be relatively easily counted. As a consequence of long-distance migrations, they can be used as indicators at the breeding grounds, on the stopover sites where they tend to concentrate with high density and in the wintering grounds (Flint, 1998). Shorebirds and wading birds represent high trophic level predators in the intertidal ecosystem (Reize, 1985) and therefore can provide integrative measure of condition of the entire ecosystem. Most shorebirds species migrate on long distances between breeding and wintering grounds (Cramp and Simmons, 1983) and therefore depend on habitats located along the flight paths in different geographical zones. Thus they are considered (Piersma & Lindstrom, 2004) to provide information about environmental change occurring over a large spatial scale. The extremely long flight distances of several species (Red Knot, Bar-tailed Godwit, Ruff, Ruddy Turnstone, Greater Knot and others) impose energy requirements thus making these species highly dependant on quality of feeding habitats, including at stopover sites (Evans, 1976; Hedenstrom, 2010; Piersma & Jukema, 1990).

1.3. Shorebirds and habitat characteristics

Shorebirds are closely related to the environmental characteristics of their habitat. Many shorebird species depend on coastal estuarine areas in different seasons, including winter, summer breeding season and resting periods during migration (Granadeiro et al., 2004).

The distribution of shorebirds in the intertidal areas depends to a large extent on the patterns of occurrence of their invertebrate prey (Piersma et al., 1993; Yates et al., 1993). This relationship exists in different spatial scales (Goss-Custard & Yates, 1992; Moreira, 1993; Yates et al., 1993).

Physical sediment parameters of the feeding areas directly influence the distribution and occurrence of birds´ invertebrate prey, and mediate the density of shorebirds (Goss-Custard & Yates, 1992; Yates et al., 1993). For example, the densities of birds are known to respond strongly to the particle size of surface sediments. This is both due to their preferences in terms of sediment penetrability and wetness (often related to their bill morphology) but also to the indirect effect that these parameters exert in the abundance of their invertebrate prey (Myers et al., 1980; Sutherland, 1982; Hicklin and Smith, 1984; Goss-Custard and Yates, 1992).

There were found responses of shorebirds to habitat properties at fine scale (Mouritsen & Jensen, 1992; Santos et al, 2009). The prey density and availability were the main factor of bird distribution, but physical sediment characteristics, such as penetrability and water content, also were important because they can influence detectability and capture rate of the prey (Mouritsen & Jensen 1992). Wader distribution at fine scale has been reported (Mouritsen & Jensen 1992) to strongly respond to microhabitat features. One of these aspects is association of waders with intertidal channels and epistructures that cross mud flats. Waders were reported to occur in higher density and apply more foraging effort in close proximity of the drainage channels (Granadeiro et al., 2005), suggesting the higher density or accessibility of their prey in close range to the channels.

Feeding habitat use of shorebirds is not restricted to intertidal areas and shores, very often birds use supratidal habitats such as salt pans. The three hypotheses for such a behavior are species specific site preference, complementary food supply and avoiding disturbance on the main foraging grounds (Masero et al., 2000).

The energetic requirements of feeding make shorebirds restrict their choice to invertebrate prey that is not only available, but also profitable, e.g. is of sufficient size (and energy content) to provide enough energy for the bird to compensate for foraging effort (Zwarts & Wanink, 1993).

This leads to the choice of prey of particular size, and therefore restricts the birds to fraction of the total available benthic biomass (Zwarts & Wanink, 1993), reducing total available food resource.

Table 1.1. Known food items of common shorebird species. Majority of benthos species identified at study sites (Appendix B) are known to be taken by observed bird species.

Bird species		**Food items**	**References**
Turnstone	*Arenaria interpres*	*Insecta, Mollusca (Hydrobia ulvae, Cerastoderma, Scrobicularia), Amphipoda, Decapoda*	Cramp, Simmons (1983)
Sanderling	*Calidris alba*	*Insecta, Crustacea (Gammaridae,), Mollusca (Dreissena, Hydrobia, Mytilus), Polychaeta, Cnidaria, fish.*	
Dunlin	*Calidris alpina*	*Polychaeta (Nereis diversicolor), Mollusca (Hydrobia ulvae, Cerastoderma, Scrobicularia), Amphipoda, Decapoda (Carcinus maenas), Diptera*	Luis et al (2002) Cramp, Simmons (1983)
Kentish Plover	*Charadrius alexandrinus*	*Polychaeta (Nereidae), Crustacea (incl. Carcinus maenas), Mollusks (Hydrobia, Littorina, Cardium), Amphipoda, Insecta*	Cramp, Simmons (1983)
Ringed Plover	*Charadrius hiaticula*	*Polychaeta (incl. Nereis, Notomastus, Scolopsos, Arenicola, Phyllodoce)* *Amphipoda (incl. Corophium, Batyporeia), Isopoda, Mollusca (Hydrobia, Littorina, Macoma, etc.)* *Oligochaeta, Insecta*	
Bar-tailed Godwit	*Limosa lapponica*	*Mollusca (Littorina, Macoma, Hidrobia,)* *Crustacea (Balanus, Gammarus, Jaera, Corofhium, Carcinus, Crangon, Talitrus)* *Polychaeta (Arenicola, Nereis, Lumbricus, Heteromastus)*	
Whimbrel	*Numenius phaeopus*	*Decapoda, Mollusca (Litorina, Nucella), Polychaeta (Arenicola)*	Cramp, Simmons (1983)
Grey Plover	*Pluvialis squatarola*	*Polychaeta (incl. Notomastus, Arenicola, Phyllodoce, Nereis), Mollusca (Gastropoda, Hydrobia, Macoma, Cerastoderma edule, Scrobicularia plana, etc.), Crustacea (incl. Amphipoda)*	Cramp, Simmons (1983) Lopes et al. (1998) Moreira (1996)
Greenshank	*Tringa nebularia*	*Insecta, Polychaeta, Mollusca, Crustacea, fish*	Cramp, Simmons (1983) Moreira (1996)
Redshank	*Tringa totanus*	*Insecta, Polychaeta, Mollusca, Crustacea*	

Invertebrate prey activity can influence foraging behavior of waders (especially visual foragers) on the intertidal flats (Evans & Dugan, 1984). Sediment drainage has effect on the activity of invertebrate prey and therefore influences the foraging behavior and intertidal flat use by waders (Rosa et al, 2007). Many species were reported to follow the tidal water line during feeding (Granadeiro et al, 2006).

Food items of the most common shorebirds in the study area obtained from the literature are presented in Table 1.1.

1.4. Ria Formosa lagoon

The Ria Formosa lagoon is situated in the South-West of Iberian Peninsula in the Algarve region of Portugal. It is located between the coordinates of 37° 02’ N latitude and 36° 57’ and 7° 31’ and 7° 52’ W longitude (Figure 1.1). The Ria Formosa is a shallow, mesotidal barrier lagoon system. This is one of the largest coastal lagoons of this type in Europe; it has the length of 55 km and 6 km maximum width (Mudge and Bebianno, 1997). The area of the lagoon is approximately 100 km^2.

Figure 1.1. Geographical position and satellite image of the Ria Formosa lagoon (map base: Instituto Geografico Portugues).

The lagoon has 14,522 ha of wetlands, including 11,800 ha of intertidal area (Mudge *et* al., 1997). The average depth is 3.5 m overall and 2 m in the shallow Eastern part. The watershed covers an area of approximately 864 km^2 (Duarte et al, 2008).

This coastal lagoon is separated from the Atlantic Ocean by several barrier islands (Barreta, Culatra, Armona, Tavira and Cabanas) and peninsulas (Ancão and Cacela). Water is exchanged by the tides with the oceanic waters through six inlets, four natural (Armona, Fuzeta, Cacela and Tavira) and two artificial (Faro-Olhão, INDIA) (Cristina, 2006; Garcia, 2002). The artificial INDIA inlet was artificially constructed in 1997 during the Inlet Dynamics Initiative project for the Algarve (INDIA), partly with the aim of improving the water quality in the Ancao Basin.

The lagoon is mesotidal, with tidal range from 1 to 3.5 m. Tides are semi-diurnal, water residence times normally vary from 6 hours to 2 days, but can be as high as 4 – 6 days in zones of restricted exchange (Mudge *et* al., 2008, Loureiro, *et al*, 2006). During each tide 50 – 75% of water mass exchanges with ocean. Intertidal area consists of salt marshes, intertidal flats, sand spits. The sediments type are mostly sandy on the barrier islands, and sandy-muddy or muddy in the intertidal area (Figure 1.2).

The Ria Formosa coastal lagoon is classified as coastal water according to the Water Framework Directive (European commission, 2000). There are no significant freshwater inputs sources and the salinity gradient in the lagoon is insufficient to be classified as transitional water (Newton & Icely, 2002). Freshwater inflow system consists of one river and 14 temporal streams. The one small permanent river Rio Gilão flows into Eastern part of the lagoon. The other fourteen small, torrential streams flow into the lagoon only after heavy rainfall, mainly in the Western part. Another permanent stream, Ribeira de São Lourenço, used to flow into the West of the lagoon (Ancão basin), but was completely dammed in the 1800s with a dyke, and now is an irregular source of freshwater inflow. The salinity of the water ranges 35.5 – 37.8 ‰, depending on the tidal stage and season (Mudge *et* al, 2007).

The Algarve region is characterized by a Mediterranean climate, with hot dry summers and warm wet winters. Based on annual and monthly data there seems to be an increase in irregularity in annual precipitation in the basin, the average annual precipitation values are between 600 and 800 mm. The month with most precipitation is December with about 17% of total annual, followed by November and January with about 15% (Duarte et al., 2008).

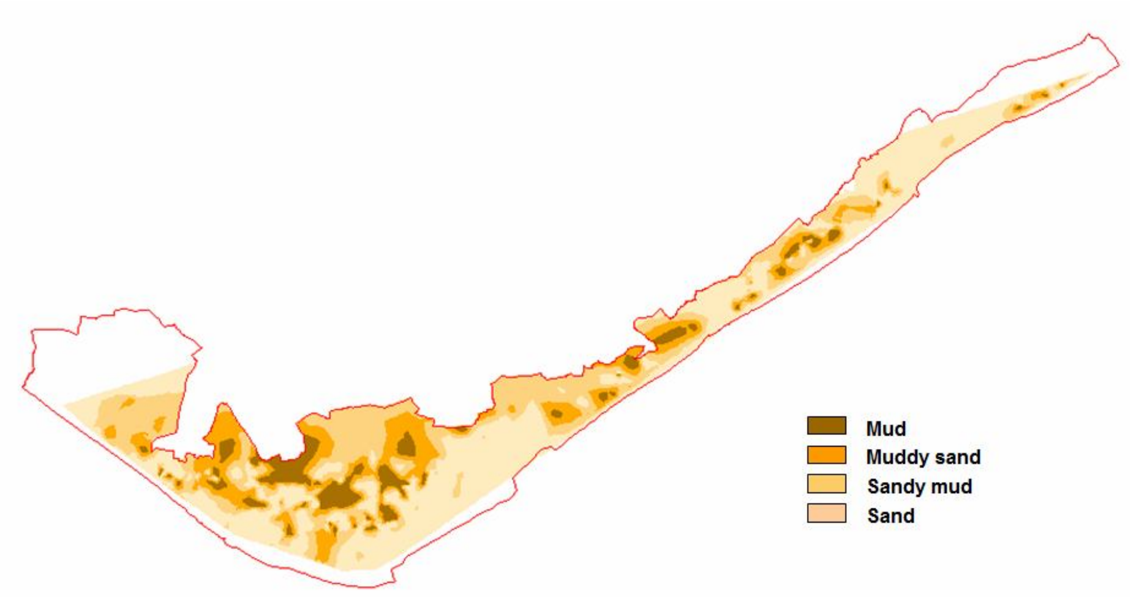


Figure 1.2. Sediment grain size composition in the Ria Formosa lagoon (Environmental Systems Analysis group, FCT/UNL)

1.5. Ecological importance and main pressures

The Ria Formosa has been designated as a Natural Park in 1987. Internationally, it forms part of the Natura 2000 European network for nature conservation, it is a Ramsar wetland and it is the Special Bird Protection Area (European Directive 79/409/EEC). The Ria Formosa Natural Park has 78,000 ha in area, with 10,000 ha of lagoon, 5,000 of salt marsh and aquaculture ponds (Caetano et al., 2002).

This lagoon is exposed to different types of anthropogenic impact of various degrees. The main pressures affecting the ecological conditions are urbanization, sewage discharge, coastal alterations, tourist activities, aquaculture and shellfishery (Gamito, 2008; Serpa *et* al, 2005; Cristina et al., 2006).

Major contaminants include untreated sewage and discharges from industrial units, agriculture, ports, marinas, aquaculture, and the associated road network. The discharge of sewage is one of the major anthropogenic impacts affecting the lagoon. During the summer months tourism increases the population from 150,000 to 450,000 people. Most sewage only receives primary screening, especially during summer months when capacity at the treatment plants is exceeded. Consequently, there are numerous sources of sewage-derived organic matter identified in the western part of the lagoon (Mudge and Bebianno, 1997). Despite these pressures, because of the high water exchange and the absence of industry the Ria Formosa can be classified as showing no or only very minor human impact (Newton & Icely, 2002).

1.6. Ecological Status according to the Water Framework Directive

The Water Framework Directive (2000/60/EC) is a common framework of measures undertaken by the EU member states, and aims at achieving a good ecological qualitative and quantitative status by 2015. Under the Common Implementation Strategy of the WFD, the mesotidal Ria Formosa lagoon has been classed as a very sheltered, shallow, coastal water due to absence of significant freshwater inflow (Newton et al., 2003).

Table 1.2. Ecological status of Ponte and Ramalhete intercalibration sites in terms of Water Framework Directive, based on different Biological Quality Elements (BQE) and Environmental Quality Ratios (EQR)

BQE	EQR	Ecological status	EQR	Ecological status	Source
	Ponte		Ramalhete		
General	0.8	High	0.6	Good	Commision Decision 2005/646/EC
Physico-chemical and Phytoplankton	0.8	High	0.6	Good	Loureiro et al., (2006)
Phytoplankton	0.7	Good	0.7	Good	Newton *et al.* (2007)
Benthic invertebrate	0.66	Good	0.73	Good	Newton *et al.* (2007)
Macroalgae	-	Moderate/ Poor	-	High	Parvatkar (2008)
Seagrass	0.90	High	0.97	High	Marin (2008)

On the basis of historical and current research data, two sites in the Ria Formosa lagoon have been identified as intercalibration sites for WFD (Table 1.2): Ponte de Praia de Faro (High/Good boundary conditions, Code C3978, Type NEA26) and Ramalhete (Good/Moderate boundary status, Code 3979, Type NEA26).

1.7. Benthic fauna community

Austin et al. (1989) examined both macrofauna and meiofauna communities along a pollutant gradient in Ramalhete channel and the channel leading to the main inlet of the lagoon. Indicators of community structure were determined for two size benthos groups and compared between each other. The macrofauna and meiofauna are distinct ecologic components of the benthos (Warwick, 1984) and so may respond to pollution in a different way. In the study by Austin et al. (1989) it was suggested that not sewage but shellfisher's digging affected the macrofauna community by physical disturbance. Meiofauna and macrofauna communities did not respond to the organic enrichment in the same way and ecological factors affecting these benthos groups were concluded to be different.

The existence of sewage discharge sources in the Ancão basin in the extreme western part of the lagoon affects the meiofaunal communities. The research of meiofauna by Hewitt and Mudge (2004) showed that there were 2 regions in the basin where meiobenthos community structure responded to organic enrichment of sewage origin (Figure 1.3). One was located in the NW part close to Quinta do Lago complex (points 39, 50, 57) and another near the Esteiro do Maria Nova stream, contaminated with sewage (points 41, 42). Points 40 and 26 located at the study site Ancão were charachterized by signature of a community influenced by organic matter of a clean community (Figure 1.3).

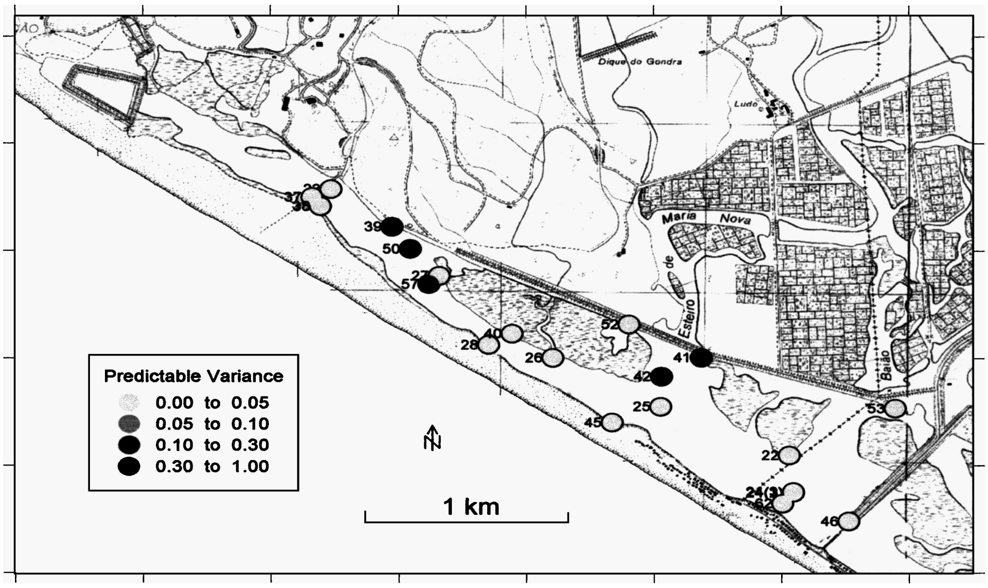


Figure 1.3. Effect of sewage discharge on meiobenthos communities. The amount of variance predicted for each site by the signature of a community influenced by organic matter of a sewage origin, PLS model (Hewitt, Mudge, 2004).

The same work suggests that meiofauna, due to its small size does not respond to mechanical stress caused on the sediments by the shellfisheries disturbance. It is concluded that it makes meiofaunal communities well suited to diagnostic application in assessment of organic (including sewage) and chemical pollution. At the same time, univariate measures such as diversity indexes, evenness, and species richness were found to show little response to physical and pollution differences between the sites (Hewitt & Mudge, 2004).

1.8. Shorebirds of the Ria Formosa

Ria Formosa coastal lagoon is one of the most important sites in the Iberian peninsula used by shorebirds during wintering and migration. It is situated in the East-Atlantic migratory flyway of the migrating birds, and represent important stopover point as well as wintering ground for many species, it supports up to 20,000 wintering birds every year, and considerable proportion of European population of several of them (Rufino, 1979). Considering the number of overwintering birds it satisfies the 1% criterion of the site of international importance by the Ramsar Convention (Costa & Rufino, 1997). The lagoon is part of Natura 2000 Network, Special Protection Area for birds, Ramsar site and a National park.

Based on morphometric characteristics (wing length in adult and juvenile birds) Batty (1993) found that Dunlins *Calidris alpina* migrating through Ria Formosa belong to different races: juveniles during autumn migration belonged to race *C. a. schinzii.*

Fonseca et al. (2005) recorded following species breeding on the salt pans near Tavira wetland: Black-winged Stilt *Himantopus himantopus*, Pied Avocet *Recurvirostra avosetta*, Kentish Plover *Charadrius alexandrinus*, Little Tern *Sterna albifrons*, Mallard *Anas platyrhynchos* and Little Grebe *Tachybaptus ruficollis*.

1.9. Aims and objectives of this study

This study is focused on comparison of environmental quality of the intertidal flats habitats of the Ria Formosa lagoon using shorebirds assemblages, physical sediment properties and benthic communities, considering known environmental status defined in terms of Water Framework Directive. The habitat quality and bird assemblages were compared between two sites in the western part of the lagoon, with presumably different ecological status (Newton et al., 2007; Loureiro et al., 2006) and consequently difference between sediment quality and shorebird communities was expected.

The main goals of the study are:

1. To study shorebirds assemblages in relation with intertidal habitat environmental properties, including benthic prey component, on the sites with different ecological status;
2. To compare the birds communities between sites;
3. To consider the possibility to use shorebirds communities as an indicator for assessment of ecological status of intertidal habitats of the Ria Formosa lagoon.

To achieve these goals, the following specific objectives were addressed:

1. To study shorebirds assemblages of the intertidal habitats on sites with different ecological status, including spatial and seasonal variation;
2. To study physico-chemical parameters of the intertidal flat habitats: sediment particle size distribution, total organic content and reduction-oxidation potential;
3. To study meiobenthic and macrobenthic communities of the sediments at the same study sites as birds assemblages, including abundance and species diversity;
4. To compare the study sites in terms of environmental parameters obtained;
5. To compare birds communities of the study sites;

6. To consider the relation between biotic components (birds and benthos) and environmental parameters of the ecosystems;
7. To discuss feasibility of use the shorebirds assemblages as indicator for ecological status of intertidal habitat.

2. METHODS

2.1. Study sites

Three intertidal study sites were chosen in the Western part of the lagoon (Fig.3.1). To test the hypothesis of a relationship between the birds' community with the lagoon ecological status, the study sites were located close to the known intercalibration sites for Water Framework Directive (WFD), for which ecological status has been defined (Table 1.1).

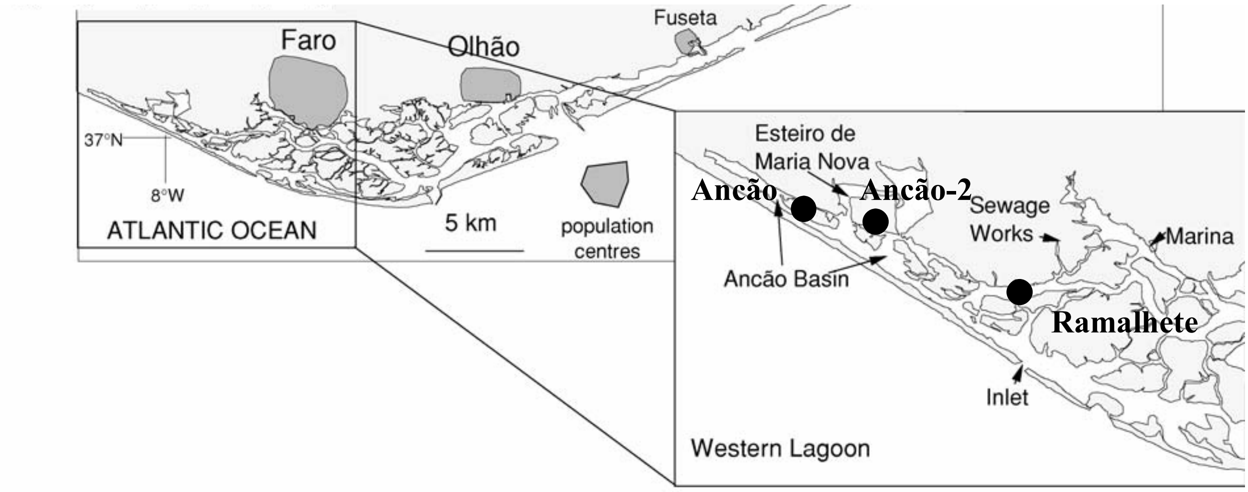


Figure 2.1. Location of the study sites in relation to main population centers and sewage discharges (Map base adapted from Wayland *et al.*, 2008).

The choice of sites was made in order to have similar tidal regime, extension of the mudflats and exposition from one side to the water edge during low tide. Accessibility was taken into account, so that both sites could be visually inspected from the salt marsh or dyke without disturbing birds feeding on the mudflats.

Figure 2.2. Study site Ramalhete is located in the vicinity of the Water Framework Directive intercalibration site. (Map base: Google Maps, 2009)

Figure 2.3. Study sites in the Ancão basin. Ancão site consists of the transect of 8 square sampling units, with two benthos sampling points. Ancão-2 site is not divided into quadrats, has one benthos sampling point.

Ramalhete site (Fig. 2.2) is situated on the Northern side of the channel with the same name, at the place of intercalibration site, used for monitoring within the WFD. The boundary status for the site was defined as Good/Moderate (Table 2.1). The Ramalhete channel has restricted water exchange compared to outer parts of the lagoon, with higher water residence times, especially of the deep layer of water (Mudge *et* al., 2008). This can lead to a decrease in the level of dissolved oxygen, and affect the sediment benthic fauna.

The Faro Noroeste waste water treatment plant is located in 1.5 km from the Ramalhete channel, and though it does not discharge directly into the channel, the water contaminated with sewage organic matter may enter the channel due to tidal movements (Mudge et al., 2008; Pires, 2004). This can lead to organic matter accumulation and nutrient enrichment combined with restricted water exchange, causing increased oxygen demand and nutrient enrichment.

Ancão site is situated in the upper part of the Ancão channel, on its Northern bank and is adjacent to the salt marsh and former fish ponds recently converted into shellfish grounds (Fig.3.3). The Ancão and Ancão-2 sites are located in the part of the lagoon which belongs to the same water body as the Ponte de Praia de Faro WFD intercalibration site with High ecological status, and boundary conditions being defined as High/Good (Commission Decision 2005/646/EC, Newton *et al*., 2007). The choice of the sampling area directly at the Ponte WFD site was not feasible because of the busy road and bridge proximity that influence the abundance of birds due to disturbance effect.

Ancão-2 site was chosen for comparison with the values obtained on the two main sites, being different in location relatively to low water mark and having a different configuration, mud flat extent and exposition to the tide (Fig. 2.3). It was not practical to establish square sampling units there, because of complicated shape, larger size and absence of suitable landmarks at the site. The observer could not move along the shore as in case of two other sites, hence the counts were done from two vantage points at distance up to 200 m.

Ancão and Ramalhete sites were divided into 8 square sampling units each, used for bird counts. The quadrats were grouped into transects, parallel to the shore and with one side exposed to the water edge at low tides. The transects length on both sites was 400 m, with sampling quadrates 50 x 50 – 80 m in size, depending on the mud flat extension. The borders of the quadrates in many cases coincided with the landmarks of shellfishery possessions, and therefore differed in the rate of sediment disturbance, because the activity of different fishermen was not the same. In some cases the intertidal creeks intercepted transects, increasing the length of water edge in the quadrates, which may have important consequences on ecological conditions, including particle size and accessibility of the prey for birds (Zwarts and Wanink, 1993; Lourenço et al, 2005). Benthic samples were taken from two quadrates on Ancão (A2, A3) and Ramalhete (R2, R7) sites, one sample was taken at the Ancão-2 site.

Distance to the suitably located high-tide roosts can limit the access of shorebirds to feeding habitats (Dias et al., 2006). At Ramalhete the available roosting sites were salt pans adjacent to the site and salt marsh at distance 50 – 200 m (Fig.3.2). The distance to sandy shore was 1,800 m. At Ancão site (Fig.3.2), the closest roosts were salt marsh (adjacent to the transect), fishponds (50 – 400 m), salt pans (1 km), freshwater wetland (400 m) and sandy shore (200 m). Ancao-2 site was located close to salt marsh (50 m), salt pans and freshwater wetland (200 m), and sandy shore (500 m).

2.2. Granulometry analysis

Granulometry (particle size distribution) of the mud flat sediments is known to affect the benthic fauna communities and as a result, spatial distribution of shorebirds assemblages (Yates et al, 1993; Granadeiro et al, 2007).

Sediment granulometry samples were taken approximately in the center of each quadrate, one sample per quadrat. In the quadrates A-2, A-3, R-2, R-7, Ancao-2 with the benthos sampling stations, the granulometry samples were taken within 5 m from the center of the benthos sampling station, in three replicates. The A3, R2 and R7 sites were sampled for sediment granulometry in three spatial replicates to provide coverage of mud flats surface variability. The stations were located using known landmarks and GPS unit Garmin 48. The core of 20 cm diameter was pushed to the sediment to the depth of 5 cm, the sediment was then extracted to the plastic bag, closed without air, carried to the laboratory within 2 hours and kept frozen at -20 ^{o}C before the analysis.

The sub-sample of 100 g from sandy and 150 g from muddy water-saturated samples were taken for particle size analysis. The whole sample was previously homogenized by mixing. Organic matter was destroyed by treatment with hydrogen peroxide 130V water solution. The destruction was considered completed when the gas stopped to run out and the color of the sample changed from black to mineral yellow or brown. The sample was then wet-sieved with distilled water through 0.063 mm mesh. The coarse fraction remaining in the sieve was dried at 60 ^{o}C to constant weight. It was then dry-sieved through a series of 2, 1, 0.5, 0.25, 0.125 mm sieves using mechanical shaker. Each fraction was weighted with accuracy of 0.01 gram. The fine fraction in suspension which passed through the 0.063 mm sieve was put to volumetric flask, diluted to 1 L volume with distilled water if necessary and mixed with added anticoagulant (Calgonite) during 5 minutes with spinning mixer and left to settle down for 24 hours. Then it was mixed until homogeneity and after 20 seconds a volume of 20 ml was taken from the depth of 20 cm to the glass of known mass, dried and weighed. The obtained sediment mass was multiplied by 50 to equalize it to the 1 L volume and considered as a measure for the total mass of finer than 0.063 mm fraction of silt and clay in the sample. The rest of the suspension was analyzed with laser granulometer (Malvern Instruments Mastersizer) to obtain the distribution diagrams of the fraction finer than 0.063 mm. The resulting volumetric distribution of particles was assumed to be equal to the mass distribution.

The GRADISTAT software (Blott and Pye, 2001) was used to classify the sediments and determine the type of particle size distribution.

2.3. Sediment total organic content analysis

Sediment total organic content (TOC) was measured by combustion of organic matter (Hewitt and Mudge , 2004).

Samples were taken in three spatial replicates within each quadrate. One replicate sample was subsampled from the granulometry core. Other two replicated samples were taken from cores 5 cm in diameter and 5 cm in depth, closed in a plastic bag without air and frozen before analysis. Samples were wet-sieved through 2 mm sieve to remove big invertebrates and algae, then homogenized. Approximately 25 – 30 g of the sediment was put into thick aluminum foil containers of known weight and dried in the oven to constant weight at 50 ^{o}C, then kept in desiccator to remove moisture and weighed. Samples were then combusted at 440 ^{o}C during 4 hours in the ventilated muffle furnace, then cooled in a desiccator for several hours and weighed. Total organic content was calculated as the difference in dry weight before and after combustion divided by initial dry mass and expressed as a percentage value:

TOC = [m (dry sediment) – m (combusted sediment)]*100% / mass of dry sediment.

2.4. Reduction-oxidation potential

Reduction-oxidation potential was measured to give an indication of the reducing properties of the sediments. Redox measurements were made in situ with a CellOx325 probe at three random locations within sampling quadrats. One of the replicates was made at the same point and simultaneously with granulometry and TOC sampling. Three sub-replicate measurements were made within 20 cm around the sediment cores positions. Redox was determined in the uppermost sediment layer between the surface and 2 cm depth. At stations A2 and R2 it was also measured at depth of down to 10 cm to examine the change of reduction-oxidation conditions with depth.

2.5. Birds census

Bird census was conducted from late March to early October 2008. Counts were made during periods of spring tides. In spring (March - May) and autumn (August - October) two visits were made to each site every two weeks, in summer (June – July) normally one visit was made fortnightly at Ramalhete and Ancão-2 sites because of low birds numbers. Two sites could not be visited at the same day during low tide, so visits covered four consecutive days every two weeks, according to the tidal cycle. From March to July one count was done during a visit, from late July till September two counts were normally carried out during a visit with 30 minutes interval between them. The sampling scheme resulted in 34 counts at Ancão site (9 in spring, 11 in summer and 14 during autumn), 22 counts at Ramalhete (9 in spring, 4 in summer and 9 in autumn) and 12 at Ancão-2 site (2 in spring, 6 in summer and 5 in autumn). Counts were made when the mudflats were open to their maximum extension between 2 hours before and after the predicted time of low water. All the counting quadrats at Ancão and Ramalhete sites had similar exposition time.

Birds were counted from the dyke of the salt pans on Ramalhete, and from the salt marsh on Ancão, from the distance 20 – 100 m using 12x binoculars. Local landmarks were used to determine the borders of counting units, distances were measured using GPS Garmin 48. Birds foraging near the water edge at the side of the quadrats were considered to be within the quadrat. In case when there was an intertidal channel between quadrats, birds feeding on the different banks were considered to belong to the respective quadrats. The observer slowly walked along the transect of counted quadrats making stops for counting. Birds were generally not disturbed by the observer. There were often shellfishers present on the tidal flats, usually 2 – 3 persons along the transect. The presence of shellfishers has been found to have no significant disturbing effect on feeding birds distribution (Dias et al, 2008), so this factor was not considered as obstacle for counting. The presence of fishermen was recorded for each quadrat.

2.6. Macrobenthos

(*Sampling, identification and calculations of macrobenthos were done by M. Cañedo-Arguelles.*)

Macrobenthos was sampled on 5 stations, in quadrats A2 and A3 at the Ancão site, R2 and R7 at the Ramalhete site and at Ancão-2 site. For taking benthos samples a core sampler was used. Samples were taken in three spatial replicates at each station. The sample was washed through the 0.5 mm mesh *in situ*. All the taxa were preserved in 70 % ethanol. Once in the lab samples were rinsed through 250 micrometers mesh size net, benthos individuals were shorted, identified to species level and counted for density calculation.

2.7. Meiobenthos

(*Sampling, identification and calculations of meiobenthos were done by K. Ivanova.*)

Meiobenthos was sampled on the same 5 stations as macrobenthos and simultaneously with it. Sediment samples for meiofauna analyses were collected at mudflats during low tide by plastic syringe (diameter 2 cm) with three replicates on each station, preserved in the 70% ethanol. Sediment samples were sieved through 63 mm mesh size. Retained fraction was centrifuged with Ludox as described by Heip et al. (1985). Nematodes were extracted and mounted on microscope slides following Warwick et al. (1998). Identification guides Warwick et al. (1998) and recent publications on Nematode taxonomy were used for *Nematoda* identification to genus level.

2.8. Data analysis

Bird count data was averaged according to season, meaning counts from March to May corresponding to spring pre-nuptial migration were considered “Spring”, from June and July (summer breeding period) “Summer” and from August to October (autumn migration) as “Autumn”. Bird densities were obtained by division of abundance of every species in a particular sampling quadrat by its area. The area of Ancão-2 site was determined using program ArcView version 3.2a by ESRI, based on scaled satellite image.

Classification of samples by sediment particle size distribution was done using GRADISTAT (Excel version 11) program. The relationship between fraction grain size <63 µm and TOC was explored using regression analysis. Abundance of taxa, diversity (Shannon-Wiener index H′), species richness (Margalef index d) and evenness e^H/S were calculated using PRIMER version 6.0 and Past version 2.02.

Hypothesis about significant differences between sites by environmental variables (particle size, TOC, redox) were investigated by means of Mann-Whitney U-test and Kruskal-Wallis non-parametric ANOVA (Quinn and Keogh, 2002). The non-parametric methods were used because sample size was small (n=3 for individual quadrats, n=8 for the whole sites) and data was not assumed to be normally distributed due to its nature.

Spearman rank correlation analysis was used to check relation between bird species densities with environmental parameters. The non-parametric correlation was used because of small sample size. Two independent correlations were calculated for Ancão and Ramalhete sites, using sampling quadrates (n=8) as correlation units in order to avoid the spatial factor. The location of them along the transect lines could lead to intercorrelation induced by spatial dependency of the sampling sites. This aspect was not considered an obstacle, because environmental variables were obtained from sampling locations in the centres of every quadrat at the range of not less than 50 m and though were considered independent. Bird counts were also considered independent between quadrats, having regard to their ability to move in choice of habitat without any physical barriers within site. Non-parametric analyses were done using Statistica version 6.0.

Principal Component Analysis (PCA) was used to investigate the differences between bird communities. Bird density data was previously $\log_{10}$ (x+1) transformed to downweight the contribution of abundant species. Cluster analysis and non-metric Multi-dimensional scaling

(MDS) were used to investigate environmental variables and structure of benthos and bird communities. Software used for multivariate statistics was PRIMER v. 6.0.

Partial Least Squares analysis implemented in Umetrics SIMCA-P software was used to investigate data for relations between environmental variables, benthos and birds.

The Individual Values analysis (IndVal) was conducted to determine indicator bird species associated with particular site. This analysis provides measurement of each species site fidelity and specificity, resulting indicator species are indicative of particular groups of sites (Dufrene & Legendre, 1997). Monte-Carlo randomization test (N permutations = 999) was run to determine significance level of obtained indicator values. Software used for IndVal analysis was PC-ORD version 5.

3. RESULTS

3.1. Environmental parameters

3.1.1. Sediment particle size

Table 3.1 and Figures 3.1 and 3.3 illustrate sediment particle size distribution and classification of the sediment at the study sites, according to Blott and Pye (2001).

Table 3.1. Sediment particle size distribution (%) and classification of sampling units, Ramalhete, Ancão and Ancão-2 sites (According to GRADISTAT, by Blott and Pye, 2001).

Station	Particle size, mm				Classification
	Gravel	**Sand**	**Silt**	**Clay**	
	>2.0	1.00-2.00	0.02-0.063	<0.002	
A1	3.6	55.2	32.9	8.3	Slightly Gravelly Muddy Sand
A2	-	62.8	32.4	4.8	Muddy Sand
A3	4.8	21.3	60.1	13.8	Slightly Gravelly Sandy Mud
A4	2.4	63.5	27.1	6.9	Slightly Gravelly Muddy Sand
A5	7.5	58.1	27.9	6.4	Gravelly Muddy Sand
A6	6.1	62.1	26.1	5.7	Gravelly Muddy Sand
A7	1.0	58.7	33.6	6.7	Slightly Gravelly Muddy Sand
A8	2.0	71.3	20.7	6.0	Slightly Gravelly Muddy Sand
R1	3.0	48.0	40.6	8.5	Slightly Gravelly Sandy Mud
R2	0.4	78.9	15.6	5.0	Slightly Gravelly Muddy Sand
R3	0.6	27.7	63.6	8.2	Slightly Gravelly Sandy Mud
R4	4.3	74.6	16.3	4.8	Slightly Gravelly Muddy Sand
R5	2.1	64.8	25.9	7.3	Slightly Gravelly Muddy Sand
R6	0.5	43.2	43.8	12.5	Slightly Gravelly Sandy Mud
R7	0.01	33.6	53.9	12.4	Slightly Gravelly Sandy Mud
R8	3.6	63.5	26.0	6.9	Slightly Gravelly Muddy Sand
Ancão-2	1.3	59.0	31.7	8.0	Slightly Gravelly Muddy Sand

Most quadrats on the Ancão site were classified as muddy sand with small content of gravel, the % of fraction <63 μm ranged from 27 to 42 %. The A3 quadrat was classified as sandy mud, with 85 % of clay and silt fraction. This contrast of sediment type is probably due to location at the bank of intertidal channel and absence of regular shellfish cultivation. At the Ramalhete site, four quadrats were considered sandy mud, the most muddy were quadrats R3 (72%) and R7 (66%), both located next to the intertidal channels. The other four quadrats were classified as muddy sand, with R2 being the most sandy (21% fraction <63 μm). The large sand content in the R2 quadrat can be explained by the fact that it was used actively for clam cultivation, in course of which the upper sediment layer wass mixed shellfishers and seagrass collectors or algae cover was removed. The quadrat R4 was likely affected by sand and gravel deposition during the construction of the salt tanks adjacent to the site, which can explain 28 % high sand fractions content. The silt and clay content showed a trend to increase from quadrat R4 to R7 from 21 to 66%. Mean content of the fraction GS<63 μm was 41.4 ± 18.0 for the whole Ancão site (A1 – A8), and 43.9 ± 19.9 % for Ramalhete site (R1 – R8).

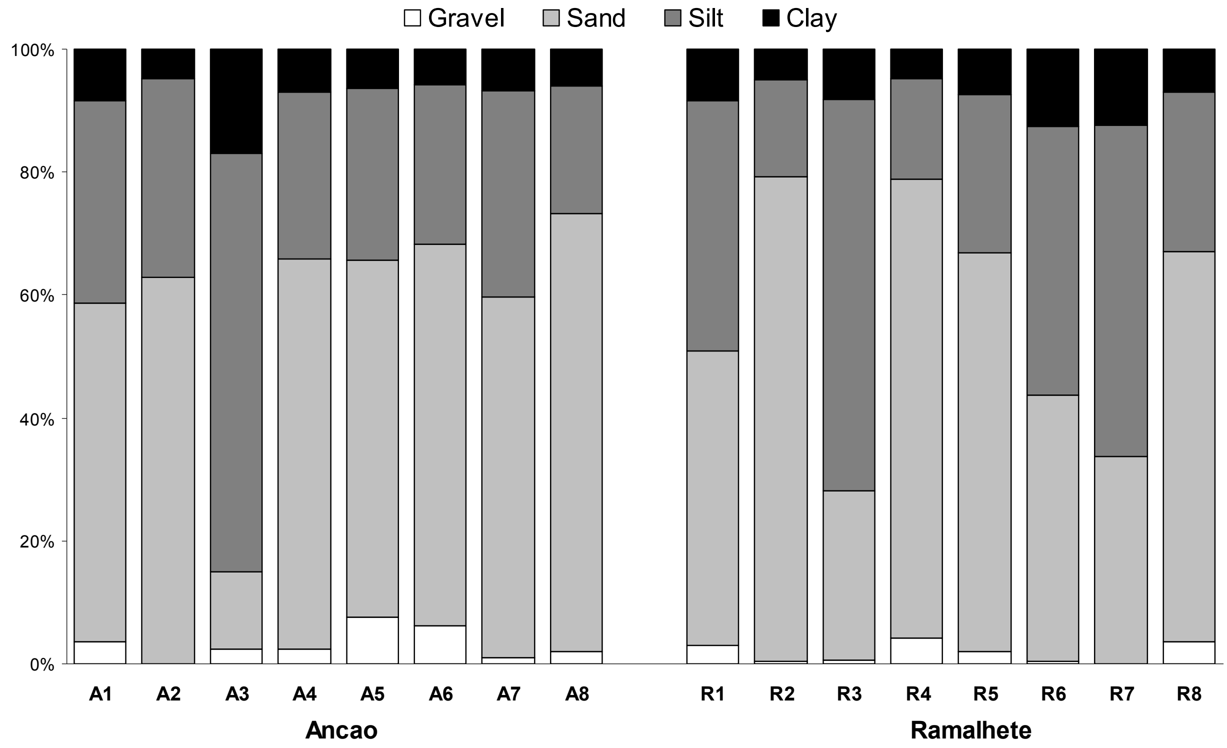


Figure 3.1. Sediment particle size distribution at Ancão (A1 to A8) and Ramalhete (R1 to R8) sites.

To compare the sediment particle size distribution between Ancão and Ramalhete sites Mann-Whitney U-test was conducted. Proportions of all defined fractions ranging from gravel (coarser than 2 mm) to silt (<2 μm), as well as total sand (0.063 – 2 mm) and mud content were compared (Table 3.2). The difference in granulometry between the two sites was significant only for the very coarse sand (1 – 2 mm) and very fine sand (0.063 – 0.125 mm) fractions (U=9, p=0.016 and U=8, p=0.012 respectively, n=8, df=7). The sites did not differ significantly by other fractions, including total sand and mud (GS<63 μm). The variation between different sampling quadrats within each site was high, as illustrated in the Figures 3.1 and 3.2. Therefore, Ancão and Ramalhete as a whole were not different statistically in terms of sediment composition, but constituting sampling units demonstrated high within-site variability.

Table 3.2. Comparison of grain size fractions distribution between Ancão and Ramalhete sites: Mann-Whitney U-test, n=8, df=7. Sites were significantly different by very coarse sand (1 – 2 mm) and very fine sand (0.063 – 0.125 mm) fractions at the level of significance $p<0.05$ (in bold).

Grain size fraction	U	z-value	p
Gravel	23.0	0.95	0.345
Very coarse sand	9.0	2.42	**0.016**
Coarse sand	25.0	0.74	0.462
Medium sand	18.0	-1.47	0.142
Fine sand	32.0	0.00	1.000
Very fine sand	8.0	2.52	**0.012**
Silt	30.0	0.21	0.834
Clay	24.0	-0.84	0.401
Total Sand %	31.0	-0.12	0.916
GS<63 µm %	32.0	0.00	1.000

Sediment particle size composition at the quadrats with benthos sampling stations (average values with classification) is presented in the Table 3.3 and Figure 3.2. and illustrates mud flats surface grain size variability.

Table 3.3. Sediment particle size distribution (%) on the benthos sampling stations

Station code	Particle size, mm				Classification
	Gravel	Sand	Silt	Clay	
	>2.0	1.00-2.00	0.02 - 0.063	<0.002	
A2	-	62.8	32.4	4.8	Muddy Sand
A3	2.5±2.1	12.5±7.8	68.1±7.1	17.0±2.8	Slightly Gravelly Sandy Mud
R2	0.4±0.4	78.9±10.1	15.6±6.8	5.0±2.9	Slightly Gravelly Muddy Sand
R7	0.01±0.02	33.6±16.1	53.9±12.9	12.4±4.0	Slightly Gravelly Sandy Mud
Ancão-2	1.3	59.0	31.7	8.0	Slightly Gravelly Muddy Sand

Comparison of sediment grain size distribution between three quadrats with benthos sampling stations was made by means of Kruskal-Wallis test using three spatial replicates within each unit (A3, R2, R7). The test revealed significant difference at $P<0.05$ ($K=6.49$, $N=9$, $df=2$, $p=0.04$) for both sand % and %GS<63µm fractions between the sites. This suggests that on the larger spatial scale the two sites (Ancão and Ramalhete) can be considered similar by grain size composition, but there was a significant variability between single sampling units of each site.

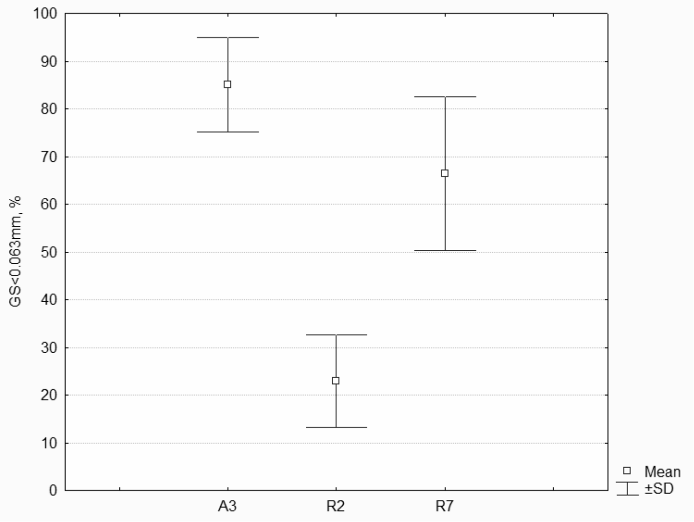


Figure 3.2. Sediment fraction GS<63 µm distribution at benthos sampling stations R2, R7and A3. Sample mean and standard deviation (SD).

3.1.2. Sediment organic content

The mean values of total organic content (TOC) varied from 1.73 to 5.73 %, being typically 2.0 – 3.5 % at Ancão, and 2.0 – 5 % at Ramalhete (Table 3.4). As expected, there was a positive relationship between sediment grain size (percent GS<63 μm) and TOC (Figure 2; R^2 = 0.80) due to the relationship between the surface area and diameter (Hopkins and Mudge, 2004).

Sites with muddy substrate tended to have more organically enriched sediments than sandy sites. Variation of TOC within one sampling quadrat was in some cases very high, up to 70 % of the mean value, demonstrating at the same time variation of silt and clay fractions proportions.

At the Ancão site mean TOC ranged from 1.7 to 4.4 % (Table 3.4), the most organically enriched were quadrats A3 and A2. Sediment at Ramalhete site had comparatively higher organic content, with TOC ranging from 2.3 to 5.7 %, while in quadrats R1, R3, R6 and R7 it exceeded 5%.

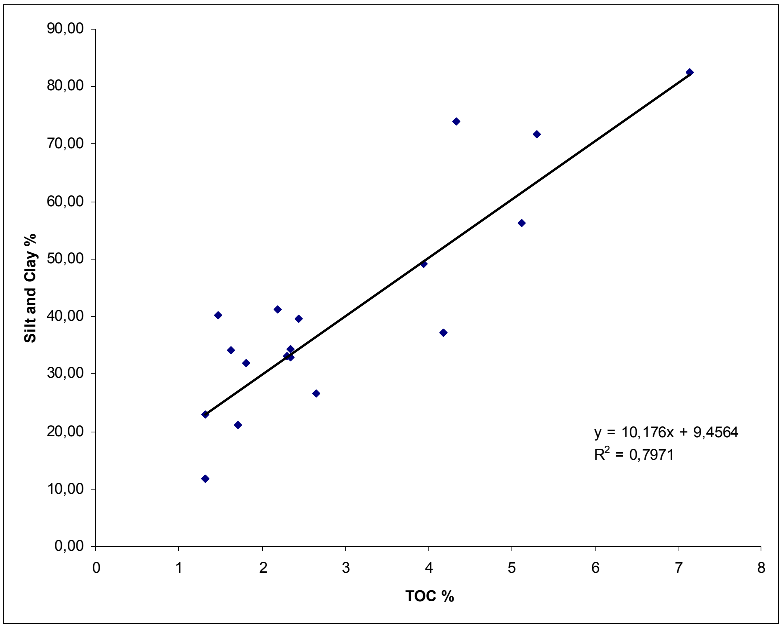


Figure 3.4. Regression between % TOC and % of fraction GS<63µm, R^2=0.80.

Table 3.4. Mean values (±SD) of total organic content (TOC) and silt-clay (fraction <63 µm) content at Ancão, Ramalhete and Ancão-2 sampling sites.

Site	Station	% TOC	% GS<63 µm
Ancão	A1	1.73±0.43	41.2
	A2	4.38±2.78	37.2
	A3	3.91±0.37	85.1
	A4	2.75±1.12	34.1
	A5	2.26±0.71	34.4
	A6	1.81±0.53	31.8
	A7	2.00±0.55	40.3
	A8	3.42±1.74	26.7
Ramalhete	R1	5.25±1.79	49.1
	R2	3.72±2.13	20.7
	R3	5.73±0.58	71.8
	R4	3.48±2.51	21.1
	R5	2.33±0.19	33.2
	R6	5.16±2.77	56.3
	R7	5.49±1.69	66.4
	R8	4.26±1.77	32.9
Ancão2	Ancão2	3.47±1.56	39.7

3.1.3. Sediment reduction-oxidation potential

The sediment reduction-oxidation potential values on all sites were found to be negative in the layer of upper 2-cm from the surface (Table 3.5). In the thin layer of water covering the sediment measured values were positive at +35 – +50 mV. It the Figures 3.5 and 3.6 the redox profiles at benthos sampling sites R2 and A2 are plotted. The reduction-oxidation values at site R2 were negative already from below the surface at values from – 47 to –133 mV. Change of Eh showed general decreasing trend until the depth of 6 cm, where the values ranged form –178 to –201 mV. At the depth of 10 cm the redox values raised to the level of –142 mV on average ranging from –120 to –172 mV. At the A2 site redox values measured in the range of –95 to –132 at the surface and maintained this level along the profiles in two cases, and decreased down to –57 - –172 mV in the third profile (Figure 3.6). The measurements characterized sediment conditions as reducing at all sites in the measured range of depths. The values given in the Table 3.5 reflect average reduction-oxidation potential, calculated based on three spatially different sampling locations in each quadrat, and therefore were considered to be representative of the sediment conditions of every quadrat in a whole.

Ramalhete and Ancão sites were found to be significantly different (U-test, n=8, df=7, U=12, p=0.035) by mean redox potential of the upper 2-cm layer. Sediments at Ramalhete were generally more reducing at average E_h= – 160 (±26.3) mV than at Ancão E_h= – 127 (±51) mV. Such values suggested anoxic conditions at both sites, which appeared from not very deep below the sediment surface (Figures 3.5 and 3.6). Visually, the upper 0.5 – 1 cm of sediment was brown, and black in the deeper layer. This indicated the reduction of iron to Fe^{2+}, which stipulated black color. At the Ancão site, in quadrats A4, A6, A7 patches of oxygenated sediment were encountered under the layer of dark anoxic sediment. They were seen as pieces of light-brown sediment with Fe^{3+} rusty orange spots.

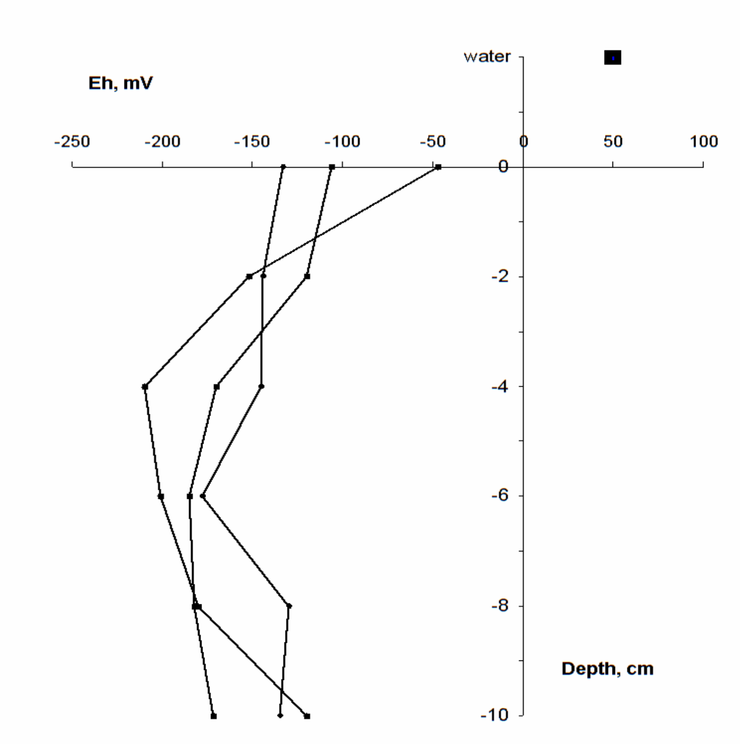


Figure 3.5. Redox profile, Eh (mV) change with depth (cm) at Ramalhete (site R2). Sediment organic content was 3.72%.

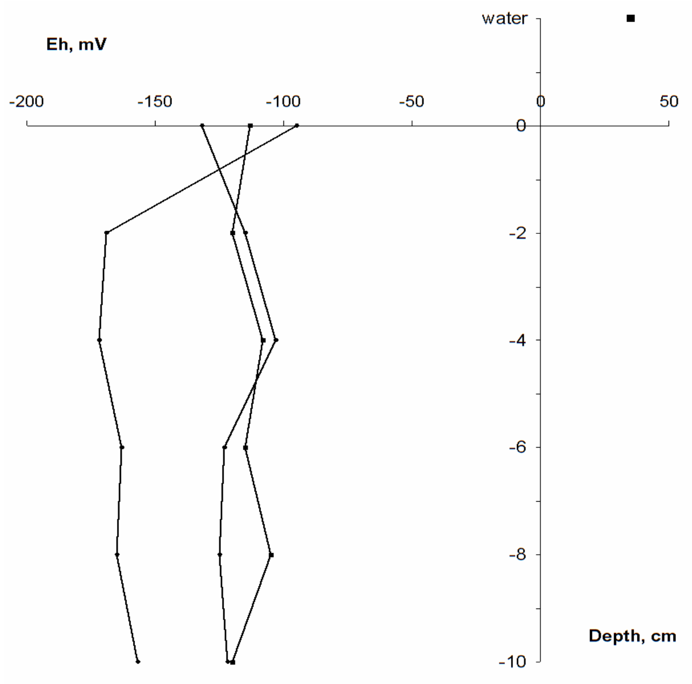


Figure 3.6. Redox profile, Eh (mV) change with depth (cm) at Ancão (site A2). Sediment organic content was 4.38 %.

Table 3.5. Redox potential site average values from three spatial sampling points (±SD) in the upper 2 cm layer of sediment.

Site	Station	E_h, mV
Ancão	A1	- 152 ±59
	A2	- 148 ±49
	A3	- 97 ±51
	A4	- 106 ±10
	A5	- 137 ±11
	A6	- 113 ±49
	A7	- 88 ±5
	A8	- 175 ±96
Ramalhete	R1	- 166 ±12
	R2	- 153 ±54
	R3	- 144 ±6
	R4	- 191 ±16
	R5	- 136 ±21
	R6	- 166 ±28
	R7	- 157 ±13
	R8	- 166 ±17
Ancão-2	Ancão-2	- 187 ±11

3.1.4. Comparison of study sites by sediment properties

Comparison in terms of physical properties of the sediments have shown that Ancão and Ramalhete sites did not differ overall in proportion of silt-clay and sand fractions (though within site variation was large), but differed significantly by total organic content and redox potential (Table 3.6).

Table 3.6. Summary of the U-tests for difference between environmental variables of Ancão and Ramalhete sites, n=8, df=7. Sites were significantly different by TOC and Redox at significance level of $P<0.05$.

Parameter	U	z	P
GS<63 µm %	31	-0.105	0.916
Total Sand %	32	0	1.000
TOC	9	-2.416	0.016
Redox (E_h)	12	-2.11	0.035

Principal Component Analysis (PCA) was used to plot the stations in the space of physical sediment variables (Figure 3.7). The PC1 axis represents gradient of TOC and grain size fractions. Positive values reflect combination of high TOC and fraction of GS<63 µm content, negative values account for large proportion of sand fractions. Stations R1, R3, R6 and R7 (Ramalhete) and A3 (Ancão) were positioned positively with the TOC and mud content. Other Ancão stations and R2, R4, R5, R8 were associated with sand fractions. Ancão site had generally sandier substrate than Ramalhete, with exception of quadrats A3 and A2. Ramalhete site was overall muddy, but quadrats R2, R4 and R5 had a high sand proportion. These conclusions were not supported by the univariate tests (Table 3.6), which compared median values for the whole Ancão and Ramalhete sites considering values of each quadrat as replicates. High content of GS<63 µm fraction at stations A3, R3, R7 and otherwise, high sand content at stations R2 and R4 contributed a large amplitude of these parameters.

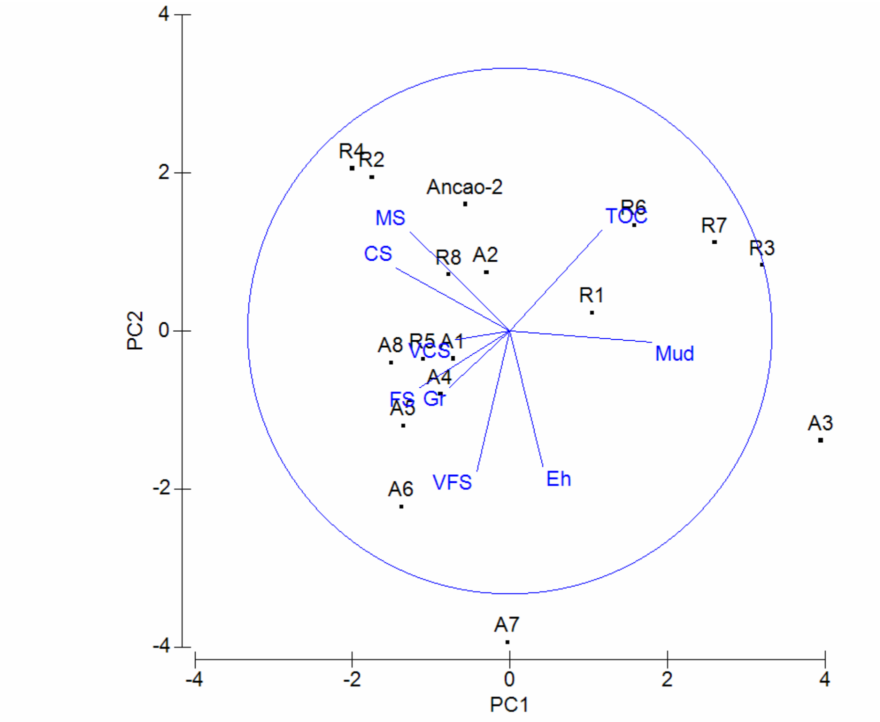


Figure 3.7. Principal Component Analysis of sediment granulometry, TOC and Redox (Log (x+1) transformed). PC1 and PC2 account for 36.7 and 28.0 % of variance, respectively. VCS – very coarse sand, CS – coarse sand, MS – medium sand, FS – fine sand, VFS – very fine sand, Mud – fraction of GS<63 µm, Eh – redox potential.

3.2. Benthos communities

3.2.1. Macrobenthos

Macrobenthos mean density varied between sites from 501 to 4922 ind/m^2 (Figure 3.8). Macrobenthos densities at A2 and A3 and Ancão-2 sites were higher than ones at Ramalhete stations. Species composition at these two groups of stations also appeared different (Appendix B).

Macrobenthos species abundance data was presented as *k*-dominance curves to indicate communities subjected to environmental stress (Clarke and Warwick, 2001). Figure 3.9 demonstrates that communities at the stations A2 and A3 and were dominated by one species (*Bittium reticulatum*), what can indicate community under pressure. Ancão-2 and R2 communities were more even in species composition. At the station R7 macrobenthos density was the lowest on average at 500 ind./m^2 (Appendix B), with gentle slope of the dominance curve. This station was located near creek connected with salt evaporator tank, and had high silt and clay content (66%). Communities at the R-2 and Ancão-2 showed high diversity and low species dominance.

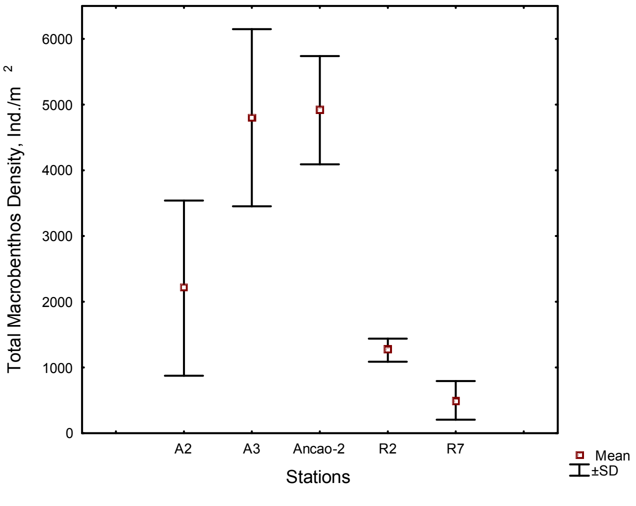


Figure 3.8. Total macrobenthos densities at the study sites, ind/m^2: mean ±SD.

The multi-dimensional scaling plot (Figure 3.10) illustrates similarity between stations of each transect (R2 and R7, A2 and A3), and difference between transects. Ancão-2 site was distinct from Ancão and Ramalhete, and had a higher range between replicate samples.

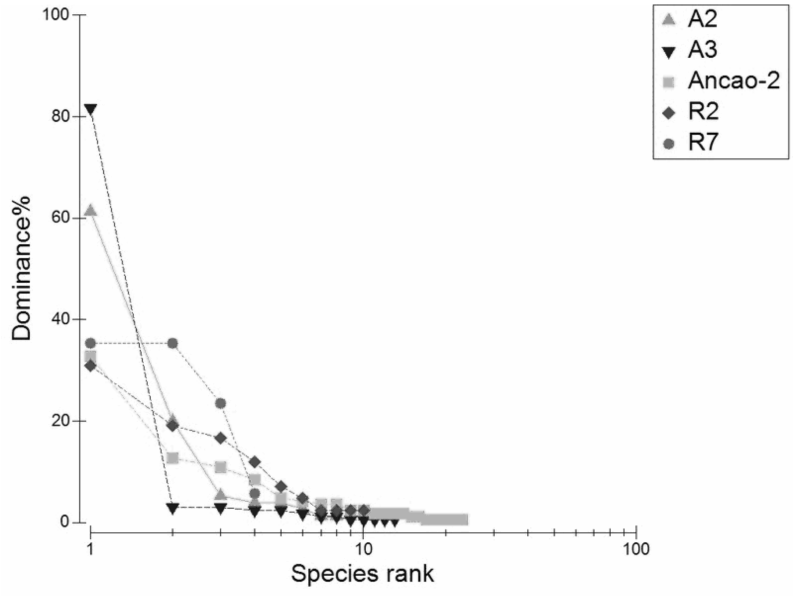


Figure 3.9. Relative *k*-dominance curves of macrobenthic communities of the study sites.

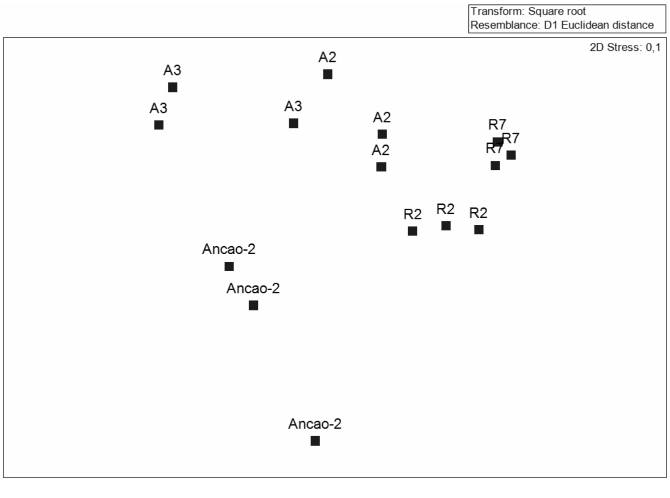


Figure 3.10. Non-metric Multi-Dimensional Scaling ordination plot based upon Euclidian distance of square-root transformed macrobenthos species density data.

Table 3.7. Diversity measures and indices for macrobenthos communities across all stations: S – species number, d – Margalef species richness, J' – Pielou's equitability, H' – Shannon diversity, AMBI – marine biotic index.

Site	**S**	**d**	**J'**	**H'**	**AMBI**
A2	5.00	0.52	0.61	1.00	0.012
A3	6.33	0.62	0.37	0.70	0.002
Ancão-2	13.33	1.45	0.81	2.09	0.013
R2	6.67	0.80	0.92	1.72	0.016
R7	2.33	0.22	0.96	0.80	0.026

Diversity measures and indices for macrobenthos communities are displayed in the Table 3.7. The macrobenthos community at Ancão-2 was the most diverse (H´=2.09), followed by R2 station.

The Marine Biotic index (AMBI) based on the ecological groups according to their sensitivity to increasing pollution gradient was calculated (Table 3.7). According to Borja (2000), the obtained values of AMBI index at all stations correspond to High ecological status. Benthic community health was characterized as “Impoverished” at the station R7, and Normal at all other stations (Borja, 2000), and sites were classified as “Unpolluted”.

3.2.2. Meiobenthos

Total meiobenthos abundance at the studied sites ranged from $4.78*10^5$ to $9.42*10^5$ individuals per m^2. Maximal densities were found at the station R7 and minimal densities at the station A2 (Fig. 4.11). Meiobenthos abundance at the other sites did not vary significantly (Appendix C).

Taxonomical composition consisted of 11 high order taxa: *Nematoda, Acarina, Polychaeta, Ostracoda, Insecta, Oligochaeta, Bivalvia, Ciliata, Foraminifera, Crustacea* and *Turbellaria*. *Nematoda* comprised 87.7 % at average of the meiofaunal composition, and therefore dominated meiobenthic community at all stations (Figure 3.12). As *Nematoda* clearly dominated meiobenthic communities, only this group was analyzed at species level and considered to be a measurement of the species diversity of meiobenthos.

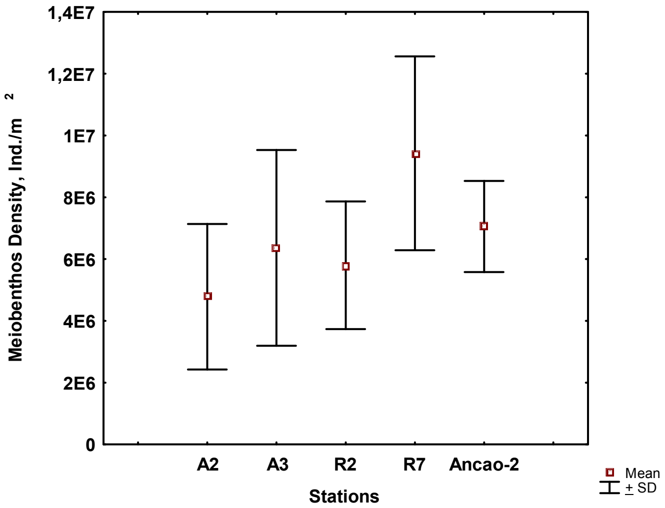


Figure 3.11. Total meiobenthos density at the study sites: mean ±SD.

Nematoda assemblages of the study sites were represented by 62 species (Appendix D). The composition included nematodes of genera *Terschellingia* spp. (all stations), *Sabatieria* spp. (stations A3 and R7), *Paracomesoma* spp. (all stations, especially numerous at A2 and R7), *Daptonema* spp. (Ancão-2, R7) and *Metoncholaimus* spp. (station A2). These nematode genera are typically found in organically rich, muddy sediment and have been proposed to be representative of a community that is well adapted to disturbed conditions (Heip et al., 1990). Nematode communities of Ancão stations were dominated by species of genera *Terschellingia* and *Metalinhomoeus*. Nematode assemblages of Ramalhete stations were dominated by *Spirinia parasitifera* and by genera *Terschellingia spp*. These genera typically inhabit muddy sediments with high organic content sediment (Heip et al., 1990; Moreno et al., 2008) and are well adapted to disturbed environments. Domination of these genera may indicate that *Nematoda* communitites at stations R2 and A2 were under higher disturbance or pollution pressure than communities at other stations.

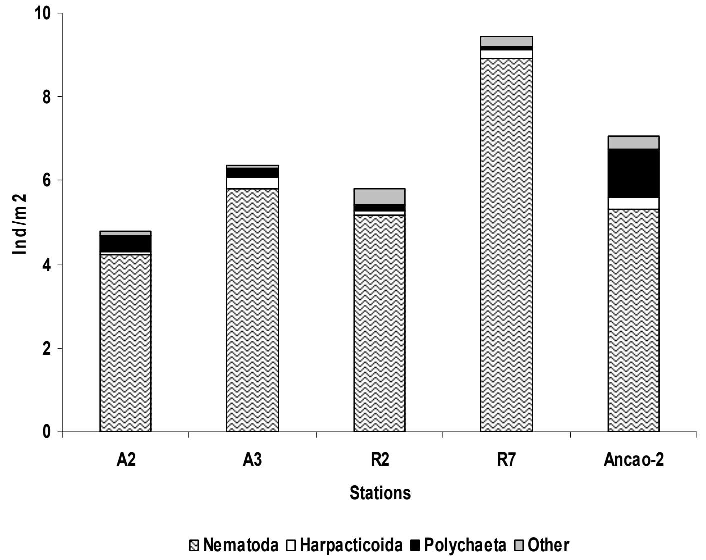


Figure 3.12. Taxonomical composition of meiobenthos by densities for high taxa level, ind/m^2.

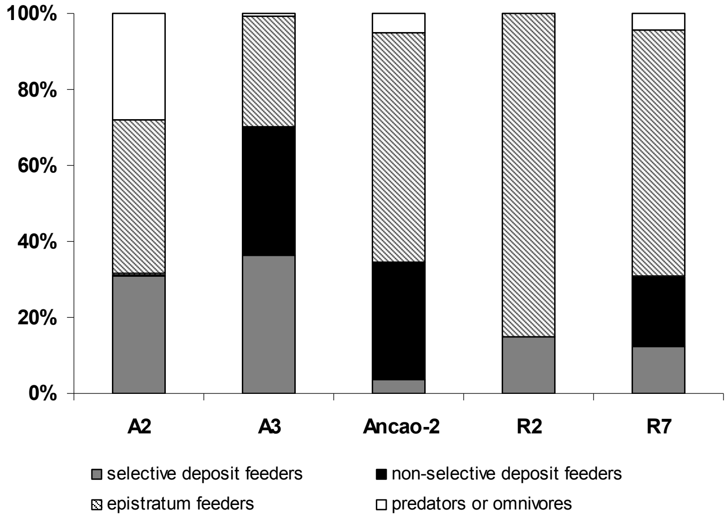


Figure 3.13. Proportions of trophic groups in the *Nematoda* communities of the study sites, %.

Proportions of the *Nematoda* trophic groups were different between stations (Figure 3.13). Selective and non-selective deposit feeders dominated community at station A3 (68.6 %), and comprised 29 to 33.4 % at stations R7, A2 and Ancão-2, where epistratum feeders and predators or omnivores were prevalent groups. Station A2 was characterized by dominance of non-selective deposit feeders and selective deposit feeders (40.53 and 30.82 %), station A3 by selective deposit feeders and epistratum feeders (35.51 and 33.05 %), at the station Ancão-2 non-selective deposit feeders (58,23%) clearly dominated trophic community structure (Figure 3.13). Stations with high TOC and substrate classified as sandy mud – A3 (3.9 % TOC, 85% GS<63 μm) and R7 (5.5 % TOC, 66 % GS<63 μm) had approximately equal proportion of selective and non-selective deposit feeders, combined with high overall density in the case of R7. The station R2 with the most sandy sediment (21 % GS<63 μm) and high TOC (3.72 %) was composed only by two groups: epistratum feeders (approximately 85 %) and selective deposit feeders (15 %). However, small number of sampling points did not give the opportunity to reliably test the relation between meiobenthic community and physical sediment properties.

Table 3.8. Diversity measures and genus-based indices for *Nematoda* communities across all stations: S – species number, d – Margalef species richness, J' – Pielou's evenness, H' – Shannon diversity, MI - Maturity index, ITD - index of trophic diversity.

Site	**S**	**d**	**J'**	**H'**	**MI**	**ITD**
A2	10	1.92	0.88	1.98	3.09	0.34
A3	11	2.30	0.85	2.03	2.50	0.31
Ancão-2	13	2.64	0.83	2.05	2.40	0.43
R2	16	3.50	0.80	2.23	3.01	0.75
R7	18	3.80	0.78	2.25	2.69	0.36

Diversity parameters and community indices are tabulated in Table 3.8. Shannon diversity index values (H') were generally slightly higher at Ramalhete stations R2 and R7 corresponding to maximum total meiofaunal density (R7). Pielou evenness index (J') was similar between Ancão and Ramalhete stations. Station A2 had minimum total and *Nematoda* densities and lower diversity. Maturity Index was highest at A2 and R2 stations, indicating less disturbed environment compared to A3 and Ancão-2. Although the differences of trophic composition between the assemblages were found, the Index of Trophic Diversity (ITD) showed similar values (0.31 – 0.43) at all Ancão basin stations. The highest ITD value was at station R2 (0.75), indicating lowest trophic diversity and dominance of one feeding group.

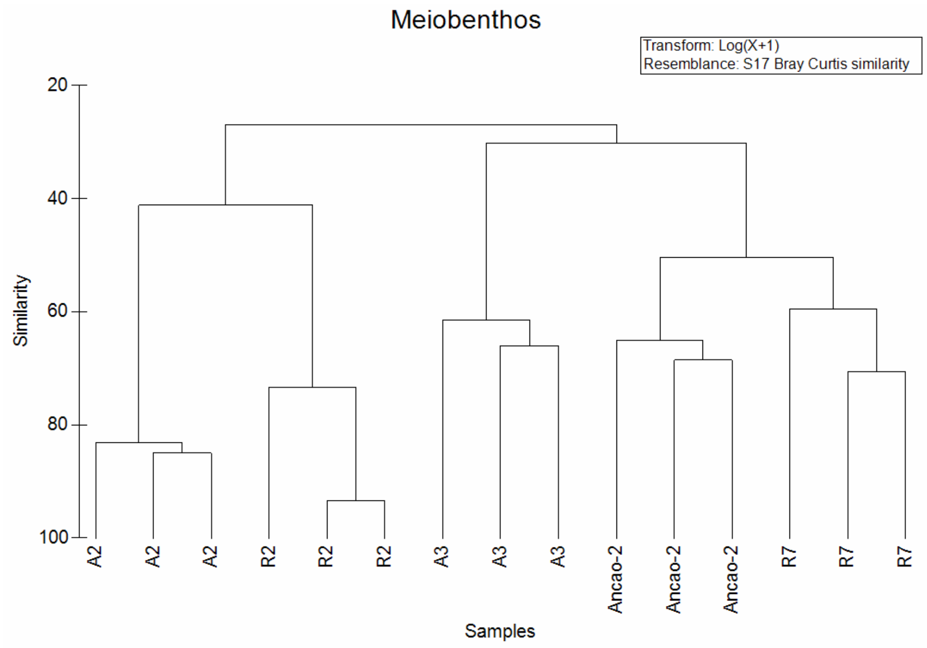


Figure 3.14. Cluster analysis dendrogram showing similarity of stations by *Nematoda* species composition. Stations were grouped by its replicates at the levels of 60 – 90%, and between stations at the levels of 50 to 27%.

Cluster analysis performed on proportions of *Nematoda* species (Figure 3.14) revealed that stations were not grouped according to the two main sites they belonged to. As expected, group average similarity was highest between replicate samples. Communities of different stations were grouped at the similarity levels of 50 to 27%. In case of stations clusters A2 – R2 and A3 – 'Ancão-2' – R7 similarity with station from different study site was greater than with adjacent station from the same site.

3.3. Bird assemblages

3.3.1. Bird densities and species composition

Bird assemblages of the intertidal flats on the study sites consisted mainly of 13 species (Table 3.9), and also of several wader species which occurred irregularly and in low numbers and though were not considered (Curlew Sandpiper *Calidris ferruginea*, Oystercatcher *Haematopus ostralegus*, Little Stint *Calidris minuta*). Excluded from the observations were gulls, ducks, White Storks *Ciconia ciconia*, and Black-winged Stint *Himantopus himantopus.* Shorebird communities of the study sites consisted from the same species, with except Red Knot, which was not present at Ancão.

Table 3.9. Main shorebird species of the study sites. Abundances recorded during all period of observations.

Species		Ancão	Ramalhete	Ancão-2
Turnstone	*Arenaria interpres*	297	229	46
Sanderling	*Calidris alba*	169	55	32
Dunlin	*Calidris alpina*	248	1645	211
Red Knot	*Calidris canutus*	0	657	3
Kentish plover	*Charadrius alexandrinus*	398	87	126
Ringed plover	*Charadrius hiaticula*	124	29	16
Bar-tailed Godwit	*Limosa lapponica*	118	12	19
Black-tailed Godwit	*Limosa limosa*	2	9	35
Curlew	*Numeius arquata*	4	6	8
Whimbrel	*Numenius phaeopus*	105	47	17
Grey plover	*Pluvialis squatarola*	123	40	34
Greenshank	*Tringa nebularia*	15	6	0
Redshank	*Tringa totanus*	49	90	41
Total		1652	2912	586

Seasonal variation of the bird densities is displayed in the Figure 3.15. Densities were high in spring and autumn and low in summer, at all sites. The highest average densities were found at Ramalhete (50 - 63 birds/ha) in spring and autumn, while at Ancão-2 densities were the lowest from the three sites at the level of 7 – 17 birds/ha. In summer, bird densities were as low as 0.8 birds/ha at Ramalhete and 7 – 10 birds/ha at Ancão and Ancão-2 sites.

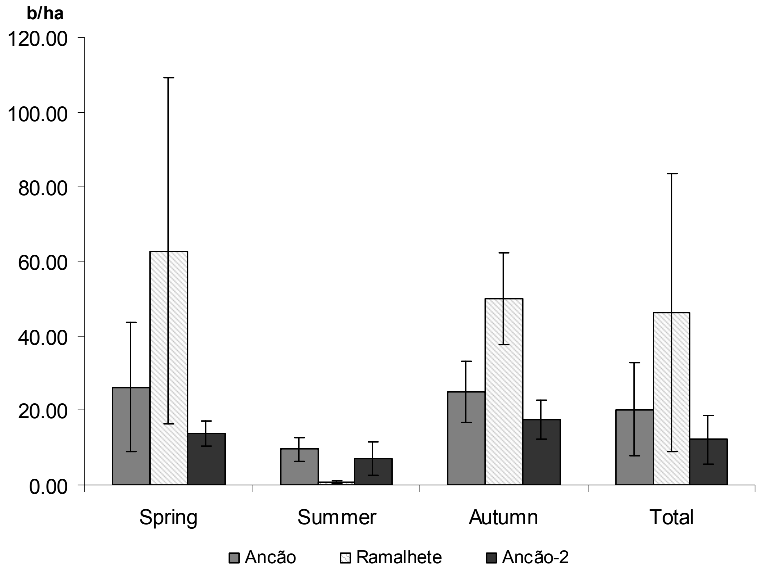
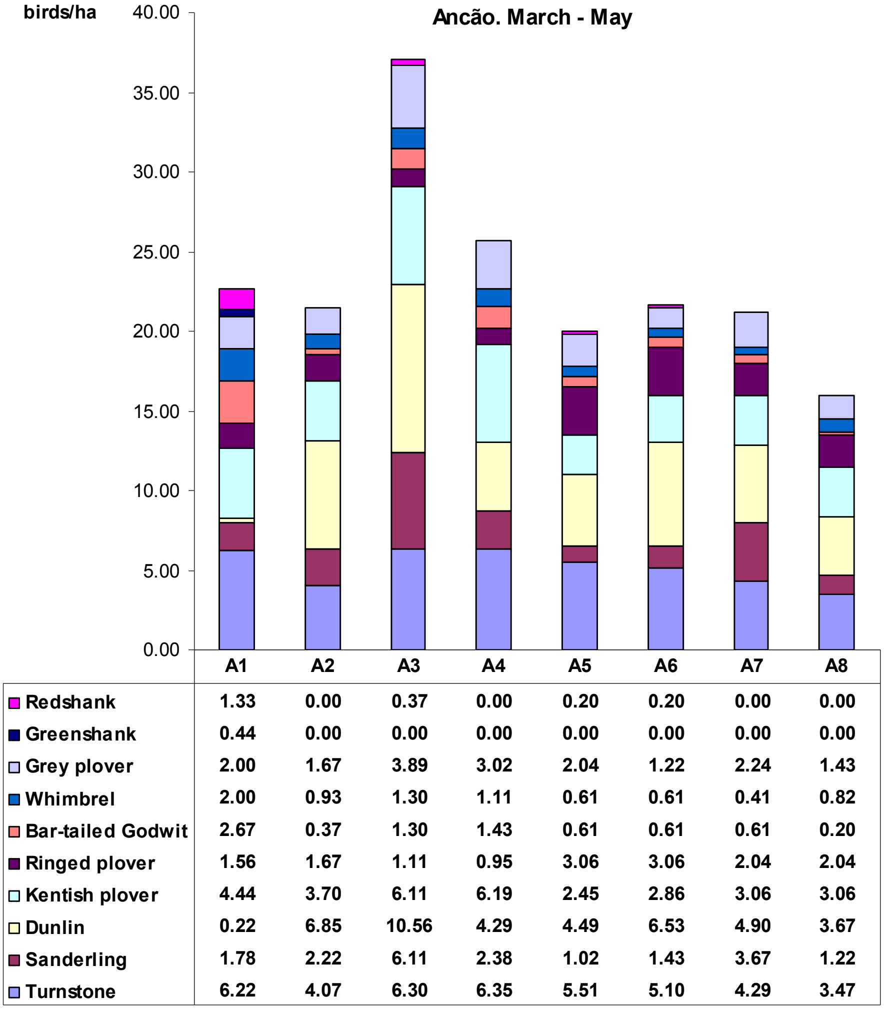


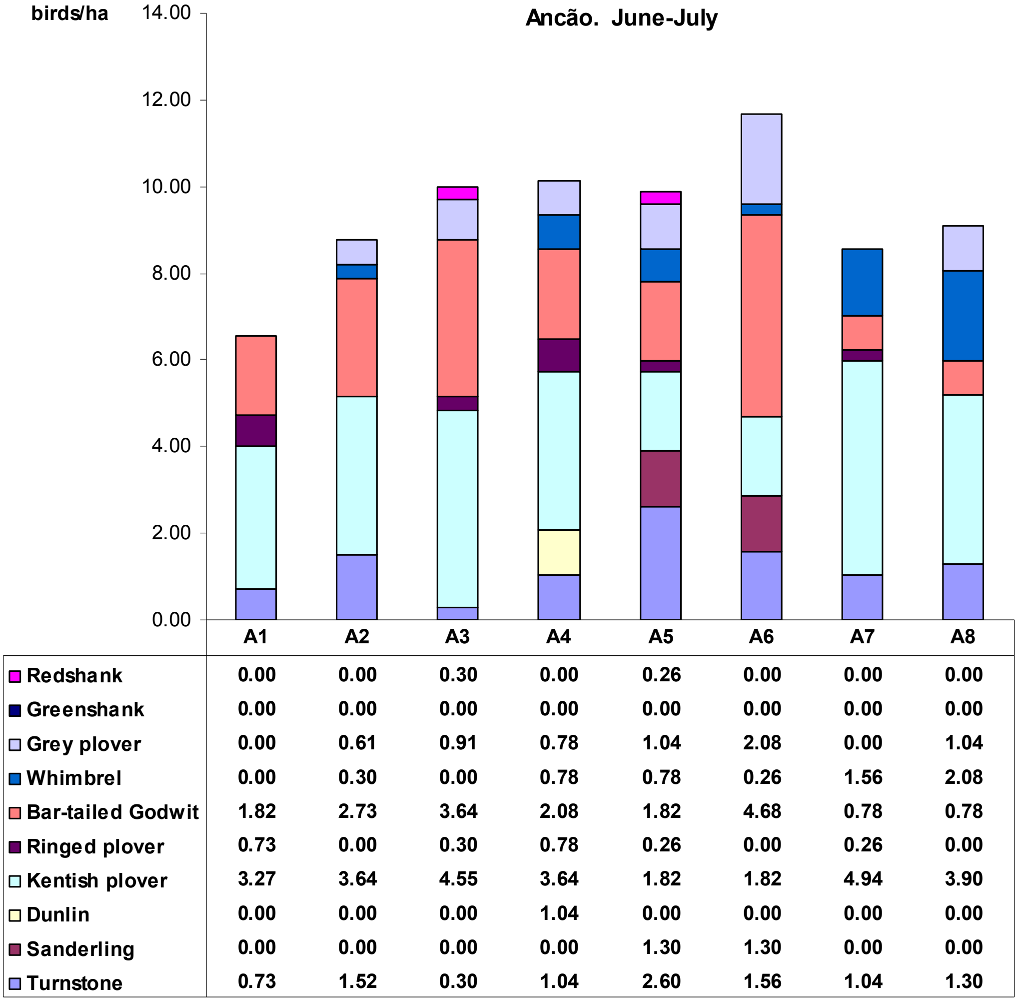
Figure 3.15. Seasonal variation of total bird densities at the study sites; mean densities ±SD.



	A1	A2	A3	A4	A5	A6	A7	A8
Redshank	1.33	0.00	0.37	0.00	0.20	0.20	0.00	0.00
Greenshank	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Grey plover	2.00	1.67	3.89	3.02	2.04	1.22	2.24	1.43
Whimbrel	2.00	0.93	1.30	1.11	0.61	0.61	0.41	0.82
Bar-tailed Godwit	2.67	0.37	1.30	1.43	0.61	0.61	0.61	0.20
Ringed plover	1.56	1.67	1.11	0.95	3.06	3.06	2.04	2.04
Kentish plover	4.44	3.70	6.11	6.19	2.45	2.86	3.06	3.06
Dunlin	0.22	6.85	10.56	4.29	4.49	6.53	4.90	3.67
Sanderling	1.78	2.22	6.11	2.38	1.02	1.43	3.67	1.22
Turnstone	6.22	4.07	6.30	6.35	5.51	5.10	4.29	3.47

Figure 3.16. Average bird densities in the sampling quadrats at Ancão in March – May, birds/ha.

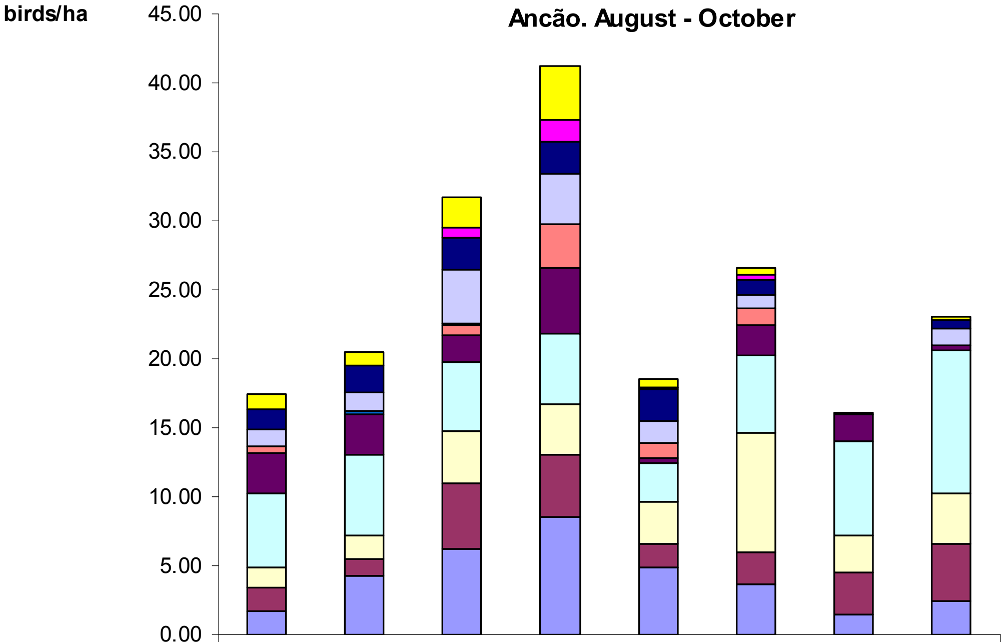
Ancão. At the Ancão site during spring pre-nuptial migration in March – May birds densities of all species varied from 12 to 37 b/ha (Fig. 3.16). The distribution of the birds was uneven between quadrats. Maximum densities occurred in the quadrat A3 (37 b/ha) and A4 (26 b/ha), both located on the banks of the wide intertidal channel. Other quadrats had densities of 13 – 23 b/ha. The structure of the assemblage was similar between counting quardats. Dunlin was dominant species (except in quadrat A1) and occurred in densities from 0.2 to 10.5 b/ha, 4.7 b/ha on average. Second most abundant species was Kentish Plover with 3.6 b/ha on average. Turnstone was equally present in each quadrat with densities of 2.7 – 6.3 b/ha. Other ordinary species were Sanderling, Grey plover, Ringed Plover, Whimbrel, Bar-tailed Godwit.



	A1	A2	A3	A4	A5	A6	A7	A8
■ Redshank	0.00	0.00	0.30	0.00	0.26	0.00	0.00	0.00
■ Greenshank	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
■ Grey plover	0.00	0.61	0.91	0.78	1.04	2.08	0.00	1.04
■ Whimbrel	0.00	0.30	0.00	0.78	0.78	0.26	1.56	2.08
■ Bar-tailed Godwit	1.82	2.73	3.64	2.08	1.82	4.68	0.78	0.78
■ Ringed plover	0.73	0.00	0.30	0.78	0.26	0.00	0.26	0.00
■ Kentish plover	3.27	3.64	4.55	3.64	1.82	1.82	4.94	3.90
■ Dunlin	0.00	0.00	0.00	1.04	0.00	0.00	0.00	0.00
■ Sanderling	0.00	0.00	0.00	0.00	1.30	1.30	0.00	0.00
■ Turnstone	0.73	1.52	0.30	1.04	2.60	1.56	1.04	1.30

Figure 3.17. Average bird densities at the sampling quadrats at Ancão in June – July, birds/ha.

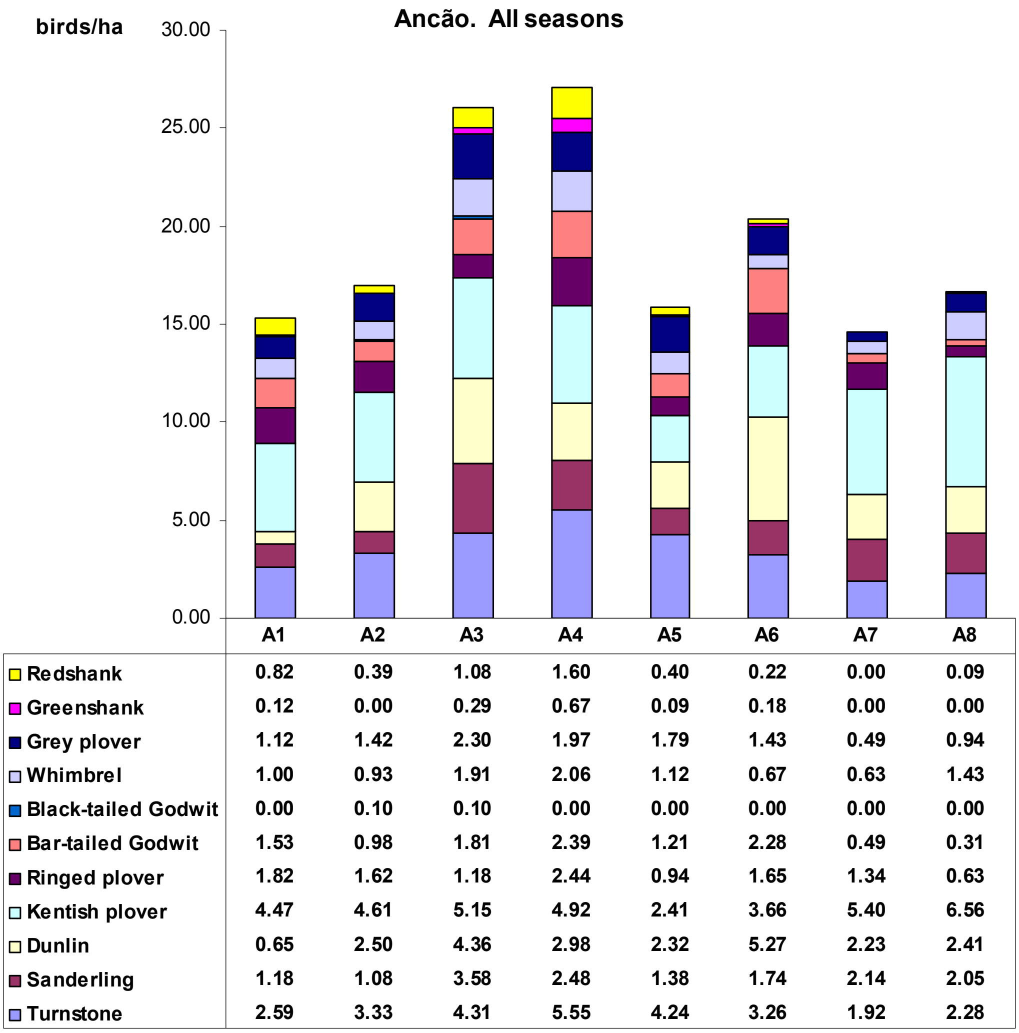
During summer the densities decreased to 6 – 12 birds/ha, and the density was more even between quadrats (Figure 3.17). The structure of assemblage also changed, most notably there were almost no Dunlins and Kentish Plover dominated together with Bar-tailed Godwit. Turnstone and Whimbrel were also present in smaller numbers than in spring.



	A1	A2	A3	A4	A5	A6	A7	A8
■ Redshank	1.14	0.95	2.14	3.88	0.61	0.41	0.00	0.20
■ Greenshank	0.00	0.00	0.71	1.63	0.20	0.41	0.00	0.00
■ Grey plover	1.43	1.90	2.38	2.24	2.24	1.02	0.00	0.61
■ Whimbrel	1.14	1.43	3.81	3.67	1.63	1.02	0.00	1.22
■ Black-tailed Godwit	0.00	0.24	0.24	0.00	0.00	0.00	0.00	0.00
■ Bar-tailed Godwit	0.57	0.00	0.71	3.27	1.02	1.22	0.20	0.00
■ Ringed plover	2.86	2.86	1.90	4.69	0.41	2.24	1.84	0.41
■ Kentish plover	5.43	5.95	5.00	5.10	2.86	5.51	6.94	10.41
■ Dunlin	1.43	1.67	3.81	3.67	3.06	8.78	2.65	3.67
■ Sanderling	1.71	1.19	4.76	4.49	1.63	2.24	3.06	4.08
■ Turnstone	1.71	4.29	6.19	8.57	4.90	3.67	1.43	2.45

Figure 3.18. Average bird densities in the sampling quadrats at Ancão in August – October, birds/ha.

During autumn migration in August – October densities increased and were from 16 to 41 b/ha (Fig. 3.18). The proportion of Dunlin in the community was much less than in spring, and the community was more diverse with no clear dominance of any species in most sampling units. The highest densities were encountered in the same quadrats A3 and A4 as in spring, 32 and 41 birds/ha, respectively.

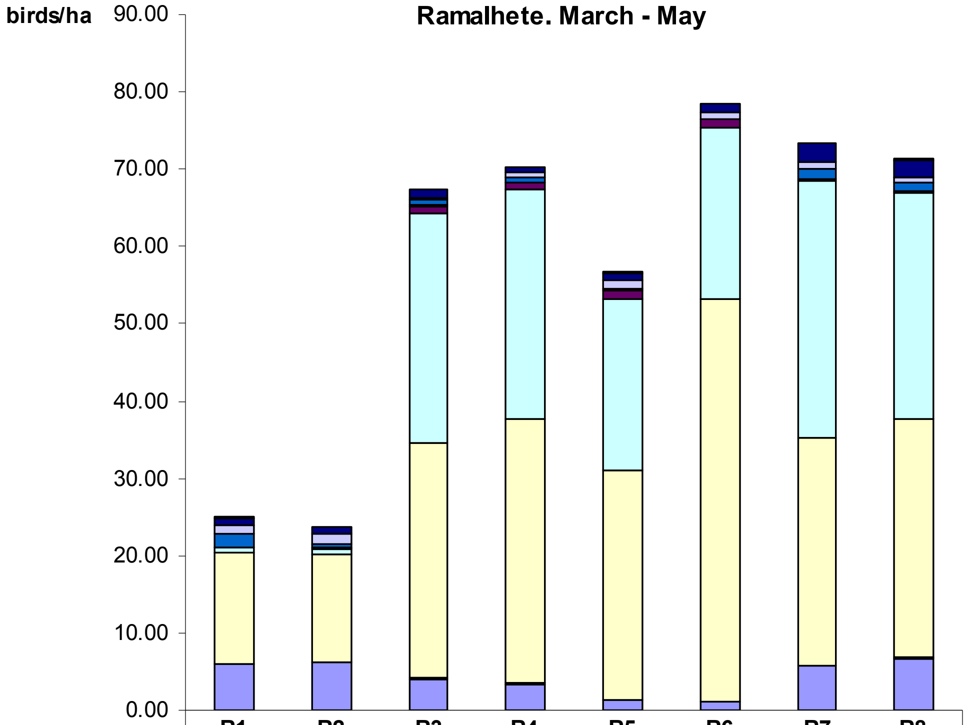


	A1	A2	A3	A4	A5	A6	A7	A8
Redshank	0.82	0.39	1.08	1.60	0.40	0.22	0.00	0.09
Greenshank	0.12	0.00	0.29	0.67	0.09	0.18	0.00	0.00
Grey plover	1.12	1.42	2.30	1.97	1.79	1.43	0.49	0.94
Whimbrel	1.00	0.93	1.91	2.06	1.12	0.67	0.63	1.43
Black-tailed Godwit	0.00	0.10	0.10	0.00	0.00	0.00	0.00	0.00
Bar-tailed Godwit	1.53	0.98	1.81	2.39	1.21	2.28	0.49	0.31
Ringed plover	1.82	1.62	1.18	2.44	0.94	1.65	1.34	0.63
Kentish plover	4.47	4.61	5.15	4.92	2.41	3.66	5.40	6.56
Dunlin	0.65	2.50	4.36	2.98	2.32	5.27	2.23	2.41
Sanderling	1.18	1.08	3.58	2.48	1.38	1.74	2.14	2.05
Turnstone	2.59	3.33	4.31	5.55	4.24	3.26	1.92	2.28

Figure 3.19. Average bird densities in the sampling quadrats at Ancão site, pooled for all seasons, birds/ha.

Figure 3.19 shows total bird community averaged for all seasons. The maximum densities were observed in quadrats A3 and A4 (26 – 27 b/ha), and lowest densities (13 – 17 birds /ha) in A7 and A5. There can be pointed out three dominating species, namely Dunlin, Turnstone and Kentish Plover.

Ramalhete. In spring densities per quadrat were higher that at Ancão (Figure 3.20), reaching 55 – 78 birds/ha, except for quadrats R1 and R2, where they did not exceed 25 birds/ha. The bird community was very different from Ancão. Dunlin dominated and accounted for 40 – 65 % of total density. The Red Knot was present on migration from end of April until May, but tended to appear in large numbers and feed in dense flocks, so contributed 35 – 50 % to the total wader density.



	R1	R2	R3	R4	R5	R6	R7	R8
■ Redshank	0.28	0.00	0.00	0.00	0.32	0.00	0.00	0.28
■ Grey plover	0.97	0.79	1.11	0.79	0.95	0.95	2.38	2.08
□ Whimbrel	1.11	1.27	0.32	0.63	0.95	0.95	0.95	0.83
■ Bar-tailed Godwit	1.81	0.48	0.63	0.63	0.00	0.00	1.27	1.11
■ Ringed plover	0.00	0.00	0.32	0.00	0.32	0.00	0.00	0.00
■ Kentish plover	0.00	0.32	0.79	0.79	1.11	1.11	0.16	0.14
□ Red Knot	0.56	0.63	29.68	29.68	22.22	22.22	33.33	29.17
□ Dunlin	14.44	13.97	30.32	34.13	29.52	52.06	29.52	30.83
■ Sanderling	0.00	0.00	0.32	0.32	0.00	0.00	0.00	0.28
■ Turnstone	5.97	6.19	3.97	3.33	1.43	1.11	5.71	6.67

Figure 3.20. Average bird densities at the Ramalhete site in spring, birds/ha.

Other species were distributed equally among the quadrats (Grey Plover, Whimbrel, Turnstone) and their densities were comparable with those at Ancão, except for Sanderling, which was almost absent from this site. In summer, the situation was very different (Figure 3.21). The site was not used by birds except by very few Kentish plovers, which used the adjacent salt pans for breeding, and several dunlins. Densities were as low as 0.5 – 2 birds/ha. At the same time, Dunlins were observed at the adjacent salt pans, albeit in small numbers.

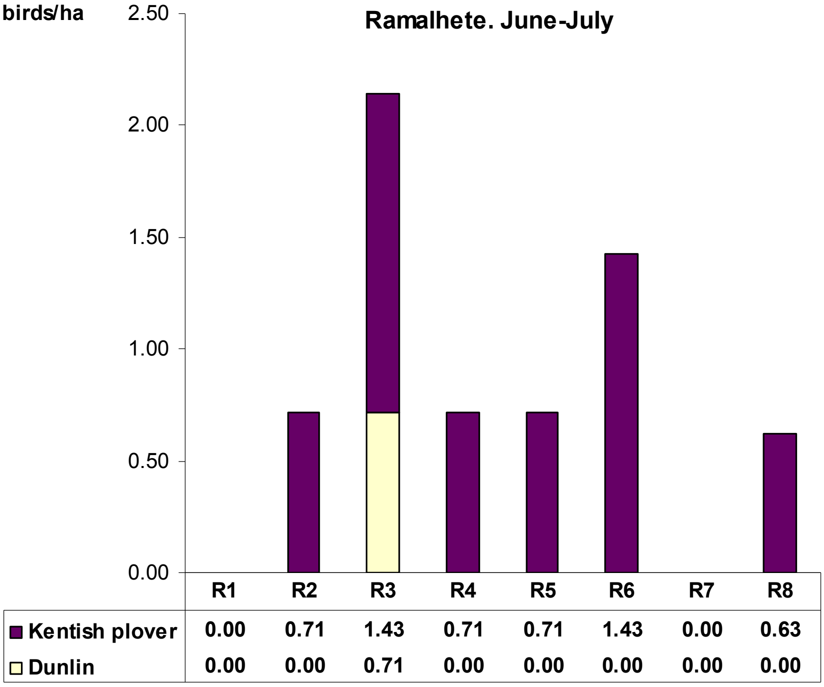
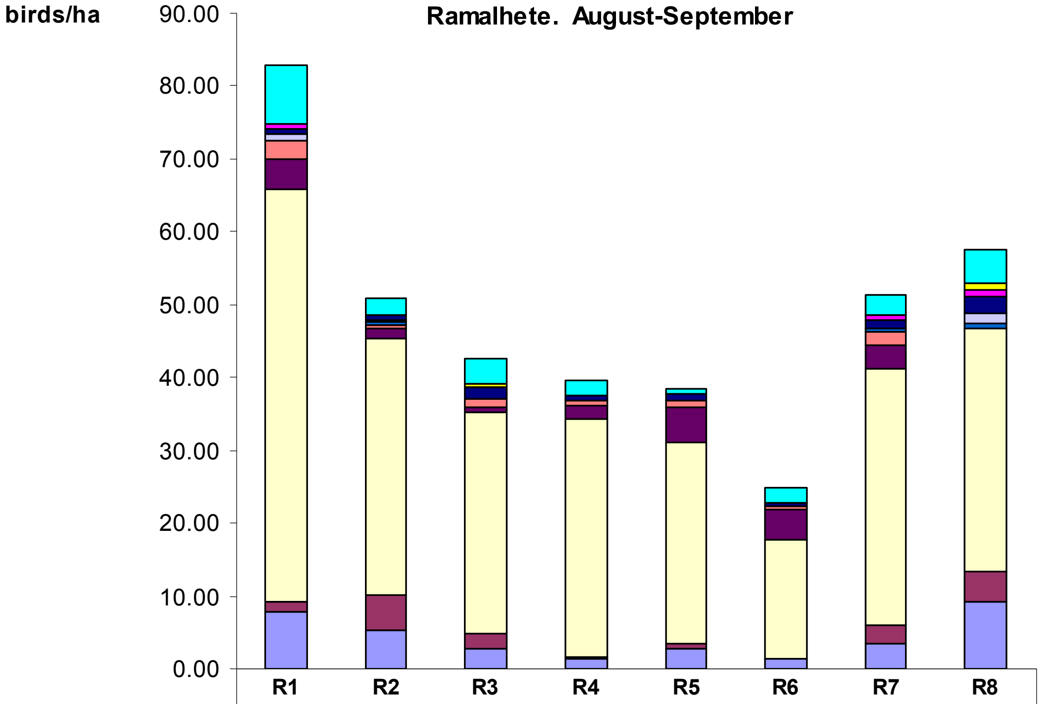


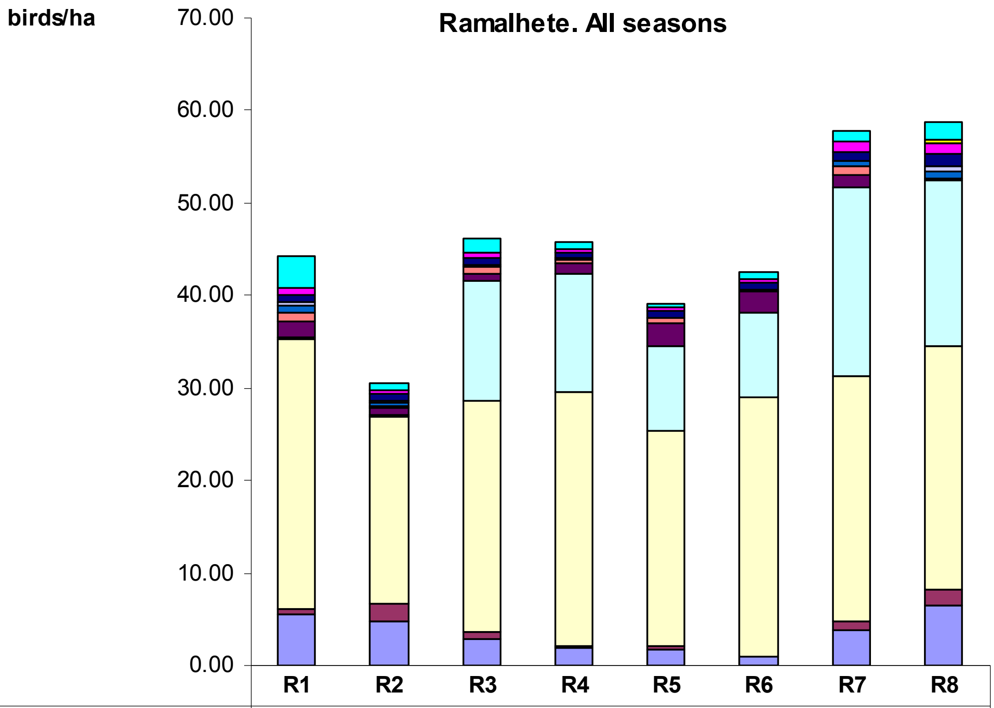
Figure 3.21. Average bird densities at the Ramalhete site in summer, birds/ha.

In August – September, during post-nuptial migration, bird densities raised again to 25 – 83 birds/ha (Figure 3.22). Quadrat R1 had the highest density, followed by R8 and R7. Typical density was around 40 birds/ha. Similarly with spring, Dunlin dominated the assemblage, comprising densities 28 – 57 birds/ha. Relatively abundant were Redshank and Turnstone, but no Red Knots were encountered. Presence of Red Knot in spring contributed greatly to the generalized density on this site (Figure 3.23).



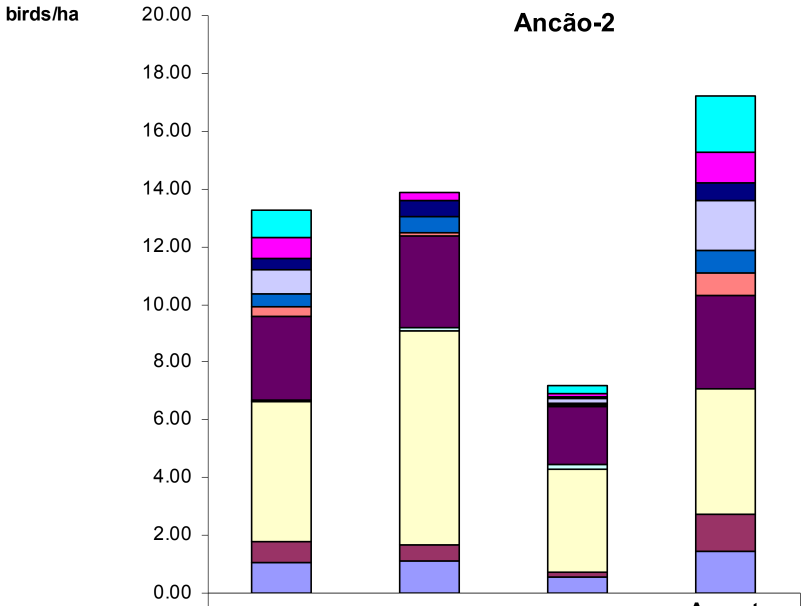
	R1	R2	R3	R4	R5	R6	R7	R8
Redshank	8.06	2.22	3.49	2.22	0.63	1.90	2.86	4.44
greenshank	0.00	0.00	0.32	0.00	0.00	0.00	0.00	1.11
Grey plover	0.56	0.00	0.00	0.00	0.00	0.00	0.63	0.83
Whimbrel	0.83	0.63	1.59	0.63	0.95	0.63	1.27	2.22
Black-tailed Godwit	0.83	0.32	0.00	0.00	0.00	0.00	0.00	1.39
Bar-tailed Godwit	0.00	0.32	0.00	0.00	0.00	0.00	0.32	0.83
Ringed plover	2.50	0.63	1.27	0.63	0.95	0.32	1.90	0.00
Kentish plover	4.17	1.27	0.63	1.90	4.76	4.13	3.17	0.00
Red Knot	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dunlin	56.67	35.24	30.48	32.70	27.62	16.51	35.24	33.33
Sanderling	1.39	4.76	1.90	0.32	0.63	0.00	2.54	4.17
Turnstone	7.78	5.40	2.86	1.27	2.86	1.27	3.49	9.17

Figure 3.22. Average bird densities at the Ramalhete site in autumn.



	R1	R2	R3	R4	R5	R6	R7	R8
■ Redshank	3.41	0.91	1.43	0.91	0.39	0.78	1.17	1.93
■ Greenshank	0.11	0.00	0.13	0.00	0.00	0.00	0.00	0.45
■ Grey plover	0.63	0.32	0.45	0.32	0.39	0.39	1.23	1.19
■ Whimbrel	0.80	0.78	0.78	0.52	0.78	0.65	0.91	1.25
■ Black-tailed Godwit	0.34	0.13	0.00	0.00	0.00	0.00	0.00	0.57
■ Bar-tailed Godwit	0.74	0.32	0.26	0.26	0.00	0.00	0.65	0.80
■ Ringed plover	1.02	0.26	0.65	0.26	0.52	0.13	0.78	0.00
■ Kentish plover	1.70	0.78	0.84	1.23	2.53	2.40	1.36	0.17
■ Red Knot	0.23	0.26	12.86	12.86	9.09	9.09	20.45	17.90
■ Dunlin	29.09	20.13	25.00	27.34	23.38	28.05	26.49	26.25
■ Sanderling	0.57	1.95	0.91	0.26	0.26	0.00	1.04	1.82
■ Turnstone	5.63	4.74	2.79	1.88	1.75	0.97	3.77	6.48

Figure 3.23. Bird densities at Ramalhete, averaged by all seasons, birds/ha.



	All period	May	June-July	August-September
Redshank	0.94	0.00	0.27	1.92
Greenshank	0.00	0.00	0.00	0.00
Grey plover	0.78	0.27	0.14	1.04
Whimbrel	0.39	0.55	0.05	0.66
Black-tailed Godwit	0.80	0.00	0.18	1.70
Bar-tailed Godwit	0.43	0.55	0.05	0.77
Ringed plover	0.37	0.14	0.05	0.77
Kentish plover	2.88	3.16	2.01	3.24
Knot	0.07	0.14	0.14	0.00
Dunlin	4.83	7.42	3.57	4.34
Sanderling	0.73	0.55	0.18	1.32
Turnstone	1.05	1.10	0.55	1.43

Figure 3.24. Bird densities at Ancão-2 in spring, summer and autumn (birds/ha).

Bird densities at the Ancão-2 site were lower and more constant during the study period, and constituted 13 – 17 birds/ha in spring and autumn and 7 birds/ha in summer. Dunlin and Kentish Plover were the most abundant species. Red Knot was present in small number during late spring and summer (June) at density 0.14 bird/ha.

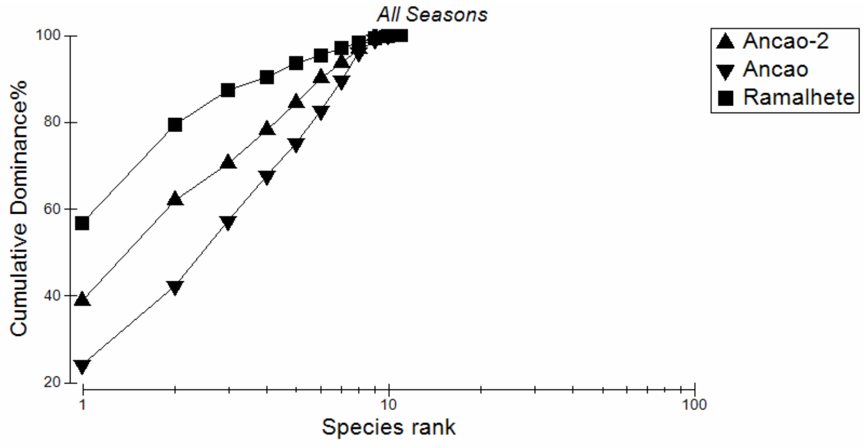


Figure 3.25. Cumulative *k*-dominance plot of bird assemblages at Ancão, Ramalhete and Ancão-2 sites during all seasons (March – October).

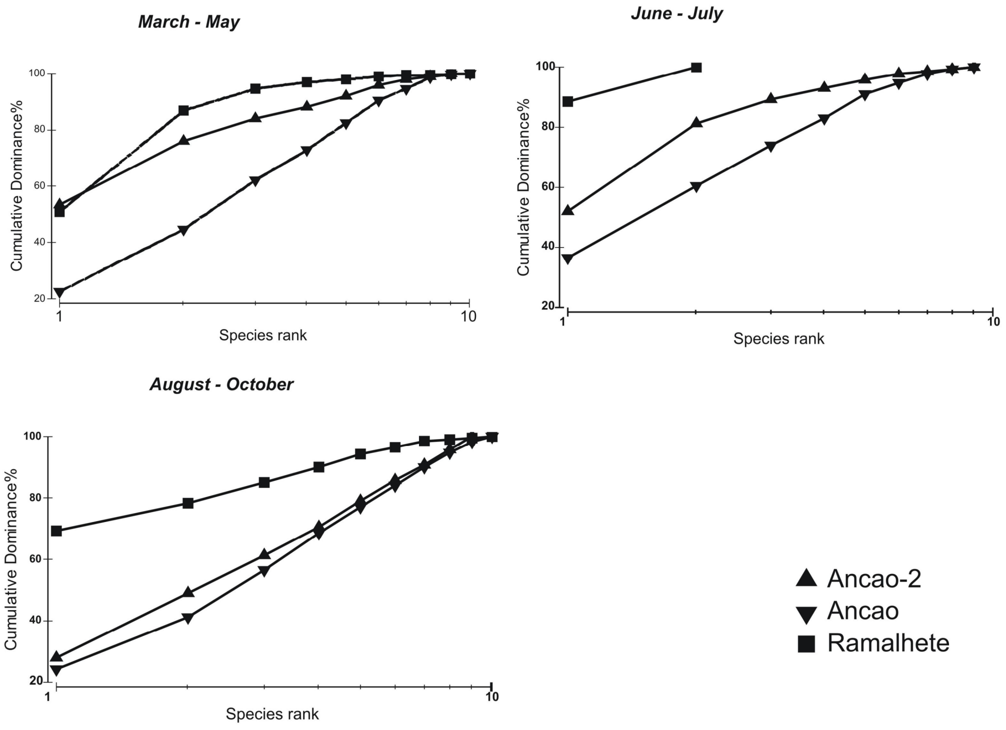


Figure 3.26. Cumulative *k*-dominance plots of bird assemblages at Ancão, Ramalhete and Ancão-2 sites in spring, summer and autumn.

The k-dominance plots (Figures 3.25 and 3.26) demonstrated that in spring (March to May) Ancão and Ramalhete sites had similarly shaped curves, but Ramalhete was heavily dominated by two species (Dunlin, Red Knot). The curve of Ancão-2 showed maximal diversity, with three first species (Dunlin, Turnstone and Kentish Plover) accounting for only 60 % of total bird density. The *k*-dominance curves showed that bird assemblages of Ramalhete site were relatively less diverse than Ancão-2 and Ancão, respectively, that was confirmed by diversity indices (Table 3.10). Between Ancão-2 and Ancão sites no considerable differences in terms of population diversity were found in summer and autumn.

Table 3.10. Number of species (S), Shannon diversity (H), Sheldon evenness $e^{H'}/S$ and species richness (d) of bird communities.

All seasons	**Number of species, S**	**Diversity, H'**	**Evenness, $e^{H'}/S$**	**Species richness, d**
Ancão-2	11	1.91	0.46	3.87
Ancão	11	2.10	0.71	3.41
Ramalhete	12	1.37	0.29	2.79
Spring				
Ancão-2	9	1.43	0.40	3.04
Ancão	10	1.96	0.63	2.97
Ramalhete	11	1.15	0.71	2.39
Summer				
Ancão-2	10	1.40	0.83	4.61
Ancão	9	1.73	0.74	3.56
Ramalhete	2	0.35	0.30	-
Autumn				
Ancão-2	10	3.16	0.61	2.11
Ancão	11	3.12	0.74	2.09
Ramalhete	11	2.48	0.33	1.21

According to diversity indices (Table 3.10) bird communities of Ancão and Ancão-2 sites were more diverse in all seasons than those of Ramalhete. This was caused by dominance of Dunlin and Red Knot at the site, while communities at Ancão sites were more even in terms of species composition (Figure 3.26). Highest diversity was reached at all sites during autumnal migration (Shannon H′ = 2.5 – 3.2).

3.3.2. Comparison of bird assemblages

To compare bird communities of the three study sites univariate and multivariate measures were used. Comparison between mean densities of the species on two main study sites was made using U-test (Table 3.11).

Table 3.11. Summary of U-tests comparing bird densities between Ancão and Ramalhete sites, n=8. Significant values at p<0.05 are in bold, at p<0.001 marked with (*).

Species	U	z	p	U	z	p
	Spring			**Summer**		
Turnstone	24	0,84	0,4008	0	3,59	**0,0003***
Sanderling	0	3,41	**0,0006***	24	1,46	0,1432
Dunlin	0	-3,36	**0,0007***	31.5	0,09	0,9272
Red Knot	0	-3,60	**0,0003***	32	-	-
Kentish Plover	0	3,37	**0,0008***	0	3,59	**0,0003***
Ringed Plover	0	3,46	**0,0005***	21	-1,17	0,2388
Bar-tailed Godwit	29	0,32	0,7516	0	3,59	**0,0003***
Black-tailed Godwit	32	-	-	32	-	-
Whimbrel	30	-0,16	0,8742	8	2,89	**0,0037**
Grey Plover	11	2,21	**0,0272**	8	2,89	**0,0037**
Greenshank	31	0,09	0,9273	32	-	-
Redshank	28	0,84	0,4008	24	1,46	0,1441
	Autumn			**All seasons**		
Turnstone	30	0,21	0,8333	31	0,11	0,9163
Sanderling	21.5	1,10	0,2697	8	2,52	**0,0116**
Dunlin	0	-3,36	**0,0007***	0	-3,36	**0,0008***
Red Knot	32	-	-	0	-3,60	**0,0003***
Kentish Plover	4	2,94	**0,0032**	1	3,25	**0,0011**
Ringed Plover	17	1,58	0,1143	4	2,94	**0,0032**
Bar-tailed Godwit	16	1,75	0,0791	7	2,63	**0,0086**
Black-tailed Godwit	25	-0,89	0,3708	25	-0,89	0,3709
Whimbrel	20	1,26	0,2062	16	1,69	0,0920
Grey Plover	7.5	2,64	**0,0081**	7	2,63	**0,0086**
Greenshank	24	0,97	0,3343	23	0,95	0,3403
Redshank	10	-2,31	**0,0207**	14	-1,89	**0,0585**

During spring (March – May) densities of Sanderling, Dunlin, Red Knot, Kentish Plover and Ringed Plover were found to be significantly different between Ancão and Ramalhete at the level <0.001, and Grey Plover at the level <0.05. Dunlin and Red Knot were considerably more abundant at Ramalhete but all other species were more numerous at Ancão. In summer communities differed by Turnstone, Kentish Plover, Bar-tailed Godwit, Whimbrel and Grey Plover, all species being attributed to Ancão, while densities at Ramalhete considerably decreased. During autumn migration shorebirds assemblages became more similar, with Dunlin, Kentish Plover, Grey Plover (at Ancão) and Redshank (at Ramalhete) being species with significantly (p<0.05) different densities. Considering total integrated population during all observed seasons we conclude that 8 of 12 bird species (75%) demonstrated significant differences in their densities between study sites. Species that were equally present at both sites were Turnstone, Whimbrel and Greenshank (the latter in very small quantity).

To quantify the observed difference between shorebird assemblages Principal Component Analysis (PCA) was performed with densities of bird species as variables, previously log (x+1) transformed (Table 3.12).

Table 3.12. Principal Component Analysis of bird communities structure. Shown is the % variation explained by the first two principal components (PC1, PC2).

Plot	**PC1**	**PC2**
All seasons	72.7	10.9
All seasons - Red Knot excluded	66.9	18.4
March-May	76.5	8.2
March-May - Red Knot excluded	65.8	13.7
June-July	65.7	12.4
August-October	49.4	24.5
August-October - Dunlin excluded	38.2	27.9

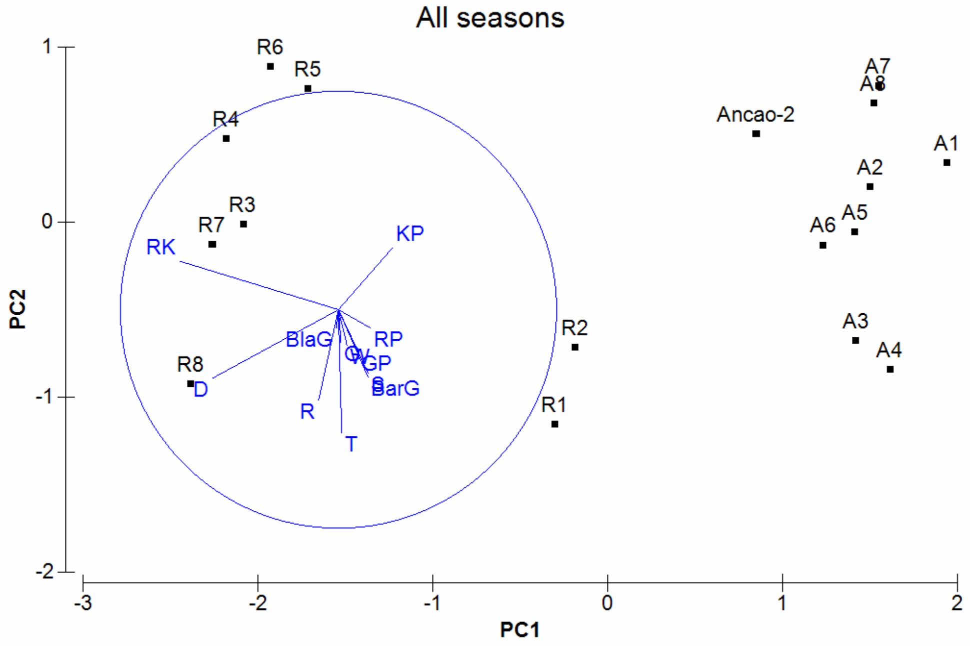


Figure 3.27. PCA plot of bird densities by species pooled for all seasons. *Bird codes*: T – Turnstone, S – Sanderling, D – Dunlin, RK – Red Knot, KP – Kentish Plover, RP – Ringed Plover, BarG – Bar-tailed Godwit, BlaG – Black-tailed Godwit, W – Whimbrel, GP – Grey Plover, G – Greenshank, R – Redshank.

Generalized plot of bird communities for all seasons and stations (Fig. 3.27) showed that the sampling quadrats from Ancão and Ramalhete were grouped into two clusters along the PC1. This is explained by greater densities of Dunlin and Red Knot at Ramalhete, also stations from Ancão (without Red Knot and moderate density of Dunlin) are negatively related to PC1. At the Ancão site Red Knot was absent during all studied seasons. Ancão-2 site had bird community similar to Ancão, but Red Knot was present there in the beginning of summer.

To diminish the influence of high densities of Red Knots in spring at the Ramalhete site on the final result, they were excluded from further analysis (Fig. 3.28). Nonetheless, the quadrats were grouped in a similar way, showing difference by proportions of other species.

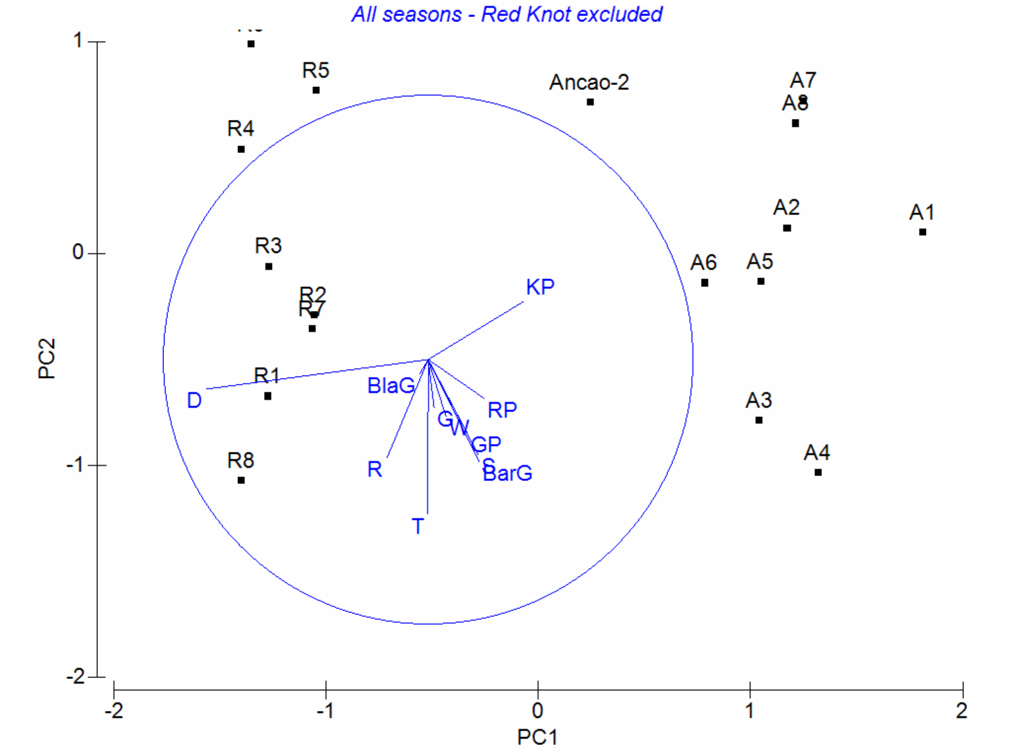


Figure 3.28. PCA plot of bird densities by species for all seasons; Red Knot excluded. *Bird codes*: see Fig. 3.27.

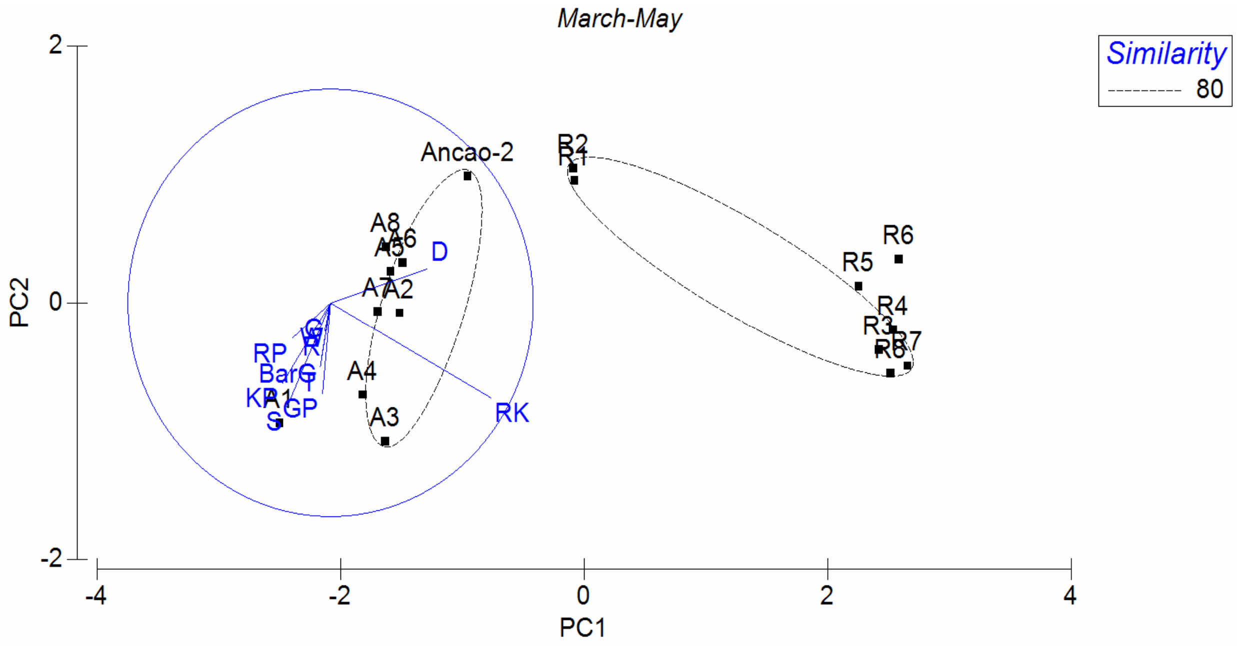


Figure 3.29. PCA plot of bird densities by species in March - May. *Bird codes*: see Fig. 3.27

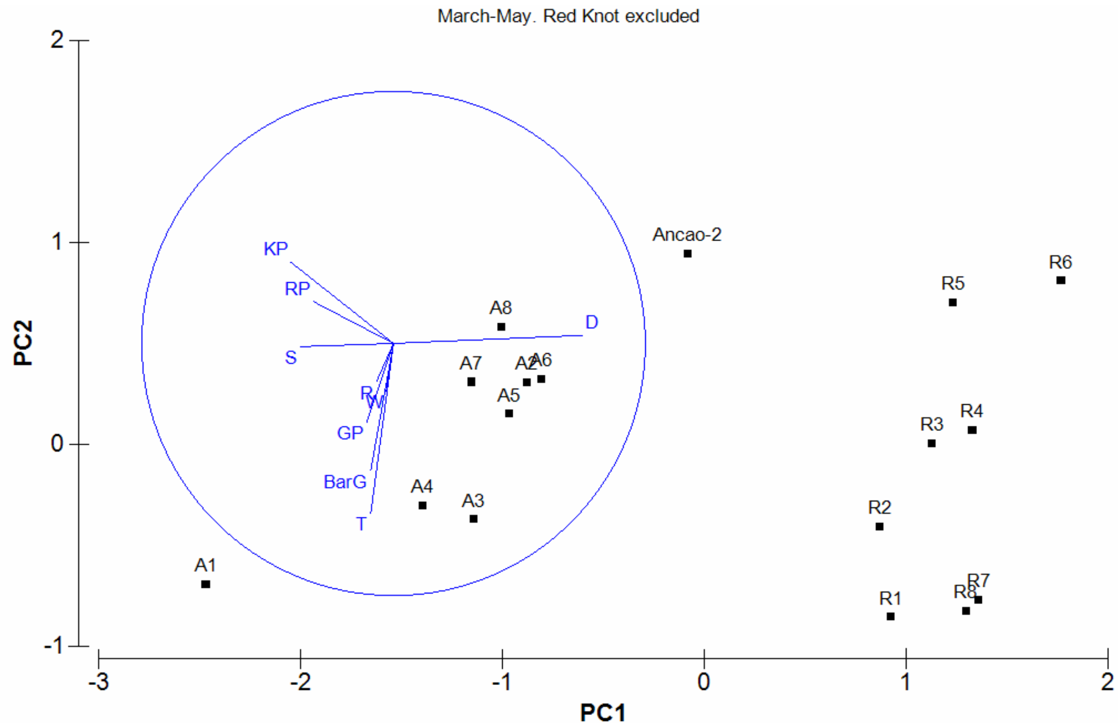


Figure 3.30. PCA plot of bird densities by species in March – May, Red Knot excluded from analysis. *Bird codes*: see Fig. 3.27.

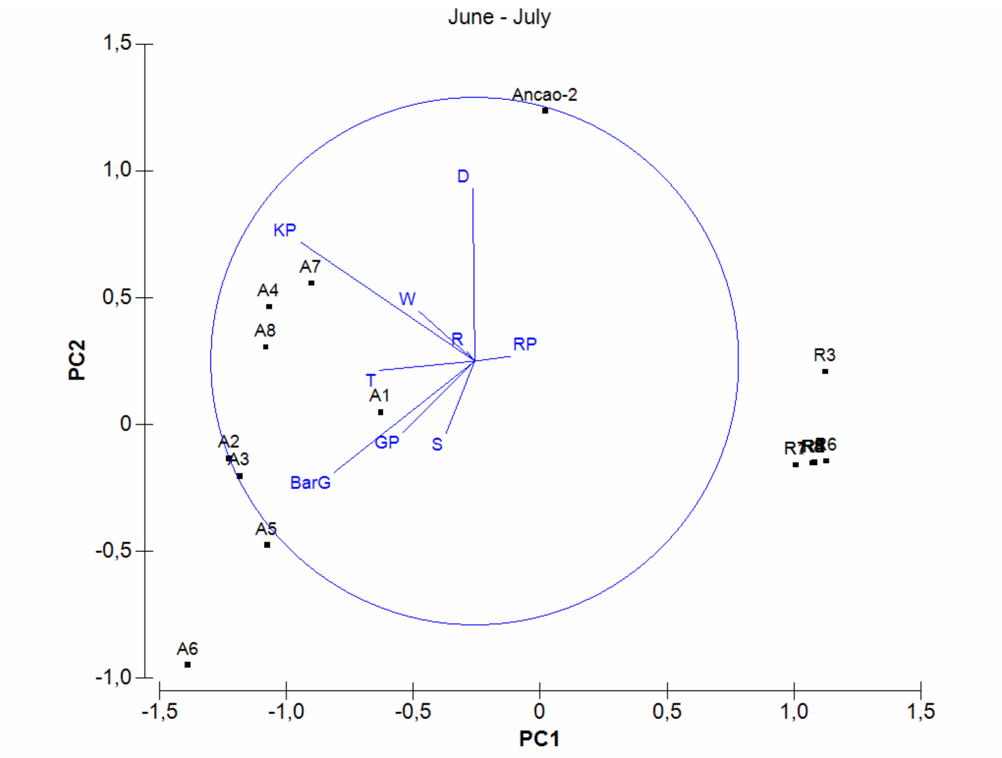


Figure 3.31. PCA plot of bird densities by species in June – July. *Bird codes*: see Fig. 3.27.

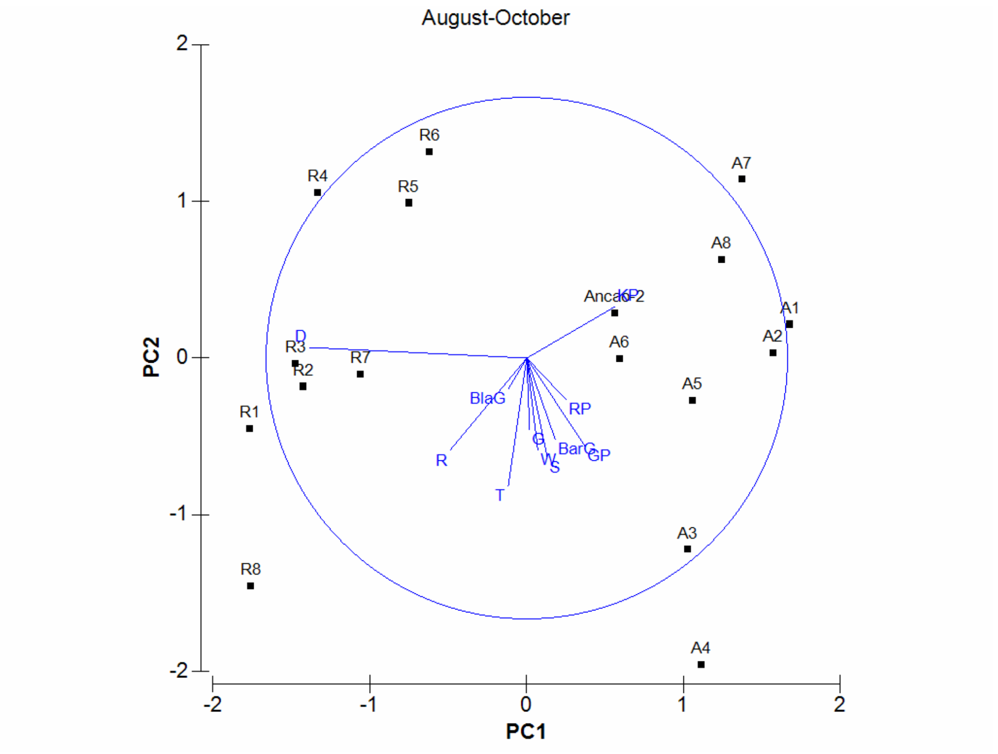


Figure 3.32. PCA plot of bird densities by species in August-October. *Bird codes*: see Fig. 3.27.

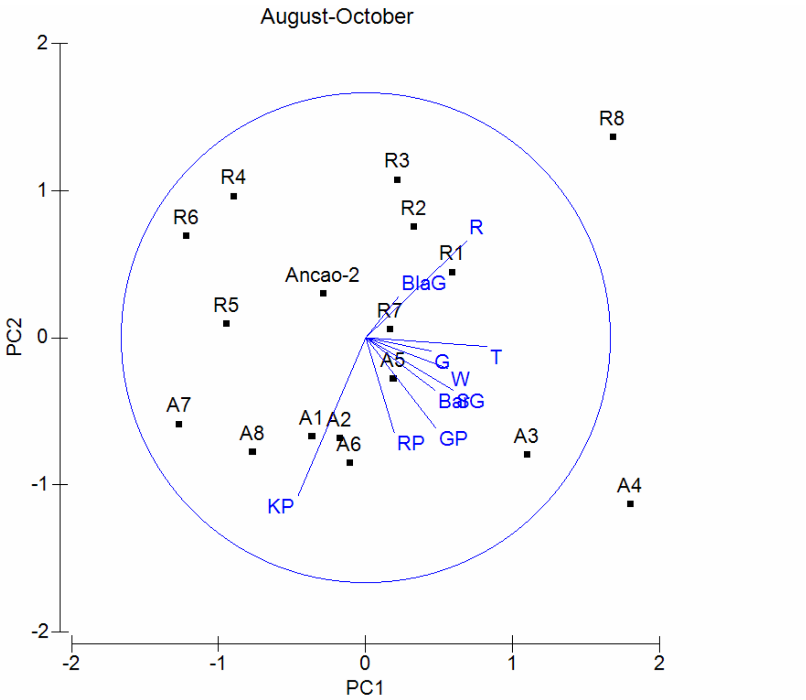


Figure 3.33. PCA plot of bird densities by species in August-October with Dunlin densities excluded. *Bird codes*: see Fig. 3.27.

In the resulting plot of PCA carried out for data from all seasons (Fig. 3.27 – 3.33) stations from Ancão and Ramalhete were grouped into two distinct site clusters. This revealed that structures of bird communities were different by relative abundance of every species. In June – July it is notable that while Ramalhete was almost free of birds, Ancão maintained several species (Figure 3.31).

Cluster analysis was performed over bird species density data for each season (Figure 3.34), with exception of Ramalhete in summer because of very low bird densities observed at that site.

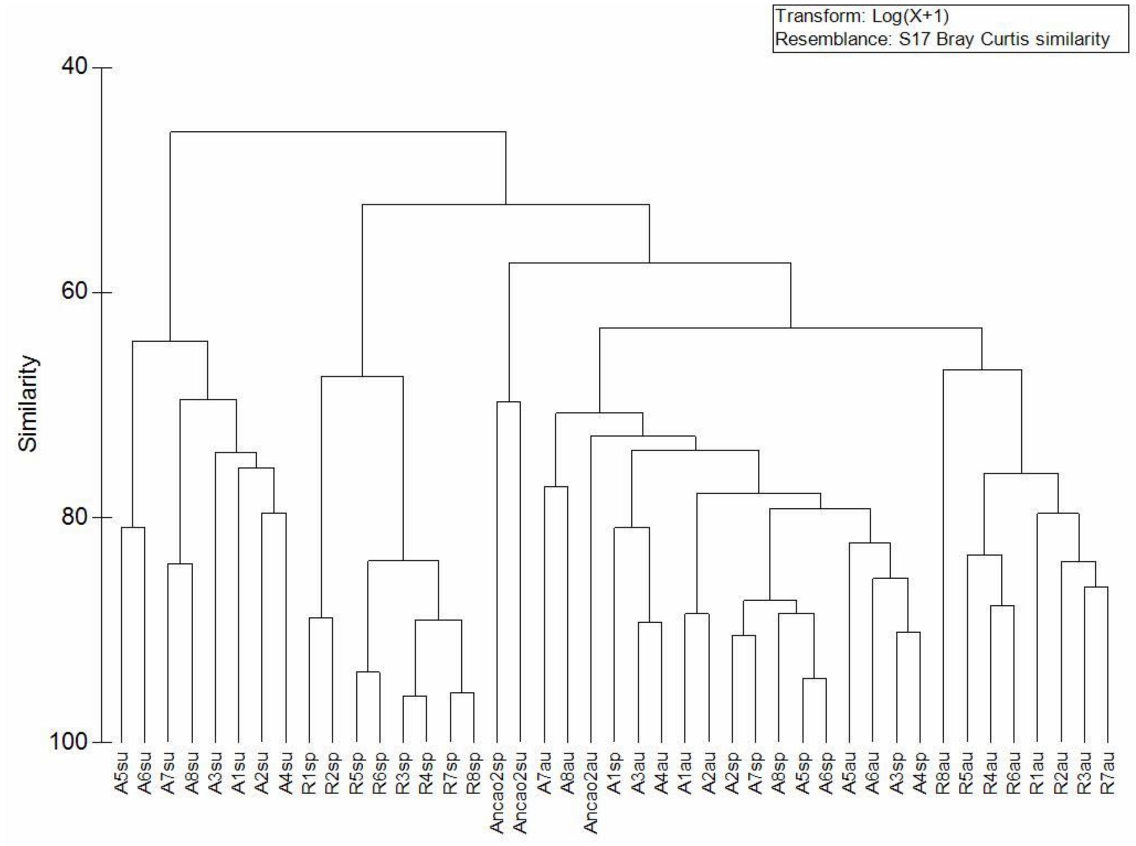


Figure 3.34. Cluster analysis dendrogram using Bray-Curtis similarity of log(x+1) transformed data showing grouping of single counting quadrats by bird densities of 12 species in spring (sp), summer (su) and autumn (au). Season and site were important factors contributing to similarity of bird assemblages.

With some exceptions, sampling units grouping were induced by site and season factors. Between-site clustering occurred on the level of similarity of not more than approximately 65 %, while within-site and within-season clustering appeared at the level of similarity between 97 and 70%. This suggests that bird communities of the study sites were different between sites and less so between seasons at one site.

3.4. Relation between sediment properties, benthos and bird densities

Tested against sediments properties (Spearman rank correlation) bird densities did not show significant correlations in most cases (Table 3.13). At Ancão, Kentish Plover had a marginal correlation with TOC (0.500, p=0.104). At Ramalhete Dunlin had a significant (r_s = -

0.756, p=0.015) negative correlation with redox, and total bird density was marginally associated with TOC and negatively with sand % (r_s = 0.500, p=0.104).

Table 3.13. Spearman rank correlation coefficient r_s between bird densities and environmental variables, significance given in parenthesis, n=8. Significant values at p<0.1 are in bold.

Species	Silt-Clay<63µm %	Sand %	TOC	E_h
Ancão				
Total bird density	-0.143 (0.736)	0.214 (0.305)	0.405 (0.320)	0.143 (0.368)
Dunlin	-0.286 (0.493)	0.190 (0.326)	0.381 (0.352)	0.286 (0.246)
Kentish Plover	0.048 (0.455)	0.262 (0.265)	**0.500 (0.104)**	0.119 (0.389)
Turnstone	0.095 (0.411)	-0.119 (0.389)	0.405 (0.320)	0.238 (0.285)
Ringed Plover	0.190 (0.326)	0.048 (0.455)	-0.238 (0.570)	0.119 (0.389)
Ramalhete				
Total bird density	0.381 (0.176)	**-0.524 (0.091)**	**0.500 (0.104)**	-0.317 (0.222)
Dunlin	0.286 (0.246)	-0.333 (0.210)	0.310 (0.228)	**-0.756 (0.015)**
Kentish Plover	0.333 (0.210)	-0.167 (0.347)	-0.143 (0.368)	0.146 (0.365)
Red Knot	0.386 (0.173)	-0.422 (0.149)	0.289 (0.244)	-0.037 (0.465)

These correlations should be interpreted with caution because of the low number of sampling units (N=8) and adjacent spatial location of quadrates, which could produce intercorrelation induced by spatial aspect (Hurlbert, 1984).

Multivariate analysis by means of Partial Least Squares was used to illustrate the effect of the complex of factors on bird density (Figures 3.35 – 3.37). The explanatory variables used were sediment characteristics (TOC, Eh, %GS<63µm) and benthos community parameters (total macrobenthos density, meibenthos high taxa densities).

Physical sediment charactesistics were used as explanatory variables in relation with total bird densities during all study period (Fig. 3.35). On the 2-dimensional plot, bird species formed two groupes along the w*c[1] axis. The first group was assosiated with high TOC content and included such variables as total bird densities, Dunlin, Red Knot, Redshank and Black-tailed Godwit. This species group was more abundant in community of the Ramalhete site, and as TOC at that site was generally higher than on Ancão, this may have caused such grouping. The second group consisted of the rest of species (except Turnstone) and was positively related to redox potential. As redox values were overall higher on the Ancão site (Table 3.6), the group can be considered corresponding to species which prefered Ancão. All bird species except Black-tailed Godwit were positively plotted with silt and clay (%GS<63 µm) content along the w*c[2] axis. The mark of Turnstone was located in the center of both axis, so this species did not show clear relation with sediment characteristics.

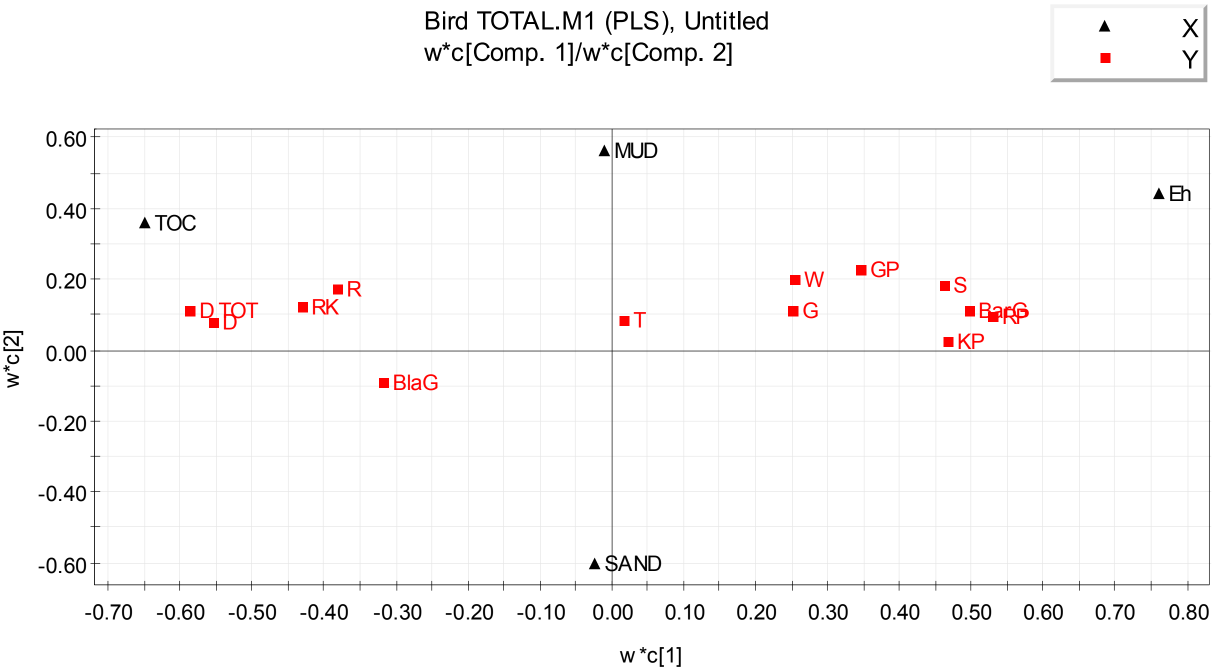


Figure 3.35. PLS plot showing birds average densities in relation with sediment variables, n=5. Independent variables (X): % GS<63 µm (Mud), TOC, E_h. Dependent variables (Y): average bird density during all study period, n=17. Bird codes as in Figure 3.27.

Macrobenthos species identified at study sites (Appendix B) were mainly represented by *Polychaeta*, *Gastropoda*, *Bivalvia* and *Crustacea*. All of them can potentially be a food resource for wading birds (Table 1.1), some, like *Hydrobia ulvae* and *Cerastoderma edule* are main food resource available to waders in temperate estuaries (Zwarts and Wanink, 1993; Yates et al., 1993). For this reason, and also because the number of benthos sampling stations was low (five), all identified in the sample benthos species were considered available for birds and their densities and total density of macrobenthos were used for analysis.

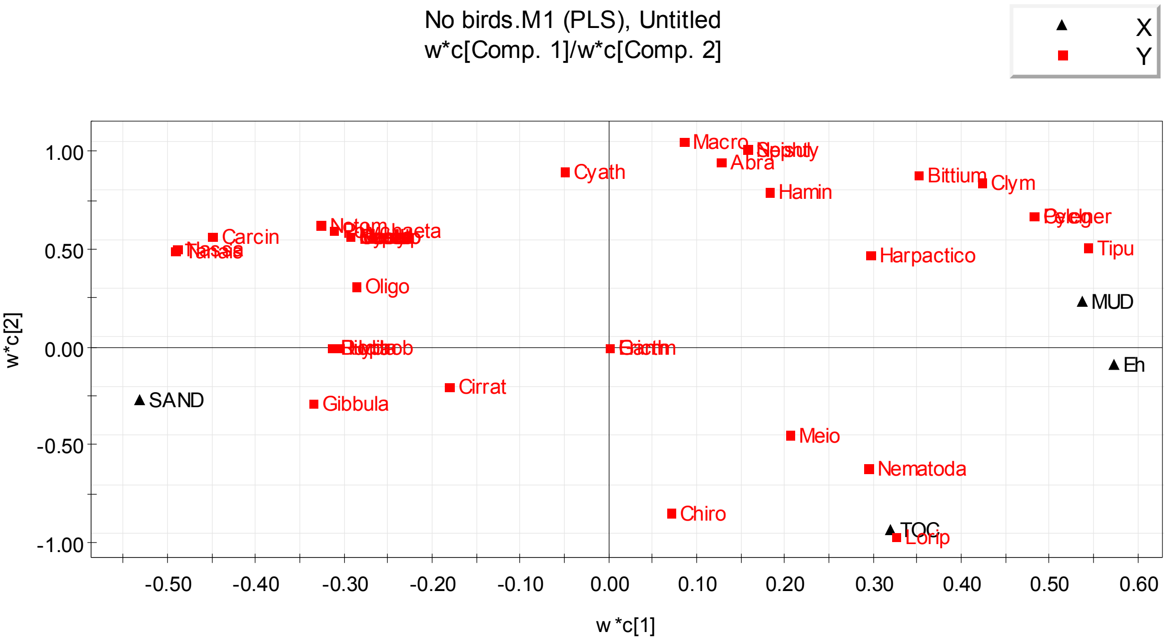


Figure 3.36. PLS plot showing benthos densities in relation with sediment variables, n=5. Independent variables (X): % GS<63 µm (Mud), TOC, E_h. Dependent variables (Y): macrobenthos species densities and meiobenthos high taxa densities.

Analysis using sediment variables at five benthos sampling stations as explanatory for macrobenthos species densities and meiobenthos high taxa densities is shown in the Figure 3.36. The environmental variables were separated along the w*c[1] axis. Mud content, redox and TOC were located positively and sand negatively along this axis. Total meiobenthos, *Harpacticoida* and *Nematoda* densities were located positively on the w*c[1] axis, showing association with TOC, mud content and high redox potential. Grouping of macrobenthos species was likely caused by site specificity of many species, taking into consideration small number of study sites (n=5), and that majority of species were found only in one or two of 5 sites. Total macrobenthos density vector was located near the center of w*c[1] axis, and thus did not show relation with either high mud content and TOC or sand proportion.

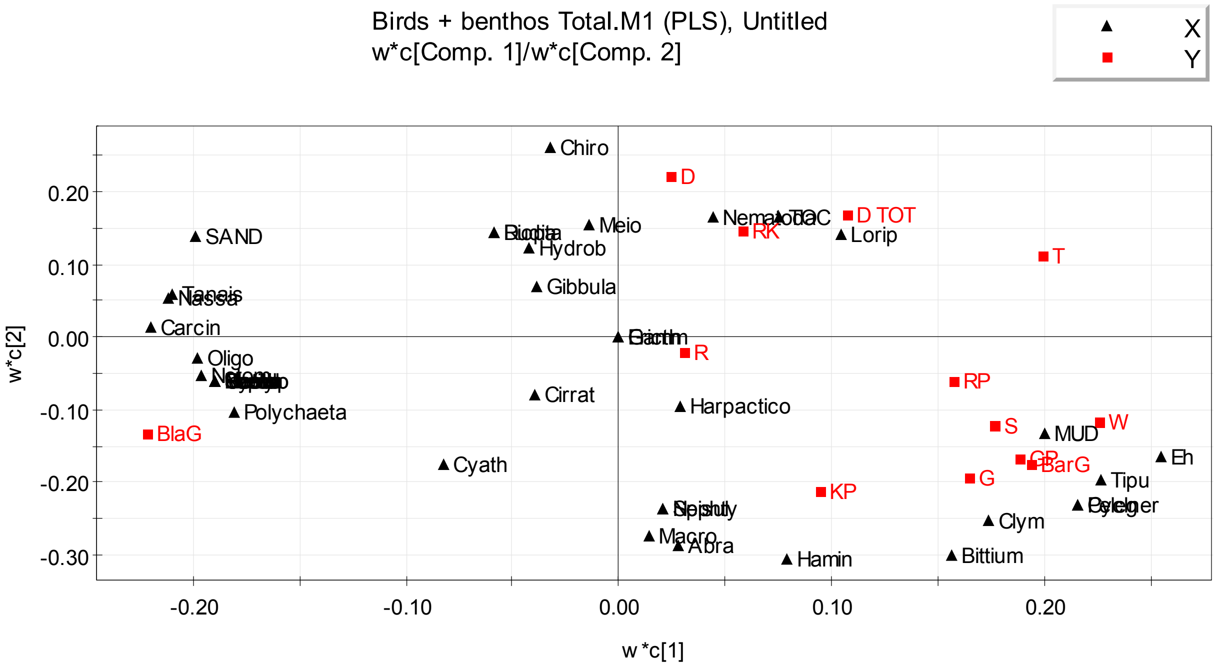


Figure 3.37. PLS plot showing average bird densities in relation with sediment and benthos variables, n=5. Independent variables (X): % GS<63 μm (MUD), TOC, E_h, meiobenthos taxa density (*Harpacticoida, Nematoda, Polychaeta*, Other taxa), total macrobenthos density (Individuals) and species densities. Dependent variables (Y): average bird density during all period. Bird codes as in Figure 3.27.

The results of analysis of bird densities using environmental parameters and benthos densities as explanatory variables are shown in Figure 3.37. Majority of bird species (Whimbrel, Grey Plover, Bar-Tailed Godwit, Sanderling, Ringed Plover, Kentish Plover, Greenshank) were plotted positively at w*c[1] axis, together with mud content, high redox values and corresponding benthos species. This group of bird species was located negatively on the w*c[2] axis similarly with total macrobenthos density and several Mollusks (*Abra sp, Bittium sp., Haminoea sp.*) and Polychaeta (*Clymenura* sp.). Densities of Dunlin, Redshank and Red Knot were associated with TOC. Along the w*c[2] axis they were plotted close to Gastropods *Hydrobia ulvae*, *Gybbula sp.*, and bivalve *Loripes lacteus*. Black-Tailed Godwit was plotted negatively at w*c[1] axis together with sand fraction and macrobenthic species found on the Ancão-2 site.

The small number of sampling units (5 stations) did not provide enough replication to draw reliable conclusions. Due to small number of benthos sampling points association between bird densities and benthos characteristics could have been caused accidentally. Therefore, the obtained results must be viewed with caution.

3.5. Indicator species

The Indicator Value analysis (IndVal) by Dufrene & Legendre (1997) was conducted to determine bird species associated with particular site (Table 3.14). This analysis provided combined measurement of each species´ site fidelity and specificity.

When the combined bird abundances for all observation period were analyzed, four indicative species were identified at the level of significance $p<0.05$: Kentish Plover for Ancão, Dunlin and Red Knot for Ramalhete and Black-tailed Godwit for Ancão-2. At the lower significance level ($p<0.1$) Ringed Plover, Bar-tailed Godwit and Sanderling were shown to have some indicator value at the Ancão site.

In spring indicator values for all species were lower, and did not exceed $p<0.1$ limit. At this level Ringed Plover and Sanderling were indicator species at the Ancão site, Dunlin at the Ramalhete site and none of the species was found indicative for the Ancão-2 site. Red Knot was not a significant indicator species at Ramalhete, although it had high indicator value (28), that could be explained by high frequency (50%) of this species at Ancão-2 site, in its turn stipulated by low number of counts (N=2) on this site in spring.

Table 3.14. Indicator values generated by IndVal analysis. The highest values correspond to indicator species for the site. Significance is according to Monte-Carlo test for observed maximum indicator value of species. Significant values at p<0.1 are in bold, at p<0.05 marked with (*).

Species	Site			Observed Indicator Value	Indicator Value from randomized groups		Significance
	Ancão	**Ramalhete**	**Ancão-2**		Mean	SD	p
All seasons							
Turnstone	24	23	11	23.8	23.4	4.2	0.337
Sanderling	**20**	3	12	20.0	13.8	4.2	**0.079**
Dunlin	3	**47**	14	47.4	21.5	5.5	**0.002***
Red Knot	0	**11**	0	11.3	4.6	3.1	**0.046***
Kentish Plover	**41**	5	31	41.3	25.4	4.6	**0.011***
Ringed Plover	**21**	2	7	21.4	13.0	4.5	**0.064**
Bar-tailed Godwit	**17**	2	9	16.7	11.6	4.2	**0.096**
Black-tailed Godwit	0	0	**36**	35.6	2.4	2.0	**0.001***
Whimbrel	18	10	7	18.1	16.0	4.2	0.207
Grey Plover	21	5	12	21.5	15.6	4.4	0.109
Greenshank	4	1	0	3.7	4.2	2.8	0.300
Redshank	3	12	14	14.2	10.4	4.2	0.117
Spring							
Turnstone	30	30	11	30.3	35.4	9.3	0.657
Sanderling	**35**	0	9	35.5	21.3	12.3	**0.092**
Dunlin	6	**55**	18	55.1	36.4	11.5	**0.080**
Red Knot	0	28	0	27.6	14.7	11.2	0.151
Kentish Plover	37	2	43	42.8	29.4	11.4	0.143
Ringed Plover	**37**	0	4	36.8	20.7	12.5	**0.070**
Bar-tailed Godwit	10	7	25	24.9	20.6	11.7	0.161
Whimbrel	15	15	12	15.2	26.5	13.1	0.922
Grey Plover	35	16	4	34.6	31.1	12.1	0.282
Greenshank	1	0	0	1.4	2.2	6.1	1.000
Redshank	5	1	0	4.8	9.0	12.1	0.490
Summer							
Turnstone	22	0	25	25.3	17.0	7.1	0.115
Sanderling	2	0	12	12.0	6.0	4.6	0.127
Dunlin	0	0	**94**	94.2	7.7	5.2	**0.001***
Red Knot	0	0	**17**	16.7	2.3	3.1	**0.043***
Kentish Plover	**38**	2	33	38.1	27.9	6.4	**0.085**
Ringed Plover	7	0	2	6.9	7.9	4.8	0.508
Bar-tailed Godwit	**28**	0	0	27.9	14.9	6.7	**0.057**
Black-tailed Godwit	0	0	**17**	16.7	2.3	3.2	**0.045***
Whimbrel	15	0	1	15.0	10.7	6.0	0.170
Grey Plover	16	0	7	15.5	12.4	6.6	0.216
Redshank	11	0	18	13.3	4.6	4.5	0.114
Autumn							
Turnstone	26	25	15	26.4	29.3	7.2	0.579
Sanderling	24	8	21	24.3	23.5	8.5	0.299
Dunlin	3	**56**	10	56.1	27.5	8.4	**0.016***
Kentish Plover	**41**	9	28	41.2	30.7	6.8	**0.084**
Ringed Plover	24	7	16	24.3	21.5	8.5	0.232
Bar-tailed Godwit	11	1	**25**	25.3	13.3	7.5	**0.056**
Black-tailed Godwit	0	0	**74**	73.8	5.6	5.2	**0.001***
Whimbrel	23	11	15	23.2	22.6	7.3	0.329
Grey Plover	20	1	**30**	30.1	17.5	7.7	**0.097**
Greenshank	8	2	0	7.8	10.1	6.6	0.579
Redshank	5	28	30	30.4	21.3	7.5	0.106

In summer Dunlin has become an indicator species at Ancão-2 ($p<0.05$), together with Red Knot and Black-tailed Godwit ($p<0.1$), which was caused by several recoveries of these species on site, while they were not present at the others. Kentish Plover and Bar-tailed Godwits were found indicative at the Ancão site, while Kentish Plover also had high indicator value at Ancão-2. The Ramalhete site during summer maintained only small population of breeding Kentish Plovers with no other species present, however Kentish Plover was not found to be indicator obviously because of its higher occurrence at the other two sites.

In autumn bird assemblages at the Ancão site were only indicated by Kentish Plover at $p<0.1$ and at Ramalhete by Dunlin ($p=0.016$). Black-tailed Godwit, Bar-tailed Godwit and Grey Plover had significantly high indicator values at the Ancão-2 site.

4. DISCUSSION

The results obtained by this study revealed a difference in physico-chemical abiotic parameters of the mud flat habitat between Ancão and Ramalhete sites. Communities of meiobenthos and macrobenthos were different between stations. Shorebirds assemblages of all sites consisted from the same species but were different by their relative densities between two main study sites.

4.1. Sediment particle size and TOC

Sediments of the study sites in the Western part of the Ria Formosa lagoon were classified as muddy sand or sandy mud. Statistical analysis did not reveal significant difference in granulometry (except for some sand fractions) between Ramalhete and Ancão sites. This was predictable, since the study sites were chosen in a way to have similar physico-geographical parameters, e.g. extent of the mud flat and exposition to the tidal line along one of the sides. There was variability between single sampling quadrats in granulometry and TOC, caused by natural geomorphological factors and shellfishery activities. Quadrats A2, A3 and R7 were more muddy due to location on the banks of intertidal streams, which contributed to deposition of silt and clay particles. Quadrates A4, A5 and especially R2 were used for shellfishery, meaning that sediment was often mechanically disturbed. It is likely that high sand fraction content (80 %) on the site R2 was to great degree caused by shellfishery activities. Cleaning of seagrass and algae on Ramalhete site in quadrats R6 and R7 might have affected grain size composition. Fishermen were observed clearing the surface of mud flat using metal grabber (algae and seagrass cleaning also confirmed in Parvatkar, 2008). There was found an expected positive relation between % grain size < 63 µm and % TOC (Hewitt and Mudge, 2004), explained by the increase of grain surface area with decrease of particle diameter, and increased capability of the sediment to adsorb organic matter.

4.2. Reduction-oxidation potential

The reduction-oxidation potential of the sediments is related with the chemical processes of reduction of nitrogen, iron, manganese and sulphate which occur in the conditions of restricted oxygen exchange with atmosphere. These reactions take place under various physico-chemical and biological processes, including sediment-atmosphere gas exchange, decomposition of organic matter and bioturbation. The reduction of O_2 to H_2O and NO_3^- to NO_2^- occurs in the E_h range of +250 to +100 mV. Iron is reduced from Fe^{3+} to Fe^{2+} in the range of +100 to 0 mV, sulphate reduction from SO_4^{2-} to H_2S or HS^- has the range of 0 to -200 mV, methane-hydrogen reduction occurs at E_h values of below - 200 mV (Barlett and James, 1993).

The results obtained are different from those of previous studies (Neves, 2003). The sediment reduction-oxidation potential was found to be negative (-50 to -200 mV) on all stations and along shallow 10 cm depth profile. Other studies (Neves, 2003, Clarke, 2001) observed often positive values as high as +50 – +200 mV. One possible explanation could be seasonality (measurements were done in August – September 2008 in the daytime), as decomposition of organic matter may become more active with high temperature and provoke reducing conditions and anoxia in the upper sediment layer. The redox values in the thin layer (2 – 3 cm) of surface water covering the sediment were positive at +35 – +50 mV. This can be explained by physico-chemical interaction between water and sediment, when reducing conditions in the sediment due to organic matter decomposition consume oxygen from the water column. Suspension of particulate organic matter to the water column as a result of sediment surface disturbance during tide rise (or mechanical perturbation caused by shellfishery activities) may lead to fast oxidation of substances exposed to oxygen-rich environment, and consume free oxygen from water (Mudge & Duce, 2005). Another reason can be increase in deposition of organic matter since the time previous studies were conducted.

There was a statistically significant difference between Ancão and Ramalhete in redox potential and mud content of the sediment. TOC was higher and redox values lower at

Ramalhete site than on Ancão. High TOC values were related with high silt and clay content. Low redox values could be caused by oxygen consumption associated with decomposition of organic matter in the sediment (Libes, 1992).

4.3. Meiobenthos

The composition of meiobenthic communities included Nematodes of genera *Terschellingia* spp. (all stations), *Sabatieria* spp. (stations A3 and R7), *Paracomesoma* spp. (all stations, especially numerous at A2 and R7), *Daptonema* spp. (Ancão-2, R7) and *Metoncholaimus* spp. (station A2). These nematode genera are typically found in organically rich, muddy sediment and have been proposed to be representative of a community that is well adapted to disturbed conditions (Heip et al., 1990). In particular, the species *Sabatieria pulchra* survives low oxygen and high sulphide concentrations and often persists under conditions that are unsuitable for most other nematode species. Some species found were suggested to be used as indicators for implementation of the WFD in Hiscock et al., 2005.

Meiobenthos communities are dependant to great degree on the physical conditions of the sediment. The most important factors are particle size distribution and organic matter content (Giere, 2009). All studied stations were different by *Nematoda* trophic structure, with the most muddy site A3 having the biggest proportion of deposit feeding *Nematodes*, and the most sandy and mechanically disturbed site R2 being dominated by omnivorous species. However, the Maturity Index of meiobenthic communities was higher at stations A2 and R2, indicating less disturbed community and therefore contradicted to Index of Trophic Diversity. Cluster analysis performed over species composition grouped stations A2 and R2 together, consistently with Maturity Index, so this index may be appropriate to assess difference in species composition between stations. Difference between meiobenthic communities between stations can be explained by physical sediment condition, primarily grain size composition and total organic content.

4.4. Macrobenthos

Habitats with the highest densities and diversity of macrobenthos in the Ria Formosa are seagrass beds (Almeida et al., 2008). Station A2 was located on the seagrass covered sediment, but did not differ significantly from other stations in terms of abundance or diversity. Benthic densities obtained in this study were significantly higher than previously reported from Ramalhete site (Almeida et al., 2008). This can be explained by the patchy distribution of the macrobenthos on the flats (Santos et al., 2009). Heterogeneity of the mud flat sediment physical properties observed in this study could also attributed to uneven distribution of macrobenthos.

Groups of species obtained on the Multi-Dimensional Scaling ordination diagrams were likely caused by specificity of benthic species to the study sites. Therefore, measures of population diversity and trophic indexes were considered more appropriate means of identifying response of benthos communities to environmental conditions. The marine biotic index AMBI suggested to be used within WFD to assess ecological status based on macrobenthic community when applied to the data obtained, characterized all stations as having high ecological status (classification according to Borja et al, 2000). The study sites were not directly exposed to strong anthropogenic pollution and therefore the main pressure on benthic communities was physical disturbance of the sediment caused by shellfishery. This kind of disturbance was present at site R2 and in a lesser degree at sites A3 and Ancão-2.

All the macrobenthic species identified in this study have been reported to be a food resource for birds (Appendix B, Table 1.1), and were included in multivariate analysis. Bird diet study was not conducted therefore any specific macrobenthic species taken by birds were not identified.

4.5. Bird assemblages

Total birds densities (Appendix E) were found to be higher than in previous researches in Ria Formosa (Batty, 1992; Rufino& Araujo 1987) and Tagus estuary (Granadeiro et al, 2004, 2006). Location of the counting areas along the shoreline has influenced the results of counts, since birds tend to concentrate at the water edge because of higher prey availability (Lourenço et al, 2005). In some cases the intertidal creeks intercepted transects, increasing the length of water edge in the quadrates, which may have important consequences on foraging conditions, including particle size and accessibility of the prey for birds (Lourenço et al, 2005).

Shorebirds communities of the study sites consisted from the same species but with different densities thus were structurally different. Densities of most bird species (Table 3.11) were statistically different between Ancão and Ramalhete. This variability in proportions caused stations which belong to two sites to clearly differentiate at the PCA plots.

Tested against sediment environmental variables, bird species grouped into two clusters similar by sediment quality preference. These two groups mainly consisted of species with densities previously found to be different between Ancão and Ramalhete sites (Figure 3.35). Therefore, relation of the bird species to the sediment characteristics was difficult to separate from the effect of site-specific distribution.

4.7. Relation between benthos and bird densities

The sampling design of the study was time and resource limited, what made it impossible to reach large enough sample size in benthos survey to allow for reliable testing of relation between birds and benthos components. Study design was better suited for comparison of the two study sites. Therefore no statistically significant relation was found between bird densities and that of their invertebrate prey. Multivariate PLS analysis did reveal some linkage between groups of bird and benthos species (Fig. 3.37), but there was high probability of casual coincidence in the analysis based on 5 samples and more that 50 variables, so results should be viewed with caution. However, absence of relation between bird and prey densities is a common finding, especially when other factors, such as prey availability rather then prey total density are taken into account. The invertebrate prey of shorebirds may not always be accessible of detectable by them (Evans & Dugan, 1984). The absence of correlation between the abundance of invertebrate prey and that of birds has been reported by Dierschke et al. (1999) for Dunlin in the Baltic Sea. The same source estimated the preference of Dunlins to feed in the shallow water along the shoreline (66 % of birds), and to follow the water line. It has been reported that Dunlins were able to respond to differences in the sediment penetrability in choice of feeding habitat (Mouritsen and Jensen, 1992). Ens et al. (2005) reported absence of significant correlation between the amounts of invertebrate pray and bird densities in the mud flats of the Wadden Sea. It was concluded that the driver of birds distribution was prey availability rather than prey abundance in habitat choice within a certain staging area.

This study was done on relatively small spatial scale, with birds and sediment data collected at a resolution of 50 m. Studies of relation between bird densities and physical parameters of the intertidal flats done on the large spatial scale (and naturally using many more sample replicates) did reveal relation between bird densities, sediment type (Yates et al., 1993) and density of macroinvertebrate prey items (Goss-Custard & Yates, 1992; Granadeiro et al, 2007). The percentage of bird variance explained by only sediment physical variables was usually low, except in cases when invertebrate prey density or accessibility correlated significantly with environmental variables (Yates et al., 1993). In such cases it was possible to predict bird densities using solely environmental variables ignoring benthos data. The structure of shorebirds assemblages likely reflected distribution of birds according to their site-specific habitat requirements (Moreira, 1993), namely density and availability of their invertebrate prey. Because of the complex nature of combination of factors that influence the availability of the prey and shorebirds foraging success, simple models usually are not able to give accurate estimation of their relation (Evans & Dugan, 1984).

4.8. High-tide roosts and supplementary feeding areas

Distance to the suitably located high-tide roosts can limit the access of shorebirds to feeding habitats (Dias et al., 2006). It is known that some areas of the habitats can be divided into spatial functional units groups of feeding areas and high-tide roosts used by a group of birds, during a certain period of time (Luís & Goss-Custard, 2005). Important aspect of the distribution of the shorebirds between study areas is distance to high-tide roosts and supplementary feeding habitat, salt pans in case of this study. There has been reported for Ria de Aveiro (Portugal) that supplementary feeding on salt pans can be important part of the feeding strategy of Dunlins (Luis et al., 2002). In this study, that conclusion is supported by observations of Dunlins feeding on the active salt pans on Ramalhete in June – July, while on the closely adjacent mudflats there were only Kentish Plovers feeding. Sanderlings also were observed frequently at salt pans near the Ramalhete channel (Fig. 2.1), even if they were not present on the intertidal study site itself. At the Ancão site available alternative feeding sites were fishponds and freshwater marshes at distance 50 – 400 m, sandy shore at 50 – 150 m and salt pans at approximately 1 km distance. Location close to the sandy beach could have caused high densities of Kentish Plovers (Figures 3.16 – 3.24) and identification of them as indicator species at the Ancão site (Table 3.14). High and middle salt marshes with developed network of intertidal channels were equally available at both sites in close range.

Ancão-2 site was located close to salt marsh (50 m), salt pans and freshwater wetland (200 m), and sandy shore (500 m), e.g. to all available kinds of high tide roosting or supplementary feeding areas. This can offer the explanation of high occurrence of Sanderlings that are related to sandy shore habitat and Kentish Plovers in all seasons. Bar-tailed Godwits and Black-tailed Godwits (in summer and autumn) were probably associated with salt pans habitat (Cramp & Simmons, 1984), as they were typical species found on adjacent salt pans during different seasons (Batty, 1993).

4.9. Small-scale distribution of shorebirds on the intertidal flats

The shorebirds were not evenly distributed between counting quadrats at each site (Figures 3.16 – 3.24). Despite that quadrats were of approximately the same size and had similar exposition to the water mark, bird densities were different between them. These differences were persistent across the studied seasons. On Ancão site quadrats with the highest densities were A4 and A3. They were located at the banks of big intertidal channel which drained the internal part of salt marsh and was connected to the aquaculture ponds. These two quadrats were different by silt and clay content, A3 was considerably more muddy (85 %) and had high TOC (3.9 %) while A4 was sandy (34 % mud) and its sediment was respectively less organically enriched (2.8 %). The high mud and TOC content at A3 was likely induced by the deposits of the intertidal channel, surface of the quadrat was generally undisturbed by shellfishery and at the big extent covered by seagrass meadow. Quadrat A4 was actively used for clam production, vegetation was removed in the most part and surface has been mixed by digging effort of the fishermen. The mixing of upper sediment layer induced fine sediment particles to suspend into water and removing of seagrass bed and algae contributed to it. Despite such evident difference in sediment properties, both quadrats maintained high bird populations, due to intersection with intertidal channel, which increased the length of the water line twofold compared to other parts of the transect.

At the Ramalhete site uneven distribution in spring was caused by the preference of Dunlin and Red Knot to feed in quadrats from R3 to R8. In autumn, birds were most abundant in the quadrats R1, R2, R7 and R8, all of them were intercepted with the intertidal creeks. Station R2 was used intensively for clam cultivation, its surface was uneven and in most part without seagrass or macroalgae cover.

There was a fine scale within-quadrat variation of bird distribution. Most of the species preferred to forage along the water line in the lower part of each quadrat (consistently with

Granadeiro et al., 2006), or along the intertidal channels. This is also consistent with the findings of Lourenço et al. (2005) in the Tagus estuary, where wader densities and foraging effort were found considerably higher in close proximity of the drainage channels than in the surrounding mudflat. Kentish and Ringed Plovers preferred central or upper parts of the mud flats, close to the border of the salt marsh. These species forage mostly visually (Cramp & Simmons, 1983) and prefer more hard and dry sediment.

Typically Whimbrel, Curlew, Grey Plover, Bar-tailed Godwit and Black-tailed Godwit foraged along the water edge and over the sediment surface covered with water. Dunlin, Sanderling, Red Knot were foraging across the entire mud flats, Kentish Plover and Ringed Plover preferred central and upper parts. Turnstone, Redshank and Greenshank were usually associated with intertidal channels, mud flat surface irregularities and areas covered by seagrass and algae.

4.10. Indicator species

Indicator Values analysis (Dufrene & Legendre, 1997) appeared to identify the most common abundant shorebirds as site-specific indicators, given that the same species were less abundant at the other sites. The identified indicator species were different in most cases between seasons at the same sites. However, the most typical species are not always best objects of monitoring, since other, less numerous species can respond sensibly to more local or small scale environmental change, according to their specific habitat requirements (Hutto, 1998). For instance, the analysis did not suggest Kentish Plover to be indicator at Ramalhete in summer, when it was the only one shorebird species present, and logically should be considered important component of the lagoon ecosystem in its given seasonal condition. The IndVal findings were consistent with results displayed on the PCA ordination plots (Figures 3.26 – 3.31), where birds with long vectors on PCA plots (high densities) were found to be indicators at the respective group of sites. Red Knot can be considered meaningful indicator for Ramalhete site, since it is the only site where it was found in high densities and specific conservational status of this species (Piersma, 2004), but it occurred on site only during short time in spring migration.

Seasonal factor should be considered when defining indicator species, because birds communities in the lagoon change greatly between seasons (Rufino & Araujo, 1987; Batty, 1992). During spring migration period indicator species for the Ancão site can be Sanderling and Ringed Plover, on the Ramalhete site Dunlin and Red Knot, and Kentish Plover at Ancão-2. In summer Kentish Plover and Bar-tailed Godwit were representative of Ancão site, Dunlin and Black-tailed Godwit at Ancão-2. Whilst Red Knot obtained high indicator value on Ancão-2 in summer, it might have been caused by coincidence and low number of counts; also Red Knots are not permanent species in Ria Formosa in summer, so it should not be considered indicator in this season. At Ramalhete in summer Kentish Plover was the only species present and can be used as indicator. During autumnal migration indicator species can be Kentish Plover at Ancão, Dunlin at Ramalhete and Black-tailed Godwit at Ancão-2 sites. The named indicator species are assumed to reflect integrative conditions of the habitat, including foraging conditions and position relatively to roosting sites and complementary habitats. Total bird density also can be considered as integrative indicator of carrying capacity of the intertidal habitat (Piersma & Lindstrom, 2004).

5. CONCLUSIONS

The objectives to study parameters of avian communities, meiobenthos, macrobenthos and sediment environmental properties in the Ancão basin and Ramalhete channel in the Western part of the Ria Formosa lagoon were achieved.

1) The study sites were found to be significantly different in terms of environmental characteristics, such as total organic content (TOC) and reduction-oxidation potential. Within site variation of these parameters was high, due to natural (geomorphological and biological) and anthropogenic reasons. The integrated analysis (by means of PCA) of all environmental variables together did not clearly divide sampling units into groups corresponding to sites. This indicates that there was high variability of environmental conditions within main study sites.

2) Shorebirds assemblages were different between sites by species densities. Principal Component Analysis revealed grouping of sampling units between Ancão and Ramalhete, in all seasons. The most abundant common species Dunlin, Red Knot (on spring migration) tended to contribute most to the principal axis and therefore determined the differentiation. With abundant species excluded from analysis the communities were still different by relative densities, in spite of being composed from the same bird species. Distinct division of birds' communities was also confirmed by Cluster analysis, and was true for three seasons observed (spring migration, summer, autumn migration).

3) Macrobenthic and meiobenthic communities were found to be different between study sites. Meiofauna demonstrated more distinct difference between sites, by such parameters as species composition and feeding groups proportions.

4) Indicator species analysis suggested that several bird species could be considered indicative species for each of the studied sites. During all seasons those were Kentish Plover for Ancão, Dunlin for Ramalhete and Black-tailed Godwit for Ancão-2.

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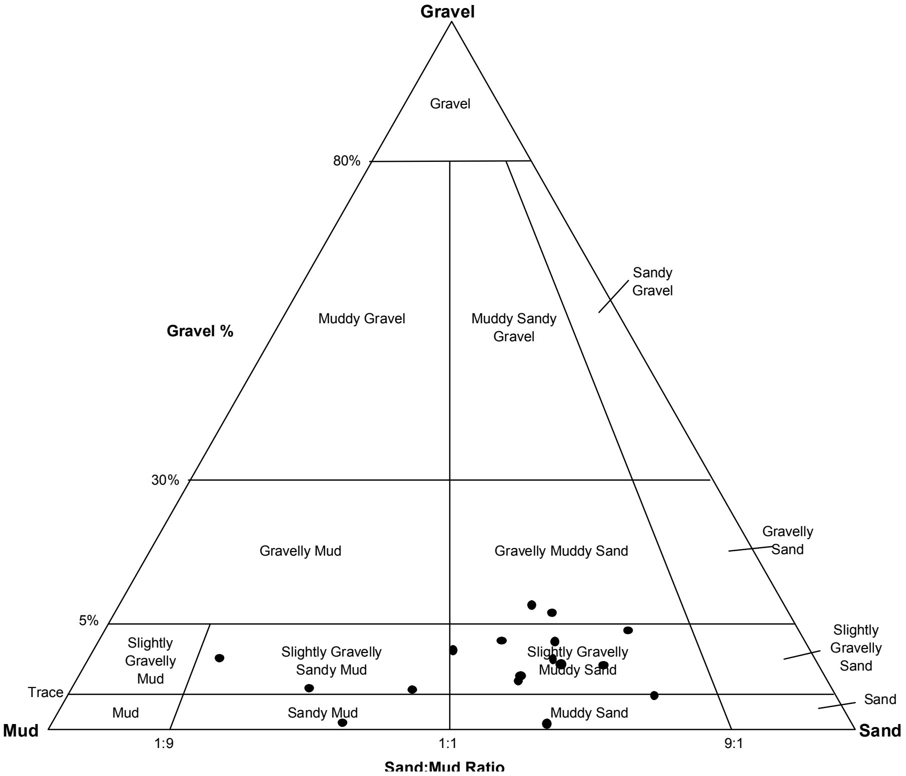
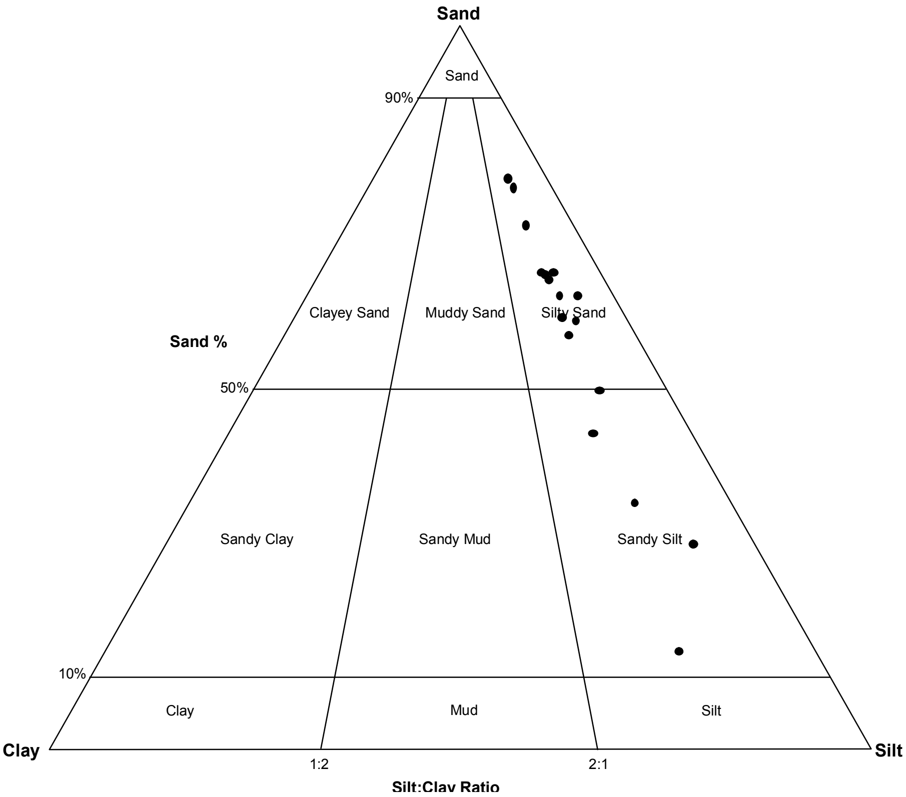
Appendix A

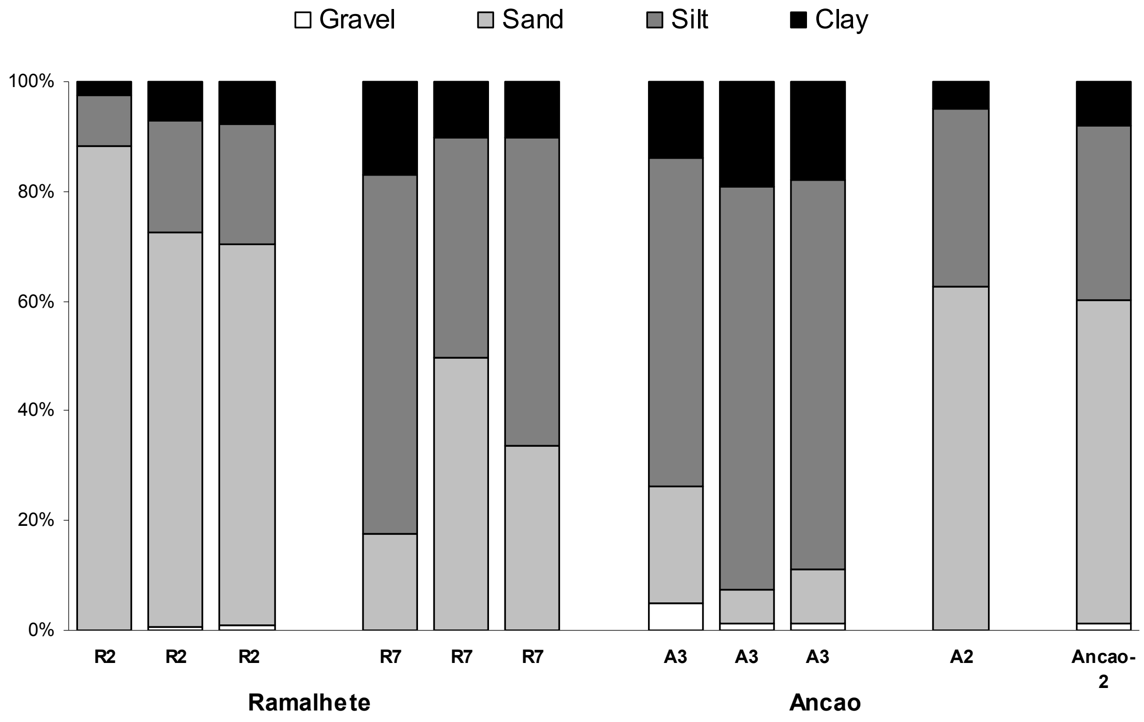
Particle size classification diagrams





Appendix A (continued)

Sediment particle size distribution at benthos sampling stations R2, R7, A2, A3, Ancão-2. Three replicates are shown for stations R2, R7 and A3.



Appendix B

Mean abundance of macrobenthic species and high taxa, ind/m^2. (Cañedo-Arguelles, M. Unpublished data).

	Site				
Species	**A2**	**A3**	**Ancão-2**	**R2**	**R7**
Cirratulus cirratus	442	-	118	-	-
Clymenura clypeata	-	118	29	-	-
Diopatra neapolitana	-	-	-	29	-
Euclymene oerstedi	-	-	88	-	-
Nanthes caudata	-	-	29	-	-
Nephty cirrosa	-	29	29	-	-
Notomastus latericeus	-	29	619	88	-
Scolepsis cirratulus	-	-	413	-	-
Bittium reticulatum	1356	3920	1592	236	-
Cerithium vulgatum	-	-	118	-	-
Cyclope neritea	-	147	-	-	-
Cyclope donovania	-	-	88	-	-
Gibbula umbilicalis	-	-	-	29	-
Haminoea hydatis	-	118	88	-	-
Hydrobia ulvae	-	29	-	383	-
Leptochiton asellus	-	-	59	-	-
Mesalia brevialis	-	-	88	-	-
Nassarius pfeifferi	-	-	177	147	-
Abra alba	29	59	59	-	-
Cerastoderma edule	-	-	29	-	-
Loripes lacteus	88	59	-	29	177
Ruditapes decussatus	-	-	-	29	-
Spisula subtruncata	-	29	29	-	-
Cyathura carinata	-	88	177	-	-
Ericthonius difformis	-	-	-	-	-
Gammarus insensibilis	-	-	-	-	-
Idotea chelipes	-	-	29	-	-
Palaemon elegans	-	29	-	-	-
Palaemonetes varians	-	-	206	-	-
Tanais dulongii	-	-	236	206	-
Carcinus maenas	-	-	59	29	-
Chironomidae	-	-	29	59	177
Tipulidae	-	147	-	-	29
Oligochaeta	118	-	531	-	118
Total	**2210**	**4804**	**4922**	**1267**	**501**

Appendix C

Abundance of meiofaunal taxa at the study sites, ind.*$10^5/m^2$
(Ivanova, K. Unpublished data).

	Site				
	A2	**A3**	**Ancao-2**	**R2**	**R7**
Acarina	-	-	0,001	0,001	0,001
Amphipoda	-	0,002	0,002	0,001	-
Bivalvia juv.	0,005	0,016	0,002	0,173	0,019
Ciliata	0,013	0,002	0,079	0,021	0,011
Decapoda juv	0,011	0,011	-	0,005	0,038
Foraminifera	0,012	0,011	0,049	0,055	0,055
Gastropoda	-	-	-	0,001	-
Harpacticoida	0,070	0,264	0,269	0,092	0,229
Insecta	0,004	-	0,002	0,033	-
Nauplii	0,005	0,021	0,054	0,016	0,062
Nematoda	4,229	5,815	5,330	5,176	8,900
Oligochaeta	0,033	0,018	0,059	0,062	0,029
Ostracoda	0,006	0,001	0,028	0,014	0,011
Unidentified	-	-	0,006	-	-
Polychaeta	0,389	0,201	1,152	0,147	0,068
Turbellaria	0,003	0,001	0,022	0,002	0,001
TOTAL	**4,780**	**6,363**	**7,055**	**5,799**	**9,423**

Appendix D

Species proportions of *Nematoda*, %.

Trophic groups: 1A – selective deposit feeders, 1B – non-selective deposit feeders, 2A – epistratum feeders, 2B – predators or omnivores. (Ivanova, K. Unpublished data).

Species	Trophic groups	Stations				
		A2	A3	Ancao-2	R2	R7
Acanthonhus sp.1	2A	0	0	0.83	0	0
Adoncholaimus papilatus	2A	0	0.42	0	0	0
Anoplostoma viviparum	1B	0	2.54	9.06	0	2.37
Anticoma sp.1	1A	0	0	0.45	0	0
Axonolaimus sp.1	1B	0	0	0.90	0	0
Bathyeurystomina sp.1	2A	0	0.43	0	0	0
Bathylaimus sp.1	1B	0	0	0.45	0	0.38
Camacolaimus ampullocaudatus	2A	0	0	0.76	0	0
Chromadorella sp.1	2A	0.33	0	0	0	0
Crenopharynx sp.1	1A	0	0	0.45	0	0
Daptonema sp.2	1B	0	0	9.06	0	10.32
Doliolaimus sp1	2B	0	0	0.69	0	0
Draconema claporedii	1A	0	0	0	0	0.38
Euchromadora sp.1	2A	0	0	0	0	0.77
Halalaimus sp.1	1A	0	0.83	0	0.59	0.76
Halichoanalaimus sp.1	2B	0	0	0.76	0	0
Leptolaimus sp.3	1A	0	0	0	0	0.40
Longicyatholaimus sp.1	2A	0	0	0	0	0.37
Metachromadora remanei	2A	0.33	15.94	32.04	0	7.48
Metadesmodora sp.1	2A	0	0	0.38	0	0
Metadesmolaimus sp.1	1B	0	0.43	1.28	0	0.78
Metadesmolaimus sp.2	1B	0	2.03	0	0	0
Metacomesoma sp.1	1B	0.76	0	0	0	0
Metalinhomoeus sp.2	1B	0	2.95	2.87	0	0
Metalinhomoeus sp.3	1B	0	0.43	1.52	0	0
Metalinhomoeus longiseta	1B	0	2.09	4.77	0	0
Microlaimus sp.2	2A	0	0	0.38	0	0.38
Microlaimus sp.2	2A	0	1.65	0	0	0
Microlaimus sp.3	2A	0	0	0.31	0	0
Microlaimus sp.3A	2A	0	4.67	1.52	0	0.37
Microlaimus sp.4	2A	0	0	0	0	0.75
Metoncholaimus albidus	2B	10.08	0	0	0	0
Neochromadora sp.1	2A	0	0.42	1.28	0	0.76
Odontophora sp.1	1B	0	2.93	0	0	0.40
Odontophora sp.2	1B	0	0	0	0	0.38
Paracomesoma sp.1	2A	12.80	2.89	9.26	1.18	18.90
Paracomesoma sp.2	2A	0	0	0	0.59	0
Paramesonchium sp.1	2A	0	0.41	0	0	0
Paralinhomoeus sp.2	1B	0	0	0	0	0.37
Prochromadora sp.1	2A	0	0	0	0	1.14
Prochromadorella sp.1	2A	0.67	0	1.83	0	2.32
Ptycholaimellus ponticus	2A	11.62	1.28	3.34	1.18	11.57
Retrotheristus sp.1	1B	0	0	0	0	0.40
Sabatieria sp.1	1B	0	18.79	0	0	0.38
Sabatieria sp.2	1B	0	0.85	0	0	0.37
Sphaerolaimus sp.1	2B	0	0.83	2.66	0	2.69
Spirinia sp.1	2A	0	0	1.52	10.63	0
Spirinia parasitifera	2A	14.78	0	6.98	71.23	23.63
Spirinia sp.3	2A	0	0	0	0.31	0.75
Terschellingia longicaudata	1A	0	0	2.29	2.17	2.63
Terschellingia longispiculata	1A	21.43	27.17	0	0	0
Terschellingia longissimacaudata	1A	4.94	3.78	0	8.55	4.68
Terschellingia sp.2	1A	4.46	0	0.38	3.57	0
Terschellingia sp.3	1A	0	2.50	0	0	0
Terschellingia sp.5	1A	0	0.85	0	0	0
Terschellingia sp.6	1A	0	2.03	0	0	0.37
Terschellingia sp.7	1A	0	0.85	0	0	0.75
Viscosia sp.1	2B	17.81	0	0.38	0	0.40
Viscosia sp.2	2B	0.00	0	0.90	0	0.75
Unknown sp.2	1A	0	0	0	0	0.40
Unknown sp.3	1A	0	0	0	0	0.40
Unknown sp.4	1A	0	0	0.31	0	0
Unknown sp.5	2B	0	0	0.38	0	0

Appendix E

Bird densities on the study sites, b/ha

Spring (March- May). Ancão

Species	A1	A2	A3	A4	A5	A6	A7	A8
Turnstone	6,22	4,07	6,30	6,35	5,51	5,10	4,29	3,47
Sanderling	1,78	2,22	6,11	2,38	1,02	1,43	3,67	1,22
Dunlin	0,22	6,85	10,56	4,29	4,49	6,53	4,90	3,67
Red Knot	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Kentish plover	4,44	3,70	6,11	6,19	2,45	2,86	3,06	3,06
Ringed plover	1,56	1,67	1,11	0,95	3,06	3,06	2,04	2,04
Bar-tailed Godwit	2,67	0,37	1,30	1,43	0,61	0,61	0,61	0,20
Black-tailed Godwit	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Whimbrel	2,00	0,93	1,30	1,11	0,61	0,61	0,41	0,82
Grey plover	2,00	1,67	3,89	3,02	2,04	1,22	2,24	1,43
Greenshank	0,44	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Redshank	1,33	0,00	0,37	0,00	0,20	0,20	0,00	0,00
Total	**22,67**	**21,48**	**37,04**	**25,71**	**20,00**	**21,63**	**21,22**	**15,92**

Spring (March- May). Ramalhete and Ancão-2

Species	R1	R2	R3	R4	R5	R6	R7	R8	Ancão-2
Turnstone	5,97	6,19	3,97	3,33	1,43	1,11	5,71	6,67	1,10
Sanderling	0,00	0,00	0,32	0,32	0,00	0,00	0,00	0,28	0,55
Dunlin	14,44	13,97	30,32	34,13	29,52	52,06	29,52	30,83	7,42
Red Knot	0,56	0,63	29,68	29,68	22,22	22,22	33,33	29,17	0,14
Kentish plover	0,00	0,32	0,79	0,79	1,11	1,11	0,16	0,14	3,16
Ringed plover	0,00	0,00	0,32	0,00	0,32	0,00	0,00	0,00	0,14
Bar-tailed Godwit	1,81	0,48	0,63	0,63	0,00	0,00	1,27	1,11	0,55
Black-tailed Godwit	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Whimbrel	1,11	1,27	0,32	0,63	0,95	0,95	0,95	0,83	0,55
Grey plover	0,97	0,79	1,11	0,79	0,95	0,95	2,38	2,08	0,27
Greenshank	0,28	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Redshank	0,28	0,00	0,00	0,00	0,32	0,00	0,00	0,28	0,00
Total	**25,42**	**23,65**	**67,46**	**70,32**	**56,83**	**78,41**	**73,33**	**71,39**	**65,19**

Appendix E (continued)

Bird densities on the study sites, b/ha

Summer (June-July). Ancão

Species	A1	A2	A3	A4	A5	A6	A7	A8
Turnstone	0,73	1,52	0,30	1,04	2,60	1,56	1,04	1,30
Sanderling	0,00	0,00	0,00	0,00	1,30	1,30	0,00	0,00
Dunlin	0,00	0,00	0,00	1,04	0,00	0,00	0,00	0,00
Knot	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Kentish plover	3,27	3,64	4,55	3,64	1,82	1,82	4,94	3,90
Ringed plover	0,73	0,00	0,30	0,78	0,26	0,00	0,26	0,00
Bar-tailed Godwit	1,82	2,73	3,64	2,08	1,82	4,68	0,78	0,78
Black-tailed Godwit	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Whimbrel	0,00	0,30	0,00	0,78	0,78	0,26	1,56	2,08
Grey plover	0,00	0,61	0,91	0,78	1,04	2,08	0,00	1,04
Greenshank	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Redshank	0,00	0,00	0,30	0,00	0,26	0,00	0,00	0,00
Total	**6,55**	**8,79**	**10,00**	**10,13**	**9,87**	**11,69**	**8,57**	**9,09**

Summer (June-July). Ramalhete and Ancão-2

Species	R1	R2	R3	R4	R5	R6	R7	R8	Ancao-2
Turnstone	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,55
Sanderling	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,18
Dunlin	0,00	0,00	0,71	0,00	0,00	0,00	0,00	0,00	3,57
Knot	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Kentish plover	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,01
Ringed plover	0,00	0,71	1,43	0,71	0,71	1,43	0,00	0,63	0,05
Bar-tailed Godwit	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,05
Black-tailed Godwit	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,18
Whimbrel	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,05
Grey plover	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,14
Greenshank	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Redshank	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,27
Total	**0,00**	**0,71**	**2,14**	**0,71**	**0,71**	**1,43**	**0,00**	**0,63**	**0,88**

Appendix E (continued)

Bird densities on the study sites, b/ha

Autumn (August-October). Ancão

Species	A1	A2	A3	A4	A5	A6	A7	A8
Turnstone	1,71	4,29	6,19	8,57	4,90	3,67	1,43	2,45
Sanderling	1,71	1,19	4,76	4,49	1,63	2,24	3,06	4,08
Dunlin	1,43	1,67	3,81	3,67	3,06	8,78	2,65	3,67
Knot	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Kentish plover	5,43	5,95	5,00	5,10	2,86	5,51	6,94	10,41
Ringed plover	2,86	2,86	1,90	4,69	0,41	2,24	1,84	0,41
Bar-tailed Godwit	0,57	0,00	0,71	3,27	1,02	1,22	0,20	0,00
Black-tailed Godwit	0,00	0,24	0,24	0,00	0,00	0,00	0,00	0,00
Whimbrel	1,14	1,43	3,81	3,67	1,63	1,02	0,00	1,22
Grey plover	1,43	1,90	2,38	2,24	2,24	1,02	0,00	0,61
Greenshank	0,00	0,00	0,71	1,63	0,20	0,41	0,00	0,00
Redshank	1,14	0,95	2,14	3,88	0,61	0,41	0,00	0,20
Total	**17,43**	**20,48**	**31,67**	**41,22**	**18,57**	**26,53**	**16,12**	**23,06**

(August-October) Ramalhete and Ancão-2

Species	R1	R2	R3	R4	R5	R6	R7	R8	Ancao-2
Turnstone	7,78	5,40	2,86	1,27	2,86	1,27	3,49	9,17	1,43
Sanderling	1,39	4,76	1,90	0,32	0,63	0,00	2,54	4,17	1,32
Dunlin	56,67	35,24	30,48	32,70	27,62	16,51	35,24	33,33	4,34
Knot	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Kentish plover	4,17	1,27	0,63	1,90	4,76	4,13	3,17	0,00	3,24
Ringed plover	2,50	0,63	1,27	0,63	0,95	0,32	1,90	0,00	0,77
Bar-tailed Godwit	0,00	0,32	0,00	0,00	0,00	0,00	0,32	0,83	0,77
Black-tailed Godwit	0,83	0,32	0,00	0,00	0,00	0,00	0,00	1,39	1,70
Whimbrel	0,83	0,63	1,59	0,63	0,95	0,63	1,27	2,22	0,66
Grey plover	0,56	0,00	0,00	0,00	0,00	0,00	0,63	0,83	1,04
Greenshank	0,00	0,00	0,32	0,00	0,00	0,00	0,00	1,11	0,00
Redshank	8,06	2,22	3,49	2,22	0,63	1,90	2,86	4,44	1,92
Total	**82,78**	**50,79**	**42,54**	**39,68**	**38,41**	**24,76**	**51,43**	**57,50**	**55,99**

Appendix E (continued)

Bird densities on the study sites, b/ha

All period. Ancão

Species	A1	A2	A3	A4	A5	A6	A7	A8
Turnstone	2,59	3,33	4,31	5,55	4,24	3,26	1,92	2,28
Sanderling	1,71	1,18	1,08	3,58	2,48	1,38	1,74	2,14
Dunlin	0,65	2,50	4,36	2,98	2,32	5,27	2,23	2,41
Knot	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Kentish plover	5,43	4,47	4,61	5,15	4,92	2,41	3,66	5,40
Ringed plover	2,86	1,82	1,62	1,18	2,44	0,94	1,65	1,34
Bar-tailed Godwit	0,57	1,53	0,98	1,81	2,39	1,21	2,28	0,49
Black-tailed Godwit	0,00	0,00	0,10	0,10	0,00	0,00	0,00	0,00
Whimbrel	1,00	0,93	1,91	2,06	1,12	0,67	0,63	1,43
Grey plover	1,43	1,12	1,42	2,30	1,97	1,79	1,43	0,49
Greenshank	0,00	0,12	0,00	0,29	0,67	0,09	0,18	0,00
Redshank	0,82	0,39	1,08	1,60	0,40	0,22	0,00	0,09
Total pooled	**15,29**	**16,96**	**26,08**	**27,06**	**15,89**	**20,36**	**14,64**	**16,70**

All period. Ramalhete and Ancão-2

Species	R1	R2	R3	R4	R5	R6	R7	R8	Ancão-2
Turnstone	4,74	2,79	1,88	1,75	0,97	3,77	6,48	1,05	4,74
Sanderling	1,95	0,91	0,26	0,26	0,00	1,04	1,82	0,73	1,95
Dunlin	20,13	25,00	27,34	23,38	28,05	26,49	26,25	4,83	20,13
Knot	0,26	12,86	12,86	9,09	9,09	20,45	17,90	0,02	0,26
Kentish plover	0,78	0,84	1,23	2,53	2,40	1,36	0,17	2,88	0,78
Ringed plover	0,26	0,65	0,26	0,52	0,13	0,78	0,00	0,37	0,26
Bar-tailed Godwit	0,32	0,26	0,26	0,00	0,00	0,65	0,80	0,43	0,32
Black-tailed Godwit	0,13	0,00	0,00	0,00	0,00	0,00	0,57	0,80	0,13
Whimbrel	0,78	0,78	0,52	0,78	0,65	0,91	1,25	0,39	0,78
Grey plover	0,32	0,45	0,32	0,39	0,39	1,23	1,19	0,78	0,32
Greenshank	0,00	0,13	0,00	0,00	0,00	0,00	0,45	0,00	0,00
Redshank	0,91	1,43	0,91	0,39	0,78	1,17	1,93	0,94	0,91
Total	**30,58**	**46,10**	**45,84**	**39,09**	**42,47**	**57,86**	**58,81**	**51,80**	**30,58**