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The role of vegetation cover and diet in explaining long-term changes in the breeding population of Little Terns (*Sternula albifrons*) in Ria Formosa, Algarve

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Table of contents

Abstract

Seabird populations are naturally regulated and their demographic fluctuations are explained by mainly two factors: the availability of suitable breeding habitats and the presence of foraging habitats with appropriate food resources. An important characteristic in determining suitability of breeding habitats for ground nesting seabirds is their vegetative cover. Suitable foraging habitats determine the availability of food resources, and are influenced by environmental variables, such as the climatic proxy, North Atlantic Oscillation (NAO) index. Environmental variables are known to affect the distribution of food resources in the marine environment which can cause declines in the availability of prey fish for seabirds.

Little Terns (*Sternula albifrons*) are known to avoid nesting on densely vegetated areas to avoid predation, however the habitats with low vegetative cover preferred by these birds to nest are subjected to the overgrowth of vegetation which leads to the abandonment of the breeding site. Little Terns are also highly susceptible to fluctuations in food availability, especially during the breeding season, and abrupt changes in their diet can lead to variations in their breeding performance which will ultimately affect the breeding population size.

This study aimed to first, evaluate if the fluctuations in Little Tern breeding population size in Ria Formosa, Algarve, could be explained by changes in vegetation cover. Secondly, it also intended to assess the role of the environmental variables and annual variations in diet, in Little Tern breeding parameters and in the number of breeding pairs in Ria Formosa, Algarve.

The percentage of vegetation cover in each sandy beach was calculated from aerial pictures of Ria Formosa barrier islands from 1976 to 2012, and related with census data from the same period. A linear regression model was performed between these two variables, after controlling for the effect of environmental variation (NAO index), and predictions of the percentage of the number of breeding pairs nesting on sandy beaches of Ria Formosa for a given percentage of vegetation cover were made. To better address this relation between the number of breeding pairs and vegetation cover, a habitat experiment was made on a site with vegetation overgrowth, with the removal of part of the vegetation before the beginning of Little Tern's breeding season. A comparison of the percentage of vegetation cover between breeding sites in salinas and on sandy beaches was also made. Little Tern diet and breeding parameters (clutch size, timing of breeding and egg size) in Ria Formosa were studied in 2012, 2013 and

2014 and added to previous published data (2002 to 2011). Annual variations in diet and the NAO index were related with breeding parameters and with census data from 1979 to 2014, to assess whether environmental variables and, as a consequence, diet could explain the fluctuations in the number of breeding pairs.

A strong negative relation was found between the number of Little Tern breeding pairs nesting on sandy beaches and the percentage of vegetation cover in this breeding habitat. In salinas, the same pattern was observed in the site subjected to the habitat experiment: the site was abandoned by breeding Little Terns when the vegetation cover became unsustainable to breed, however, birds returned to that site following vegetation removal. The comparison of the levels of vegetation between the two types of breeding habitats showed that vegetation cover in salinas was greater than that on sandy beaches, and also that sandy beaches with smaller vegetation cover had a higher number of breeding pairs. The breeding population size fluctuations over the years were also related with the environmental conditions, as captured by the NAO index. There was a relation between the negative NAO conditions and (1) earlier breeding, (2) larger clutch size, and, as a consequence, (3) higher number of breeding pairs. Diet was also related with breeding parameters and, consequently, with the number of breeding pairs.

The climatic variation measured by the NAO index did not show a significant relation with the number of breeding pairs nesting in each sandy beach colony, so we suggest that the percentage of vegetation cover is more important in explaining the breeding population size on a local scale. Similarly, in a more regional scale the food availability, determined by the climatic conditions and the NAO index, should play an important role in explaining the number of breeding Little Terns.

Keywords: Vegetation cover, environmental variables, North Atlantic Oscillation, breeding parameters, Little Tern (*Sternula albifrons*)

Resumo

As populações de aves marinhas são reguladas naturalmente e as suas flutuações demográficas são explicadas principalmente por dois fatores: a disponibilidade de habitats de reprodução adequeados e a presença de habitats de alimentação com recursos alimentares apropriados. Uma característica importante na determinação da adequabilidade dos habitats de reprodução em aves marinhas que se reproduzem no solo é a sua cobertura vegetativa e os habitats de alimentação são afetados por variáveis ambientais, tais como a Oscilação do Atlântico Norte (NAO). As variáveis ambientais são conhecidas por influenciar a distribuição dos recursos alimentares no ambiente marinho o que pode provocar declínio na abundância de pequenos peixes pelágicos para as aves marinhas se alimentarem.

A Andorinha-do-mar-anã ou Chilreta (*Sternula albifrons*) é conhecida por evitar nidificar em áreas com vegetação de forma a evitar a predação, mas os habitats com baixa cobertura vegetativa, preferidos por esta ave para nidificar, estão sujeitas ao crescimento exagerado da vegetação o que leva ao abandono do local. A Chilreta é também altamente suscetível a flutuações na disponibilidade de alimento, especialmente durante a época de reprodução, e alterações abruptas na sua dieta podem levar a variações no seu desempenho reprodutor e, em última análise, afetar o tamanho da população reprodutora.

Este estudo teve o objetivo de, primeiramente, avaliar se as flutuações no tamanho da população reprodutora de Chilreta na Ria Formosa, Algarve, poderiam ser explicadas por alterações na cobertura vegetativa. Por outro lado, também pretendeu avaliar o papel das variáveis ambientais e das variações anuais na dieta nos parâmetros reprodutores da Chilreta e no número de casais reprodutores na Ria Formosa, Algarve.

A percentagem de cobertura vegetativa em cada praia foi calculada a partir de fotografias aéreas das ilhas barreira da Ria Formosa, recolhidas entre 1976 e 2012, e relacionada com dados de censos recolhidos no mesmo período. Foi efetuado um modelo de regressão linear entre estas duas variáveis, após controlar para o efeito das variáveis ambientais (índice NAO), e foram feitas previsões da percentagem do número de casais reprodutores a nidificar nas praias da Ria Formosa para uma certa percentagem de cobertura vegetativa. De modo a avaliar melhor a relação entre o número de casais reprodutores e a cobertura vegetativa, foi realizada uma experiência de gestão de habitat, num local específico em que a vegetação cresceu exageradamente, com a remoção de parte da vegetação antes do início da época reprodutora da Chilreta.

Foi também efetuada uma comparação da percentagem de cobertura vegetativa entre os locais de reprodução nas salinas e nas praias.

Foram estudados a dieta e os parâmetros reprodutores (tamanho das posturas, tamanho dos ovos e iniciação das posturas) da Chilreta na Ria Formosa nos anos 2012, 2013 e 2014 e adicionados a dados previamente publicados (2002 a 2011). As variações anuais na dieta e o índice NAO foram relacionados com os parâmetros reprodutores e com dados de censos entre 1979 e 2014, para avaliar se as variáveis ambientais e, consequentemente, a dieta poderiam explicar as flutuações no número de casais reprodutores. Foi encontrada uma forte relação negativa entre o número de casais reprodutores de Chilreta nidificando nas praias e a percentagem de cobertura vegetativa neste habitat. Nas salinas foi observado o mesmo padrão no local sujeito à experiência de remoção da vegetação: o local foi abandonado pela Chilreta quando a cobertura vegetativa se tornou insustentável para nidificar, mas as aves voltaram ao local logo após a remoção da vegetação. A comparação dos níveis de vegetação entre os dois tipos de habitats de reprodução mostrou que a cobertura vegetativa nas salinas é maior do que a das praias e, além disso, as praias com menor cobertura vegetativa apresentaram um maior número de casais reprodutores. As flutuações no tamanho da população reprodutora ao longo dos anos estavam também relacionadas com as condições ambientais, como traduzido pelo índice NAO. Houve uma relação entre as condições negativas do NAO e (1) início das ninhadas mais precoce, (2) maiores posturas e, como consequência, (3) maior número de casais reprodutores. A dieta também esteve relacionada com os parâmetros reprodutores e, consequentemente, com o número de casais reprodutores.

A variação climática medida pelo índice NAO não mostrou uma relação significativa com o número de casais reprodutores a nidificar em cada colónia das ilhas barreira, o que nos faz sugerir que a percentagem de cobertura vegetativa é mais importante para explicar o tamanho da população reprodutora a uma escala local. Da mesma forma, numa escala mais regional, a disponibilidade de alimento, determinada pelas condições climáticas e pelo índice NAO, deverá ter um papel importante no número de Chilretas reprodutoras.

Palavras-chave: Cobertura vegetativa, variáveis ambientais, Oscilação do Atlântico Norte, parâmetros reprodutores, Chilreta (*Sternula albifrons*)

List of tables

List of figures

Chapter 1 – Introduction

1.1- Regulation of seabird population numbers

Seabirds are long-lived organisms that spend almost 90% of their lives at sea (Ballance et al., 2001), and often nest on islands in mixed-species colonies, and where terrestrial predators are often absent (Hamer et al., 2001). When breeding, they have to return to the sea to find food for themselves and for their chicks, often foraging quite far from nesting sites, which make them dependent on oceanographic conditions throughout their lives (Diamond and Devlin, 2003). Consequently, habitat use in seabirds can be divided into two main categories: nesting and foraging habitat (Hamer et al., 2001). As seabirds nest on land, most knowledge of their biology has been obtained during their breeding season, a relatively short part of their annual cycle. Seabird populations are naturally regulated by some mechanisms such as availability of food resources, availability of nesting habitat, parasites, diseases, and predation (Weimerskirch, 2001; Kildaw et al., 2005; Wakefield et al., 2014). Episodic environmental perturbations also limit some populations (Wakefield et al., 2014). However, the availability of suitable breeding habitats and foraging habitats determining food resources are considered to be the two most important factors explaining demographic fluctuation in seabirds (Fasola and Canova, 1991; Suryan and Irons, 2001).

Seabirds nest in a great variety of habitats (Hamer et al., 2001) and the distribution of their populations during the breeding season is ruled by the availability (whether the birds can or cannot use the site), quality (physical and biological attributes of the site, e.g. size, slope, location, substrate, vegetation) and suitability (all attributes of the site) of breeding sites (Potts et al., 1980; Gochfeld, 1983; Wakefield et al., 2014). In a colony of different seabird species, birds choose which areas they should use, taking into account some features such as vegetation cover, type of substrate, desired space between nests and desired space from the neighbour, influencing competition for nest sites within and between species (Hamer et al., 2001). Thus, the greater the diversity in spatial heterogeneity, the greater niche diversification is possible and the greater the probability of that place to be occupied by birds. Nest-site quality declines significantly as the population increases and, consequently, it is an important factor restraining the growth of the population (Potts et al., 1980; Duffy, 1983; Forbes et al., 2000).

The marine environment, in which seabirds rely on, is assumed to be poor, patchy and unpredictable, but also very diverse and heterogeneous, with localized rich feeding areas (Weimerskirch, 2001). Seabirds are associated with a varied range of physical features of the marine environment, such as water masses, currents, ecosystem gradients, coastline and topographical patterns (Wakefield et al., 2009). These characteristics affect physiological temperature limits and, through primary production, the general level of prey abundance and availability (Ballance et al., 2001; Paiva et al., 2008). Furthermore, over the course of the breeding season, seabirds have to adapt to the fact that their offspring grow, thus requiring larger amounts of food and different prey species, which may change in location and availability (Diamond and Devlin, 2003). This can cause dramatic spatial and temporal variations not only in breeding habitat quality but also in seabird population numbers (Suryan and Irons, 2001).

Atmospheric phenomena, such as the North Atlantic Oscillation (NAO) index, are known to affect the oceanographic conditions and allow a better visualization of the climatic variations that influence the abundance and distribution of marine taxa (Hurrell and Deser, 2010). The NAO represents a large-scale fluctuation in the air pressure difference between the subtropical Atlantic and the Artic (Hurrell, 1995; Hurrell et al., 2003) and it involves out-of-phase behaviour between the climatological low-pressure centre near Iceland (“Iceland Low”) and the high-pressure centre near Azores (“Azores High”, Stenseth et al., 2003). It is known to affect upwelling patterns (Santos et al., 2007), to induce changes in various marine trophic levels, including seabirds (Drinkwater et al., 2003; Paiva et al., 2013), to change air temperature and precipitation (Hurrell et al., 2003), and to modify sea surface temperature (SST; Visbeck et al., 2003) in the Atlantic Ocean. Fluctuations from one extreme phase of the NAO to another create variations in the wind speed and direction, in the number, intensity and paths of storms, and in their associated weather (Stenseth et al., 2003).

The availability of food resources plays an important role in regulating seabird population, in a density-dependent way (Furness, 2003; Oro et al., 2004b; Wakefield et al., 2014), considering the size of populations in relation to potential food availability around the breeding grounds or in relation to the location of other colonies of conspecifics (Furness and Birkhead, 1984; Birkhead and Furness, 1985). As a colony grows in size, prey in the surrounding waters are depleted or disturbed and, consequently, birds have to travel further away to provision their young as they grow, eventually becoming unsustainable. If the neighbour colonies compete for the same resources, then the colony size will correlate negatively with the number of neighbouring conspecifics within the potential foraging ranges (Furness and Birkhead, 1984).

Food availability affects different components of fecundity such as laying date (Safina et al., 1988; Oro et al., 1996; Shorrocks et al., 1998; Oro et al., 2004b), clutch size (Safina et al., 1988; Oro et al., 1996; Shorrocks et al., 1998; Oro et al., 2004b), egg size (Hiom et al., 1991; Bolton et al., 1992; Oro, 1996; Oro et al., 1996; Oro et al., 2004b), due to the changes in egg quality and size, hatching success is also affected (Oro et al., 1996), chick growth rate (Safina et al., 1988; Phillips et al., 1996), juvenile survival (Oro et al., 2004b) and fledgling success (Shorrocks et al., 1998). When food supply is poor, the probability of nest desertion is higher, because some females are under a critical body condition threshold and they desert after laying the first egg (Crawford and Dyer, 1995). Seabirds can also respond to low food supply by delayed breeding, abandoning a breeding attempt rather than compromise their survival and future opportunities to reproduce (Crawford and Dyer, 1995; Wernham and Bryant, 1998), as they are long-lived species.

By affecting all of these fitness components, food supply has an important impact on breeding success (Springer et al., 1984; Springer et al., 1986; Cairns, 1988; Monaghan et al., 1989; Baird, 1990; Crawford and Dyer, 1995; Phillips et al., 1996; Oro et al., 2004b). Thus, relations between seabird populations and their prey composition and abundance are very important to understand seabirds' reproductive and demographic parameters (Birkhead and Furness, 1985; Furness and Camphuysen, 1997; Le Corre and Jaquemet, 2005; Catry et al., 2006; Paiva et al., 2006b). In fact, seabirds have the potential to indicate short and long term variations in oceanographic conditions (Diamond and Devlin, 2003) through their breeding biology. Changes in their breeding numbers may be a useful indicator of fluctuations in prey abundance (Phillips et al., 1996) and consequently in the marine environment, over much larger areas than the immediate foraging grounds around the colony (Diamond and Devlin, 2003).

Seabirds depend on marine resources but they have to breed on land, resulting in an important relation between nesting and foraging habitat (Weimerskirch, 2001). While nesting, seabirds make decisions on where to feed and which prey to search for, and need to balance activities at sea (feeding, courtship and other social activities) with those on land (finding and keeping a nesting site, protecting eggs and young against predators and extreme weather, Diamond and Devlin, 2003) . Colonies are often located in proximity to productive coastal and oceanic zones and, when the distance between available breeding locations and foraging resources increases, declines in seabird population may occur (Wakefield et al., 2014). Short-ranging coastal seabirds such as terns breed very close to areas where the abundance of food resources is high, such as coastal lagoons and shallow marine areas (Paiva et al., 2008), and often nest in sand-bars, sandy-beaches or other structures that are relatively free from terrestrial and aerial predators (Medeiros et al., 2012).

1.2- Breeding habitat characteristics of Little Terns

The process of nest-site selection should maximize the birds' overall fitness, influencing their breeding success (Gochfeld, 1983; Kotliar and Burger, 1986; Medeiros et al., 2012). Coastal terns such as the Little Tern (*Sternula albifrons*) and its sibling species, the Least Tern (*Sterna antillarum*) breed often in sandy beaches, and are known to actively select locations to place their nest in order to avoid, on one hand, the most predictable threat, nest flooding by the sea, and, on the other hand, they also avoid the encroachment of vegetation coming from the other direction (Fasola and Canova, 1991; Medeiros et al., 2012). When choosing nesting habitat, in addition to the threat of flooding, birds also have to avoid the risk of predation of adults, eggs and young by natural predators and disturbance by Human-related activities (e.g. summer recreational activities) that may influence the overall nesting and breeding success (Kotliar and Burger, 1986).

Nest substrate for Little Tern could be sand, sand covered by bivalve shells, mud and gravel (Goutner, 1990; Oro et al., 2004a). Shells and other materials in the proximity of the nests are likely to provide a more cryptic background for eggs and chicks than the relatively uniform colour and consistency of pure sand, and also help adults to locate their nest within the colony (Davies, 1981; Gochfeld, 1983; Kotliar and Burger, 1986). Nests in coarse sand are more likely to succeed than those on fine sand, contributing to a greater breeding success (Medeiros et al., 2012). Vegetation cover is also an important feature of the breeding sites. Little Terns prefer to nest with low vegetative cover: less than 30% registered by Medeiros et al. (2012) in Ria Formosa and an average 15% recorded by Goutner (1990) in Evros Delta, Greece. For Least Terns, Gochfeld (1983) estimated vegetative cover as ± 10%. As the vegetation cover increases, nest occurrence decreases (Medeiros et al., 2012). Most tern species avoid nesting in vegetated areas to avoid predators that breed or hide in the vegetation: vegetation can increase predation on eggs and chicks by providing cover for predators which are less likely to venture into the open sand. This is the case of the Stone Curlew (*Burhinus oedicnemus*) that occupies and breeds in vegetation areas in the barrier sand islands of Ria Formosa, Algarve, and predates on Little Terns eggs, when birds breed in areas with more vegetation (Medeiros et al., 2012). Vegetation can also reduce the ability of terns to manoeuver (Gochfeld, 1983; Kotliar and Burger, 1986; Fasola and Canova, 1991). Alternatively, vegetation might function as a protective cover or a shelter, particularly for older chicks that are less frequently brooded, against the bad weather (strong winds and rain) and avian predators (Davies, 1981). To highlight the importance of the vegetative cover as a shelter, Davies (1981) observed at Gibraltar Point, England, that Little Terns chicks were exposed to the weather and predators and, as soon as possible (usually when chicks were two or three days old), were led by their parents from the nesting area to an adjacent saltmarsh with "protective vegetation", characterized by hummocks of cord-grass surrounded by stretches of mud. The chicks tended to remain there until they fledged, unless disturbed by tides, predators or intruders.

Habitats with low vegetative cover preferred by Little Terns to nest may be considered as ephemeral (Gochfeld, 1983) because of the natural growth of vegetation (vegetational succession) which can lead to the abandonment of the colony site as the species is said to adopt a fugitive strategy, moving readily from one site to another (Kotliar and Burger, 1984; 1986; Medeiros et al., 2007). Concerning these habitat features, Little Tern breeding habitats mainly include natural habitats, both coastal (beaches and delta marshes) and inland (lakes and rivers), and alternative/ artificial habitats (Cramp, 1985; Lloyd et al., 1991; Catry et al., 2004; Scarton, 2008). Alternative breeding habitats include artificial Human-made salt-pans (Catry et al., 2004; Medeiros et al., 2007) and fish farms (Oro et al., 2004a).

There are several studies that compare the different breeding habitats used by birds (Erwin et al., 1981; Krogh and Schweitzer, 1999; Catry et al., 2004; Scarton, 2008). In one of those studies, Catry et al. (2004) compared the Little Tern's breeding population and reproductive variables between sandy beaches (natural) and salinas (alternative) in Ria Formosa and concluded that birds nesting on salinas bred later, laid smaller eggs and smaller clutches. However, nesting success was not significantly different between the two types of habitat, which means that Little Terns can quickly adapt to breed in salinas and when both habitats are available, higher quality birds prefer to breed on sandy beaches and, when first breeding attempts failed, they may try to re-nest in salinas.

There are some causes affecting Little Tern reproductive success, both natural and anthropogenic. Natural causes are, for instance, predation, physical stability of sand, marsh or rocky substrates, flooding of eggs and chicks, strong winds, rain and natural competition for nest sites (Erwin et al., 1981; Cramp, 1985; Hong et al., 1998; Medeiros et al., 2007; Medeiros et al., 2012). In Ria Formosa, on beaches, the most common Little Terns' predators are dogs, Stone-curlews and gulls, and in salinas are dogs, cats, brown rats and birds, including Montagu's Harrier, Turnstone *Arenaria interpres* and gulls (Catry et al., 2004; Medeiros et al., 2007; Medeiros et al., 2012). Human activities related to tourism on sandy beaches, like the use of the beaches for recreation and direct perturbation by off-road vehicles, lead to the destruction, degradation and disturbance of many breeding sites and birds breeding in these locations are exposed to greater pressures (Gochfeld, 1983; Catry et al., 2004). Human influence transforms the breeding habitats compromising their availability and leading to serious consequences for the viability of Little Tern populations (Medeiros et al., 2012).

1.3- Foraging habitats and food resources for Little Terns

Variations in food supply have a strong impact on seabird breeding numbers and productivity, especially on small seabirds (Monaghan et al., 1989; Ramos, 2001; Crawford, 2003), because they spend a larger proportion of their time foraging, when compared to other seabird species (Pearson, 1968). Little Terns are, then, highly susceptible to changes in food availability, especially during the breeding season (Fasola and Bogliani, 1990) and declines in prey fish stocks can even lead to breeding failure of entire colonies (Paiva et al., 2006a; Paiva et al., 2006b). Terns are known to forage opportunistically, inshore or in nearby oceanic waters and Least and Little Terns are the most estuarine of the terns found in temperate climates, foraging closer to breeding colonies than other tern species (Cramp, 1985). A pilot study from Allcorn et al. (2003) in England concluded that 90% of the individuals foraged within 2.5 km from the coast and 99.5% in less than 2 km. Fasola and Bogliani (1990) showed that Little Terns foraged at a maximum distance of 6 km from the colony, but the majority of the foraging trips occur between 3 and 1.5 km (Davies, 1981; Cramp, 1985). Little Terns usually forage in areas characterized by shallow and transparent waters and include marine, freshwater and brackish environments (Fasola and Bogliani, 1990; Brenninkmeijer et al., 2002; Catry et al., 2006; Paiva et al., 2006a; Paiva et al., 2006b). In Algarve, they forage in natural (estuarine lagoon system and adjacent sea) and man-made (salinas and artificial channels) habitats (Paiva et al., 2006a; Paiva et al., 2006b; Paiva et al., 2008).

Adults and chicks diet consists in small fishes, crustaceans and, in a much smaller amount, insects (Cramp, 1985; Paiva et al., 2006a). Catry et al. (2006) proved that both chicks and adults diet match the abundance of the main prey, confirming the opportunistic foraging character of this species. They are, therefore, able to adjust their diet to prey availability, showing a high plasticity in their feeding behaviour. According to Paiva et al. (2006a), Little Terns breeding in Ria Formosa showed different diets regarding their breeding habitat: those breeding in salinas forage in channels and salinas near the breeding colonies and the main prey items are *Atherina* spp., *Fundulus* spp. and shrimps. Birds breeding on sandy beaches feed preferentially in the main lagoon and adjacent sea and the main prey items are *Sardina pilchardus, Atherina* spp. and *Belone belone*. Paiva et al. (2008) identified four basic needs for the selection of feeding areas by Little Terns breeding in Ria Formosa: (1) the presence of areas with abundant feeding resources; (2) the social attraction between foraging individuals: the finding of a particularly suitable place to feed should alert other foragers to that area; (3) the existence of channels with stronger currents, which should increase the availability of prey species and (4) the proximity of areas with alternative food sources (salinas), as prey availability should be more constant in the salinas where foraging activity is relatively unaffected by environmental factors such as wind speed and tidal phase (Paiva et al., 2006a).

A wide range of physical features such as wind speed, tidal phase, water clarity, salinity, water current and foraging range are known to influence feeding ecology of terns because they influence prey availability (Brenninkmeijer et al., 2002; Paiva et al., 2006a; Paiva et al., 2008). Small seabird species such as terns experience considerable difficulty maintaining stability in strong winds and these conditions also ripple the water surface and obscure visibility making it difficult to hunt by plunge diving (Shealer, 2001). Turbidity reduces visibility impairing the terns' foraging capacity (Shealer, 2001; Brenninkmeijer et al., 2002). Tide and wind speed affect the availability of certain prey and also the parents' fishing success. Therefore, these factors influenced both size and type of prey delivered to chicks (Paiva et al., 2006a).

When it comes to the relation between foraging and nesting habitat, adult Little Terns make trade-offs between foraging distance from the colony, prey availability and prey quality, influenced by the size and the energy content of prey (Catry et al., 2006). So, this relation is simple: the choice of nesting sites close to good foraging resources allows individuals to minimize travel time and energy expenditure and, thereby, to allocate a greater proportion of time and energy to their nestlings' needs (Paiva et al., 2008). In Ria Formosa, salinas are considered to be suitable habitats for Little Terns (Catry et al., 2004), but, for successful breeding, it is essential the proximity to marine/coastal lagoon areas due to the importance of marine species for chick growth. These habitats may provide important foraging areas during certain years and in periods of strong winds and decreased visibility in the lagoon habitat (Paiva et al., 2006a; Paiva et al., 2008).

1.4- Objectives

This study evaluates the importance of vegetation cover and diet in explaining long-term changes in Little Tern breeding numbers in the Algarve. This species has been censused in the Algarve since the 1970's in the different barrier islands of Ria Formosa coastal lagoon system, so we took advantage of this long-term data set on breeding numbers to evaluate whether changes in the breeding population of each barrier island can be explained by changes in vegetation cover. In each barrier island, the large majority of the Little Terns breed close to the inlets (or entrance channels), within pure sand, but progressively these areas will be covered in vegetation, which should lead to the abandonment of those sites (Kotliar and Burger, 1986; Medeiros et al., 2007). We combined data on breeding numbers collected since the 1970's with measures of vegetation cover from aerial photographs to address the fugitive strategy of Little Terns, i.e. their readily moving site to site (Kotliar and Burger, 1984; 1986; Medeiros et al., 2007) in relation to vegetation encroachment. We predict that as vegetation cover increases the numbers of breeding pairs should decrease, ultimately leading to the abandonment of a particular site. We were particularly interested in finding the level of vegetation cover that leads to the abandonment of a breeding site. In order to better address this question, in one site that was abandoned as vegetation cover increased, we removed the vegetation to see whether breeding birds returned promptly to that site.

The diet of the Little Tern has also been evaluted since 2002, and Ramos et al., (2013) presents diet data for the period 2002-2011. We added 3 more years of data on diet to evaluate the contribution of annual variations in diet in explaining annual variation in breeding numbers. This is justified because Little Terns forage on the most abundant prey items in Ria Formosa (Catry et al., 2006), and the most abundant prey item (*Atherina* spp.) influences their clutch and egg sizes (Ramos et al., 2013). Therefore, the diet of Little Tern may be regarded as a bioindicator of changes in the abundance of their main prey types.

Overall, this study will enable us to discuss the role of these two important factors, vegetation cover and food resources in explaining short- and long-term variations in the breeding population size of Little Terns in Ria Formosa, Algarve.

Chapter 2 – Methods

David Woodfall

2.1- Study area

Ria Formosa Natural Park is located on the south coast of Portugal, in the Algarve region (37°01’N, 07°48’W). The protected area of the Natural Park covers an area of approximately 18400 hectares along 60 kilometres of coastline, from Ancão to Manta Rota, through the districts of Loulé, Faro, Olhão, Tavira and Vila Real de Santo António.

The Natural Park includes a narrow strip of land and dunes, almost parallel to the coastline, constituted by peninsulas and barrier islands that form a barrier between the Atlantic Ocean and the lagoon, and protect the wide variety of habitats such as marshes, salty and fresh water lagoons, water channels, fish farms and saltpans/salinas. Most of the park comprises the lagoon system of Ria Formosa.

The barrier islands system of Ria Formosa (Fig. 1) is currently constituted by two peninsulas (Ancão e Cacela), that are respectively the western and eastern limits of the system, and five barrier islands (from west to east: Barreta or Deserta, Culatra or Farol, Armona, Tavira and Cabanas). These islands are separated by six inlets (Ancão, Faro-Olhão, Armona, Fuseta, Tavira and Lacém) that are responsible for the hydraulic, sedimentary, chemical and nutrient transport between the ocean and lagoon marshes (Ceia et al., 2010). From these referred inlets, Faro-Olhão and Tavira inlets are artificial and stabilized with jetties.

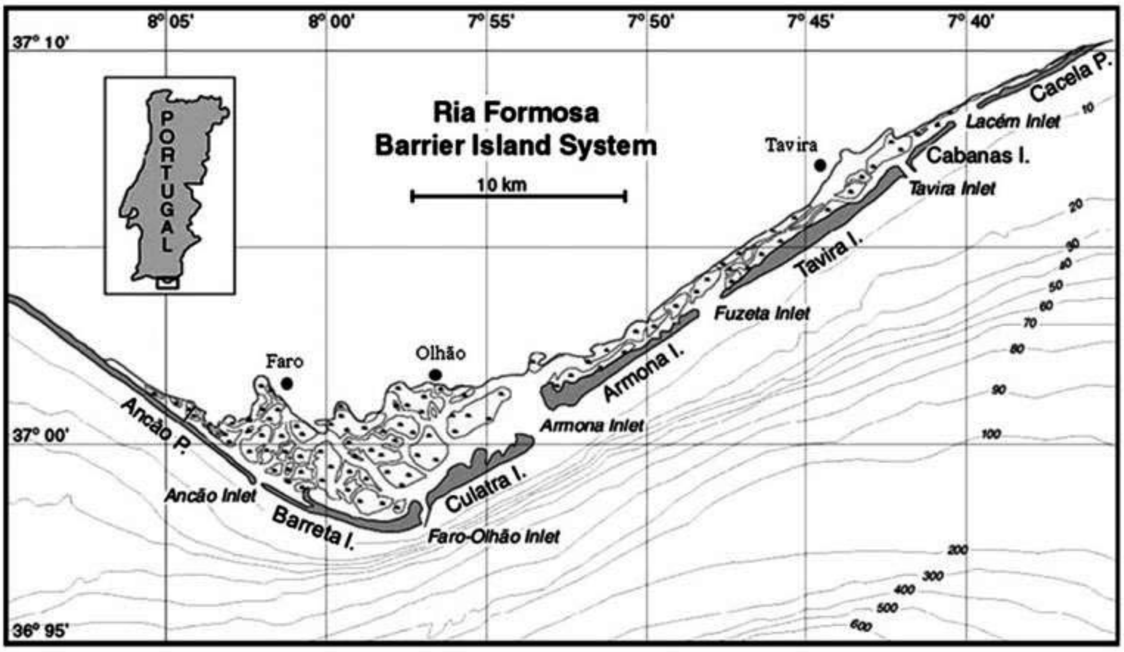

Figure 1 - Ria Formosa barrier island system, showing the peninsulas, barrier islands and inlets (adapted from Ceia et al., 2010).

This system is considered to be intensively dynamic with changes in shape and extent of the islands and also migration and/or opening of new inlets and closure of others. There are two types of migration: longitudinal migration, when occurs the accumulation of sand at the end of one of the islands and erosion of the end of the next island, and transversal migration of the system towards the continent, in response to small variations of the sea level (Dias et al., 2004). The longitudinal migration of the Ria Formosa barrier island system is considered to be cyclic: the inlets tend to migrate from west to east until they reach a limiting position when they start to infill. Then, a new inlet opens in a position close to the initial one, enabling the start of a new cycle (Weinholtz, 1978). The system dynamics varies in function of maritime agitation and tidal currents (Pilkey Jr et al., 1989), therefore it is very vulnerable and incompatible with permanent human settlement. This is especially problematic because of the tourism in several islands, with the construction of infrastructures buildings and parking lots (Ceia et al., 2010).

Ria Formosa is a national protected area, with national and international value mainly due to its avian fauna. On May 2, 1978 Ria Formosa received the status of Natural Reserve, but since 9 December 1987 it is considered a Natural Park. Ria Formosa is also included in Ramsar Convention[1], as an important wetland, and is also a Special Protection Area (SPA) under the 79/409CEE Birds Directive. This area is impacted by a variety of economic activities such as the abandonment and conversion of salinas into fish farms, sand extraction, implement of intensive aquaculture, industrial and urban pollution, illegal construction on barrier islands, tourism pressure and illegal hunting and fishing[2].

Ria Formosa has been the most important breeding site for Little Terns since the 20th century and, presently, encompasses the largest Portuguese nucleus of breeding Little Terns (is where 40% of the Portuguese population breeds, Catry et al., 2004). This site is also the only site in Portugal where Little Terns breed in natural (sandy beaches) and alternative (salinas) habitats. Little Tern colonies are distributed over all barrier islands and peninsulas previously described (natural habitat) and over the salinas that surround the lagoon system (alternative habitat).

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[1] http://www.ramsar.org accessed on 20/05/2014.

[2] http://www.icnf.pt accessed on 20/05/2014

2.2- Study species

The Little Tern (*Sternula albifrons*) (Pallas, 1764) is a colonial and migratory waterbird species of the Sternidae family. It is the smallest of the terns, with a wing-span of only 47-55cm, which corresponds to ± 2/3 the size of a Common Tern (*Sterna hirundo*), and an adult mass of 50-60g (Cramp, 1985; Schreiber and Burger, 2001). It has a worldwide, but mainly northern hemisphere distribution, breeding in every continent except in the Antarctic (Cramp, 1985). There are six sub-species that have been described: *S. a. albifrons* in Europe and Asia, *S. a. guineae* in West and central Africa, *S. a. sinensis* from South-East and East Asia to Australia, *S. a. innominata* on islands in Persian Golf, *S. a. pusilla* in North-East India and *S. a. placens* in East Australia and East Tasmania. There is also a geographical sibling species, the Least Tern (*Sterna antillarum*) constrained to the North and Centre America and the Caribbean.

In Europe, it breeds around the Baltic and North Seas and along Atlantic and Mediterranean coasts, with the largest European populations in Italy, Britain and Spain (Fig. 2; Cramp, 1985; Lloyd et al., 1991). In Portugal, the seven major Little Tern breeding areas are in: Aveiro, Tejo estuary, Sado estuary, Lagoa de Santo André, Alvor estuary, Ria Formosa and Castro Marim (Catry et al., 2004). Outside the breeding season, most of the western European population winters in West and Southern Africa and the eastern European population winters in the Red Sea and in South-East Arabia (Fig. 2; Cramp 1985).

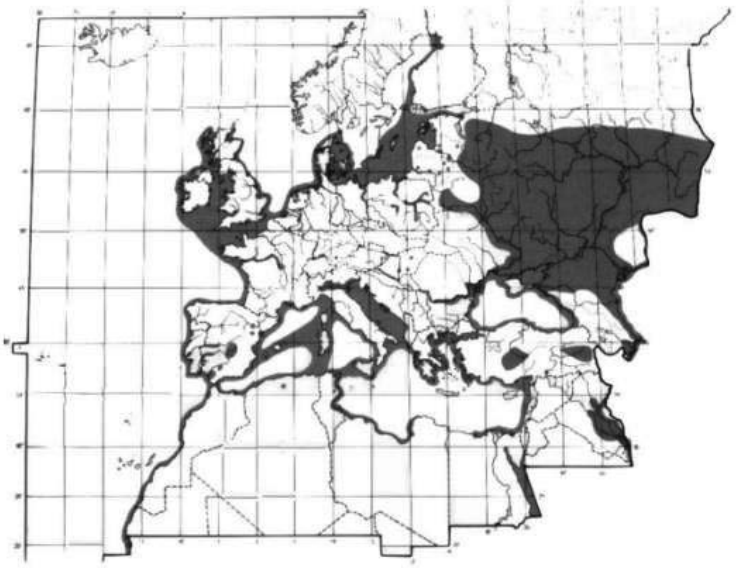

Figure 2 - Little Tern (*Sternula albifrons*) distribution in the Palearctic region (Cramp 1985).

Little Tern is a colonial seabird that tends to nest in small colonies of commonly 5 to 15 pairs and with some breeding pairs even nesting solitarily, unlike other tern species which nest in large colonies (Cramp, 1985; Fasola and Canova, 1991; Coulson, 2001; Medeiros et al., 2012). Colonially, Little Terns do not associate with Yellow-legged Gull (*Larus michahellis*), Audouin's Gull (*Larus audouinii*) or Black-headed Gull (*Larus melanocephalus*), apparently to avoid aggression from larger larid species. It commonly associates with Common Terns (*Sterna hirundo*), Gull-billed Tern (*Gelochelidon nilotica*), Avocet (*Recurvirostra avosetta*) and Kentish Plover (*Charadrius alexandrinus*) (Fasola and Canova, 1991).

Both Little Tern adults participate in choosing nest site, which is in open areas near to water (often just above the high tide mark or flood limit), typically situated on isolated islands or peninsulae, on coastal sand beaches, but it may breed also inland, on sandy islands along large rivers or on reservoirs (Cramp, 1985). Furthermore, there are some breeding pairs that nest in alternative habitats in estuaries such as salt-pans (salinas) and fish-farms (Fasola and Canova, 1991; Catry et al., 2004; Oro et al., 2004a).

The nest consists of a shallow scrape in the sand with 1-3 eggs. The incubation period, shared by both parents, occurs during 18 to 22 days and the fledgling period lasts from 19 to 20 days (Cramp, 1985; Schreiber and Burger, 2001). Gulls and terns that have three-egg clutches frequently show a pattern that could reflect a progressive decline in the female's nutritional reserves along the laying period: the last-laid egg is typically about 10% smaller than the first two and produces a smaller chick with a lower probability of survival to fledgling (Hamer et al., 2001).

Little Terns' feeding behaviour is typical from the Sternidae family: they fly over the water surface with quick wing beats and head directed downward and they feed by plunge diving from a hover, sometimes by dipping for floating prey. They usually feed singly, in small groups, or in widely scattered flocks (Cramp, 1985), and, both adults and chicks, feed on small, often juvenile, fish and invertebrates, especially crustacean and insects (Davies, 1981). Little Terns usually fish in very shallow water only a few centimetres deep (Davies, 1981), often over the advancing or receding tideline, in brackish lagoons and saltmarsh creeks, channels and lagoons (Cramp, 1985; Paiva et al., 2008). Their foraging range is very short when compared to other seabirds, with most food generally being obtained from within 4 km of the colony (Allcorn et al., 2003).

At the European level, Little Tern has experienced a long-term decline in numbers and a contraction of the breeding range (Cramp, 1985; Medeiros et al., 2007). Mitchell et al. (2004) recorded a decline of 25% in the number of breeding Little Terns, in Britain and Ireland, from 1985/88 to 1998/2002. Oro et al. (2004a) registered a decline of 2% per year (from 1961 to 2003) in the number of breeding pairs, in Ebro Delta, Spain, but from 1993 to 2003, the rate of decrease was 7.1% per year. The main reasons for Little Tern decline throughout Europe are the excessive habitat change or destruction and human disturbance (Cramp, 1985; Hong et al., 1998; Catry et al., 2004). The interference in a colony by predators or humans leads adults to fly away, leaving eggs and/or chicks exposed to weather and predators (Davies, 1981). This is especially problematic when the use of beaches by humans for recreation matches with the birds' breeding season which makes, therefore, many physically suitable habitats untenable (Medeiros et al., 2007).

BirdLife International (2004) estimated the Little Tern European Union (EU25) breeding population size as 17 000 to 23 000 pairs, which corresponds to 5 to 24% of the global breeding population. From 1970 to 1990 breeding population trend was stable and from 1990 to 2000 suffered a moderate decline. In 2004, the conservation status in the EU25 was unfavourable. The Little Tern is included in the SPEC list (Species of European Conservation Concern), being classified as SPEC 3, i.e. with an unfavourable conservation status. It is also included in the Schedule 1 of Wildlife and Countryside Act (1981), in the Annex II of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention) and in the Annex I of the Birds Directive 79/409CEE (BirdLife International 2004).

Little Tern was a common breeder in Portugal at the end of the 19th century and the beginning of the 20th century, however historical data shows important changes in breeding habitat use, mainly the abandonment or a reduction in the use of natural habitats and a colonization and increase in numbers in some areas with alternative habitats (Cramp, 1985; Catry et al., 2004). In the Portuguese Red Data Book, Little Tern is classified as vulnerable (Cabral et al., 2005).

2.3- Census data

Little Tern has been censused in the Algarve since the 1970's in the different barrier islands and salinas of Ria Formosa coastal lagoon system (Araújo & Pina, 1984; Teixeira, 1984; Calado 1995 *in* Catry et al., 2004). The number of breeding pairs in each breeding habitat censused in 1979, 1981, 1983, 1992, 1993, 2002-2007 and 2009-2014 was used to evaluate changes in the breeding population over the last 35 years, and to correlate breeding numbers with climatic variables and vegetation cover in the main breeding areas of the sand barrier islands.

From 2002 to 2014 a more in depth analysis was performed: published data on timing of breeding, clutch size, egg size and diet of Little Terns breeding in salinas of Ria Formosa from 2002 to 2004 (Catry et al., 2004) and from 2005 to 2011 (Ramos et al., 2013) were used and assembled with data collected during this study, carried out from 2012 to 2014.

2.4- Measure of vegetation cover in 2013

The vegetation cover was measured on the beaches with breeding pairs in 2013 (Praia de Faro, Culatra, Barreta and Fuseta) using a 1m^2 frame subdivided into 100 squares of 10cm by 10cm. This was obtained by establishing transects in "Z" randomly initiated in a point of the colony (Fig. 3).

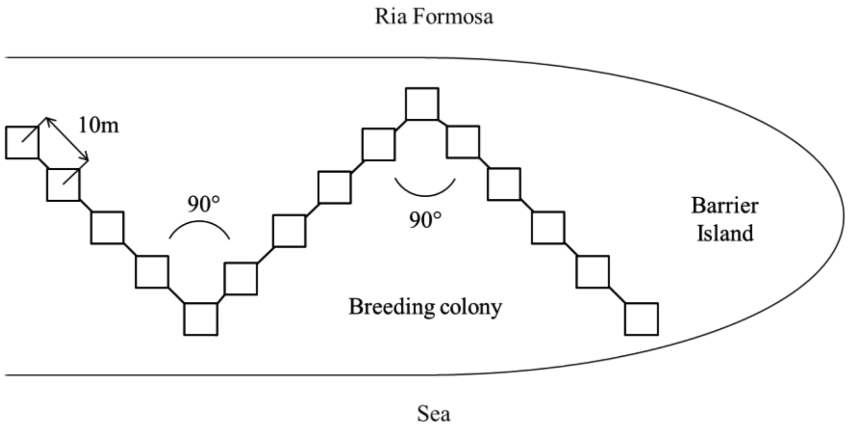

Figure 3 - Schematic representation of the process of measuring the vegetation cover in the Little Tern breeding colonies on sandy beaches. Each square represents the frame of 1m^2 and, respectively a sampling point. The centre of a sampling point distanced 10 meters from the following. The total number of sampling points was 15 in each breeding colony except in Barreta (n = 10).

From the initial point, the measurement was made by placing the frame on the sand to count the number of the 100 10x10 cm with vegetation. The sampling square was firstly placed in 5 points (including the first one), at 10 footsteps (about 10 meters) intervals, following a random direction. After these first 5 measurements, the direction changed approximately 90° and more 5 points were measured following this new direction. Lastly, the direction of the transect changed again and the last 5 points were measured, summing a total of 15 points in each colony. The exception was the colony in Barreta barrier island where only 10 points were used.

In salinas, the vegetation cover was measured only in Vale Caranguejo and Santa Luzia, in the "corridors" with breeding pairs. The number of points where the vegetation cover was measured depended on the size of the "corridor" (Vale Caranguejo n=12; Santa Luzia n=15). In Santa Luzia, vegetation cover was measured after the habitat management experiment (see section 2.6).

2.5- Measuring vegetation cover in aerial pictures

Ria Formosa Natural Park aerial images from 1976 to 2012 were analysed using ArcGIS v10.0. Little Terns breeding on sandy beaches prefer to nest on areas close to the inlets on the several barrier islands of Ria Formosa lagoon system (Catry et al., 2004). From all the aerial images available, those referring to the areas near to the inlets were selected. Starting from a known distance on each image, one kilometre was counted from the inlet to the interior of the barrier island. This was carried out for each inlet in the years with available images.

On each image of these areas two types of polygons were created along 1 km from the inlet: a) polygons with dense vegetation, representing unsuitable areas for Little Tern to nest, and b) polygons with little or no vegetation, representing the areas usable to nest (Fig. 4). A strip along the water line was also taken into account and discarded because this area should be covered in water at high tide (Medeiros et al., 2012).

Figure 4 - Analysis of the aerial images using ArcGIS10, with Fuseta inlet in the year of 2001 as an example. On the left, the original aerial image, on the right the image with the polygons created. The red polygons correspond to the unsuitable areas for Little Tern to nest and green polygons represent the suitable nesting areas without vegetation.

A total area in each barrier island was established through the use of both types of polygons, in other words, the total area is composed by both areas, suitable and unsuitable. In relation to that total area, a percentage of unsuitable area (with vegetation) was calculated. Some years with available aerial images did not correspond exactly to years with available census data, so, although the analysis of the aerial images has been performed on the images of all available years, only the years corresponding to those with census data were used.

2.6- Habitat management in Santa Luzia

Salinas of Santa Luzia are part of a group of semi-industrial salt extraction active ponds, near the city of Tavira (N 37°06’303’’; W 7°38’203’’). One area in these salinas was used for a habitat management experiment (see figure 5). This area distances about 80 m from the lagoon and 800 m from the sea, and census data from 2002 to 2012 show that the number of breeding pairs in this location decreased apparently due to the overgrowth of the vegetation, and in 2012 there were no breeding pairs nesting on that area. In March 2013, before the Little Tern’s breeding period, the vegetation cover in this area was removed, with the exception of the margins, where vegetation was maintained both for soil stabilization and to provide shelter for young chicks. Furthermore, an electric fence was placed to reduce the presence of ground predators.

Figure 5 – Location of the study site subject to the habitat management experience, inside the white circle, in relation to the lagoon, sea (Atlantic Ocean) and Tavira entrance channel (or inlet; adapted from Google Earth).

2.7- Little Tern reproductive parameters

Following methods of previous studies (Catry et al., 2004; Medeiros et al., 2012; Ramos et al., 2013) sandy beaches and salinas of Ria Formosa were surveyed in the beginning of May 2013 and 2014 to determine the number of breeding pairs in each colony and, consequently, in each breeding habitat. In order to find Little Tern nests, areas where adults were incubating and areas where birds showed nesting behaviour (courtship, nest defence, feeding activity) were searched with more detail and information on the location of the colonies in previous years was taken into account. The number of nests and the number of eggs in each nest were counted (i.e. clutch size). Information about possible egg predation and vegetation cover was also annotated.

Salinas were studied more intensively from Little Tern's laying date to fledgling. From the beginning of May to the beginning of June, these colonies were visited at least once a week. On the first visit to each colony, each nest was marked by a numbered wooden tongue, well camouflaged, placed at about 40 cm from the nest; eggs were measured with a calliper (accuracy ± 0.1 mm) and numbered using a soft pencil. Egg length (L) and egg breadth (B) were registered (in mm) and egg volume was calculated using the formula: $V\ (cm^3) = (K \times L \times B^2)/1000$, where K=0.4866 as calculated by Coulson (1963) for kittiwake (*Rissa tridactyla)* and following the analysis of other studies on Little Tern such as Holloway (1993), Hong et al. (1998) or Ramos et al. (2013). On subsequent visits, in each previously numbered nest, the possible increase in the number of eggs (clutch size) was observed and if it occurred, the new eggs were also numbered and measured. Egg status (egg intact, damaged, missing, predated, about to hatch or hatched) and possible causes of nest failure (e.g. signs of predation) were also registered in each visit. The most common predators (dogs *Canis familiaris*, gulls *Larus* spp. and brown rats *Rattus norvegicus*) were identified from footprints and eggshell fragments around failed nests. From 13 to 17 May, the nesting areas were visited daily to assess whether the clutches were laid in the first or in the second 15-day period of May, thus obtained the timing of breeding (percentage of clutches initiated in the first fortnight of May), following Ramos et al. (2013). In order to avoid counting second breeding attempts (Medeiros et al., 2007), the nesting areas were not visited after the first fortnight of June, because data from previous years showed that very few clutches were laid after this date (Ramos et al., 2013). Clutch size and egg volume were calculated only using data from complete clutches. In order to prevent pseudo-replication problems (Hurlbert, 1984) in egg measurements analysis, the mean for each clutch was first calculated and then these mean values were used to calculate the annual mean of all clutches.

Each visit to the colonies took the least time possible (10 to 20 min) in order to reduce disturbance. Birds resumed incubation soon after we left the study colony, which means that these visits did not cause disorder (Catry et al., 2004).

2.8- Environmental variables

The North Atlantic Oscillation (NAO) controls fluctuations in temperature, salinity, vertical mixing and circulation patterns that affect marine biology (Hurrell and Deser, 2010). This environmental variable is a useful proxy for meteorological and oceanographic phenomena such as wind speed, upwelling patterns, strength of ocean currents and sea-surface temperature (Hurrell et al., 2003), parameters that affect seabirds directly or indirectly, through changes in the availability of their prey. These physical conditions, especially upwelling induced by storms, favour the development of pelagic fish eggs and larvae, increasing fish recruitment (Checkley et al., 1988). Each trophic level, from phytoplankton to top predators can be affected by climate itself or by effects of climate on the trophic levels below (Sandvik et al., 2005).

Two versions of the NAO index were used to describe large scale climatic variables influencing the breeding population and reproductive parameters of Little Terns. Both indexes measure the fluctuations in the difference of atmospheric pressure at sea between Iceland (Stykkisholmur/Reykjavik) and (1) Gibraltar or (2) Azores. First version of the NAO index, measured between Iceland and Gibraltar and including the Algarve area, was chosen because it should depict a more coastal and local climatic effect on marine productivity (Jones et al., 1997). These data were available on the Internet (http://www.cru.uea.ac.uk/cru/data/vinther/nao1821.txt) from the year 1821 to 1999 and on http://www.cru.uea.ac.uk/~timo/datapages/naoi.htm from 1999 to 2013. The NAO index used for each year (from 1976 to 2013) was the mean of April and May, corresponding to the Little Tern's main laying season (Ramos et al., 2013). The second NAO index, measured between Iceland and the Azores, was chosen because it should depict a more oceanic climatic effect on marine productivity (Hurrell and Deser, 2010). It was also available on the Internet (https:// climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-station-based), monthly, from 1865 to 2013. Data for the years and months of interest (1976 to 2013, April and May) were selected and, once again, the mean of April and May was calculated.

The SST data was also downloaded from the Internet, using IGOSS (Integrated Global Ocean Services System, Reynolds et al., 2002), that blended from ship, buoy and bias-corrected satellite data. The location used for the SST data research was 7°5'W and 36°5'N, which is the closest marine area to the Ria Formosa barrier island system available on http://iridl.ldeo.columbia.edu/SOURCES/.IGOSS/ and, for this location, the SST data was only available from 1982 to 2014. As for the NAO index, we calculated the mean SST of April and May for each year, which should represent a proxy of productivity for the coastal sea, inlets and lagoon habitats of Ria Formosa during the Little Tern laying season.

2.9- Diet analysis

Little Terns are known to regurgitate along the water line of the ponds while resting. Pellets were collected in salinas of the study area in 2012, 2013 and 2014, from 2 May to 15 June, which is the main laying season, and to avoid collecting pellets from chicks. The whole area of the salinas with breeding birds was searched to guarantee that pellets were collected from as many different individuals as possible. Pellets were stored in individual paper bags or small tubes and were later analysed in the laboratory, where the hard parts of each pellet were separated from the remaining material. These hard parts, mainly sagittae otoliths, were identified to the lowest taxonomic level possible, with the help of photographs of previous analyses (Catry et al., 2006; Ramos et al., 2013) and identification guides (Assis, 2004; Campana, 2004; Tuset et al., 2008). Also, the presence of fragments of insects and crustaceans in the remaining material of each pellet was registered.

A table with the number of otoliths of each species in each pellet was obtained, but most of the pellets contained either sand-smelts (*Atherina* spp.) or gobies (*Pomatoschistus* spp.) in a larger number and the other species were present but at much lower frequencies (Catry et al., 2006). This table was, therefore, converted to a table of presence/absence of a given species in a given pellet. Thus, the diet composition of adult Little Terns was expressed as frequency of occurrence, calculated as the number of pellets with a given prey type. Diet analysis using pellets and otoliths may be biased mainly towards smaller otoliths because of the differential digestibility of otoliths of the several prey items, although it allows to compare data among different years (Duffy and Jackson, 1986; Catry et al., 2006). Our diet data obtained from 2012 – 2014 was assembled with published data from 2002 to 2011 (Ramos et al., 2013).

The sand-smelts longest otolith axis (x) was measure with a calliper to estimate length ($y = 0.2148x^2 + 21.1640x + 3.3635$, in mm) and mass ($y = 0.6726x^2 - 0.8113x + 0.2766$, in g) of ingested fishes for 2012, 2013 and 2014. These equations were established by J. Martins of the University of Algarve using fresh specimens captured in Ria Formosa (Ramos et al., 2013).

2.10- Data analysis

A multiple regression between census data on sandy beaches since 1979, and: 1) the percentage of vegetation cover in the same breeding habitat and 2) the mean NAO index for April and May, measured between Azores and Iceland, was made. We were particularly interested in the relationship between the number of breeding pairs at each site and the vegetation cover. However, to control for the climatic influence on the breeding population, we also used the NAO index in the multiple regression, and used the partial correlations of each independent variable. This analysis was carried out only for barrier islands and for the years with both data available (the number of breeding pairs and aerial images) and intends to evaluate the relation between number of breeding pairs and vegetation cover, controlling for the effect of climatic influence (using the NAO index) on the number of breeding pairs.

A regression model was constructed to model the effect of vegetation cover on the Little Tern census data and thus predict the maximum vegetation cover suitable for Little Terns to nest on sandy beaches. Firstly, we calculated the percentage of the number of breeding pairs in each year and for each study area. The total number of breeding pairs nesting in the study areas (Faro, Barreta, Culatra, Armona, Tavira and Cabanas) was calculated for each year and then the percentage of breeding birds in each study areas was calculated in relation to the total of that year. Secondly, all the study areas used previously in the partial correlations were combined to predict the percentage of the number of breeding pairs on sandy beaches for a certain level of vegetation cover.

Pearson correlations were used to test the relationship between the census data from 1979 to 2014 and (1) SST, (2) April NAO index, (3) May NAO index, (4) mean NAO index for April and May. The same correlation test was also used to measure the relationship between the census data from 2002 to 2014 and (1) environmental variables, (2) reproductive parameters and (3) diet composition. These correlations were carried out taking into account the following assumptions: environmental variables function as predictors of annual variation in the occurrence of sand-smelts in the Little Terns' diet, as this is their main prey, and the relative occurrence of sand-smelts in their diet allows to explain the variability in breeding variables (Ramos et al., 2013). Again, the NAO index for each month separately (April and May) was used in addition to the mean NAO index for these two months. The reproductive parameters used were: timing of breeding (the percentage of total clutches initiated between 1 and 15 May), mean clutch size (the number of eggs per nest, only in complete clutches) and mean egg volume. Diet variables used were the percentage of occurrence of sand-smelts in Little Terns' diet and the estimated mean *Atherina* spp. mass.

The estimated mean mass of ingested sand-smelts was compared among years, from 2007 to 2014, using a One-way ANOVA followed by a post-hoc Tukey test.

Response variables were tested for normality and, when needed, were transformed (Zuur et al., 2010). Number of breeding pairs in each habitat, mean clutch size and mean sand-smelts mass were log transformed, and the percentage of *Atherina* spp. in Little Terns’ diet and percentage of clutches initiated between 1 and 15 May were arcsine transformed. Mean egg volume and environmental variables were not transformed because they are continuous variables. In the partial correlations between the census data and the vegetation cover, data on the number of breeding pairs was log transformed. However, in the plots, in order to obtain a better visualization, non-transformed data is presented. All analysis were performed with a significance level of $p < 0.05$ and results are given in mean ± SD.

The number of breeding pairs nesting in the salinas of Santa Luzia was plotted on a graph to describe the variation in the number of breeding pairs over the years, and to visualize the relationship between the removal of vegetation and the number of breeding pairs in that area.

All statistical analyses were carried out with STATISTICA v12.0 (Statsoft 2013). Aerial images were analysed with ArcGIS v10.0 (ESRI 2010).

Chapter 3 – Results

3.1- Vegetation cover and number of breeding pairs

The total number of breeding pairs underwent changes over the years, creating a cycle: years with a lower number of breeding pairs are followed by years with a progressive increase in the number of breeding birds. However, this type of cycle ended in 2005, and since then the number of breeding pairs appears to be decreasing, which is particularly noticeable in the years of 2005 to 2006 and 2012 to 2013 (Fig. 6). In relation to the type of habitat used to nest, the number of pairs breeding in salinas has been always smaller than those nesting on sandy beaches (Fig. 7).

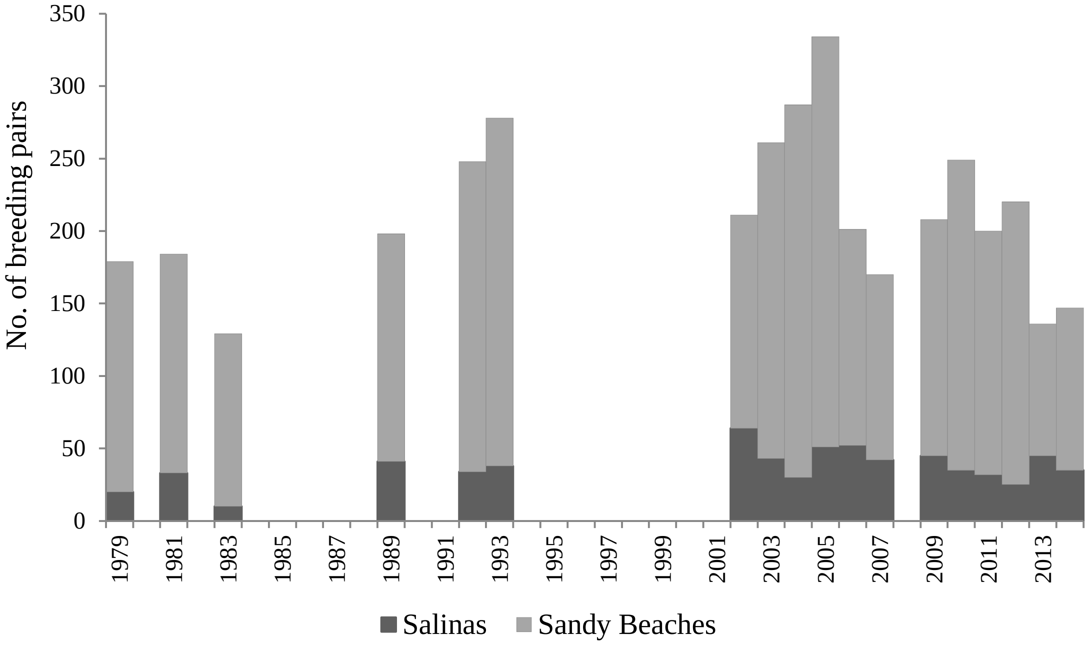

Figure 6 - Number of Little Tern (*Sternula albifrons*) breeding pairs on sandy beaches (lightest grey) and in salinas (darkest grey) of Ria Formosa (Algarve) in the years 1979, 1981, 1983, 1989, 1992, 1993, 2002-2007 and 2009-2014 (data from 1970 to 2002 was taken from Catry et al., 2004, and the remaining data are from annual census of the Little Tern breeding population in Ria Formosa).

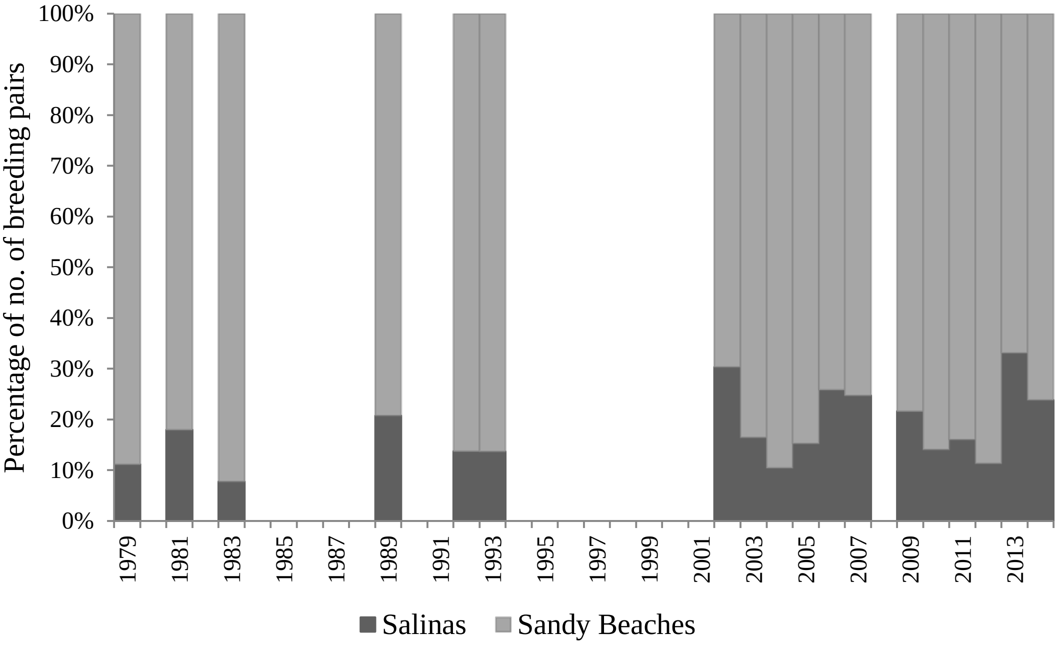

Figure 7 - Percentage of the total number of Little Tern (*Sternula albifrons*) breeding pairs on sandy beaches (lightest grey) and in salinas (darkest grey) of Ria Formosa (Algarve) in the years 1979, 1981, 1983, 1989, 1992, 1993, 2002-2007 and 2009-2014 (data from 1970 to 2002 was taken from Catry et al., 2004, and the remaining data came from annual census of the Little Tern breeding population in Ria Formosa).

The comparison of the percentage of vegetation cover between sandy beaches (Praia de Faro, Barreta, Culatra, Fuseta) and salinas (Vale Caranguejo) is shown in figure 8. Although the vegetation cover was measured in salinas of Santa Luzia, it is not present in figure 8 because this site was subjected to a habitat experience and the vegetation was measured after that experience, which means that the percentage of vegetation cover measured in this site was exceptionally low.

On sandy beaches, the levels of vegetation are smaller than those in salinas. For high levels of vegetation cover, the number of breeding pairs is small, such for Vale Caranguejo, and, on sandy beaches where the levels of vegetation cover are smaller, there are more breeding pairs nesting in this habitat (Fig. 8).

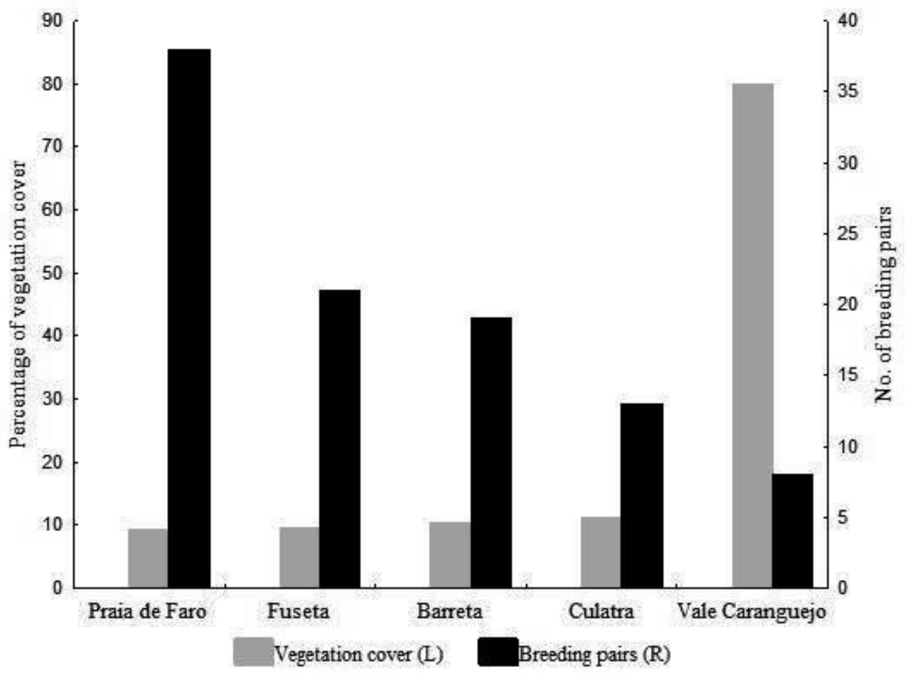

Figure 8 - Mean percentage of vegetation cover measured in 2013 (grey bars, scale on the left, L) and the number of Little Tern (*Sternula albifrons*) breeding pairs (black bars, scale on the right, R) nesting on sandy beaches (Praia de Faro, Fuseta, Barreta and Culatra) and in salinas (Vale Caranguejo) of Ria Formosa (Algarve).

The partial correlations of the number of breeding pairs on each barrier island with both the percentage of vegetation cover area and the general environmental conditions for that year indicate no relationship with NAO ($r < 0.37$, $t < 1.06$; $p > 0.32$ for the sandy beaches of all barrier islands). However, the number of breeding pairs showed a strong negative relationship with vegetation cover (Table I), after controlling for the effect of climatic variation: the higher the vegetation cover, the smaller the number of breeding pairs nesting in a certain barrier island. This was significant for each sandy beach, always with associated probabilities smaller than 0.009.

Table I – Partial correlations (with r, t and p values) between the number of Little Tern (*Sternula albifrons*) breeding pairs and the percentage of vegetation cover in each sandy beach. The analysis was performed only for sandy beaches and for the years with both the number of breeding pairs and aerial images available. The number of years used for each sandy beach is represented between parentheses. Data for the number of breeding pairs was log transformed to attain normality.

Sandy beach	Partial Correlation (*r*)	t	p
Faro (n = 11)	-0.76	-3.50	0.007
Barreta (n = 10)	-0.86	-4.68	0.002
Culatra (n = 11)	-0.76	-3.54	0.006
Armona (n = 11)	-0.81	-4.12	0.003
Tavira (n = 10)	-0.82	-4.00	0.004
Cabanas (n = 9)	-0.81	-3.61	0.009

In order to predict the maximum percentage of vegetation cover suitable for Little Terns to nest on sandy beaches, a regression model was performed, combining the data of all areas. As expected, the effect of the mean NAO index for April and May measured between Azores and Iceland did not show a significant relation with the number of breeding pairs (partial correlations, $p = 0.83$, when NAO index was included in the multiple regression).

The regression model is given by the equation $y = -0.44(\pm 0.05)x + 43.52(\pm 3.00)$, where x stands for the percentage of vegetation cover and y stands for the percentage of the number of breeding pairs, calculated in relation to the total number of breeding birds in the study areas in each year. The regression coefficient associated is $r = -0.77$ and the probability associated is $p < 0.001$ (Fig. 9). With this model, predictions about the percentage of the number of breeding pairs nesting on a particular sandy beach of Ria Formosa for a certain percentage of vegetation cover were made (Table II).

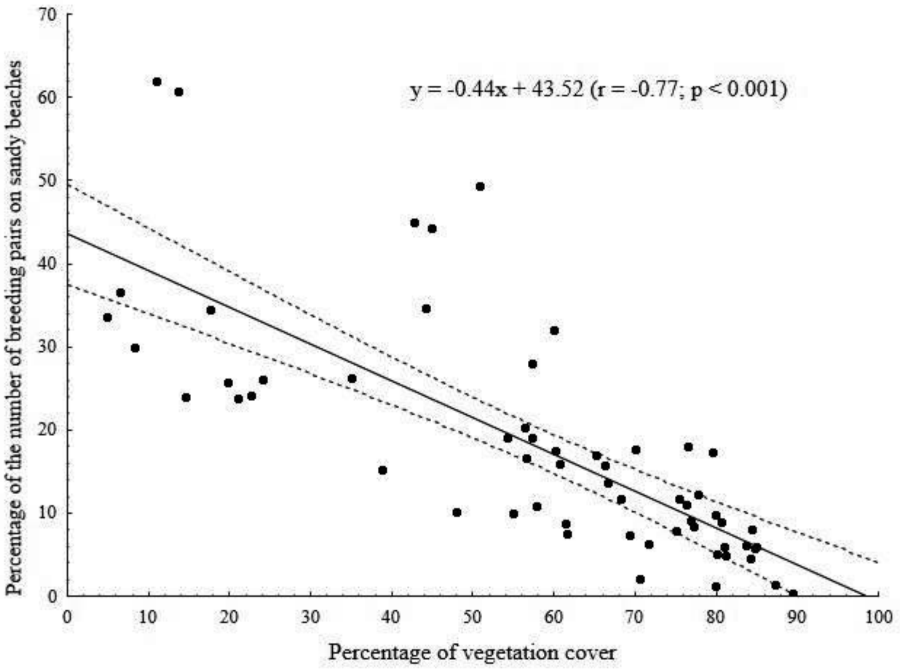

Figure 9 - Scatterplot showing the effect of the vegetation cover on number of Little Tern (*Sternula albifrons*) breeding pairs on sandy beaches of Ria Formosa, Algarve (Faro, Barreta, Culatra, Armona, Tavira, Cabanas), calculated in relation to the total number of breeding birds in each year.

Table II – Predictions of the percentage of the number of Little Tern (*Sternula albifrons*) breeding pairs nesting on a particular sandy beach of Ria Formosa taking into account five different percentages of vegetation cover (0, 25, 50, 75 and 90%) provided by the regression model and the maximum and minimum values associated to those previsions.

% Vegetation cover	Minimum % of breeding pairs	% of breeding pairs	Maximum % of breeding pairs
0	37.51	43.52	49.52
25	28.60	32.51	36.42
50	19.06	21.50	23.94
75	7.64	10.49	13.33
90	0.03	3.88	7.73

From 2002 to 2005, the number of pairs nesting in salinas of Santa Luzia increased until holding 32 breeding pairs. Since 2005 to 2012, the number of individuals decreased until that in 2012 there were no breeding pairs nesting in these salinas (Fig. 10). After the removal of part of the vegetation cover in March 2013, before the main Little Terns' laying season, there was a small increase in the number of breeding pairs in the study area (from 0 breeding pairs in 2012 to 5 breeding pairs in 2013). However, in 2014 the whole area surrounding the tank used in this study (including the tank itself) was flooded and there were no conditions for Little Tern to breed.

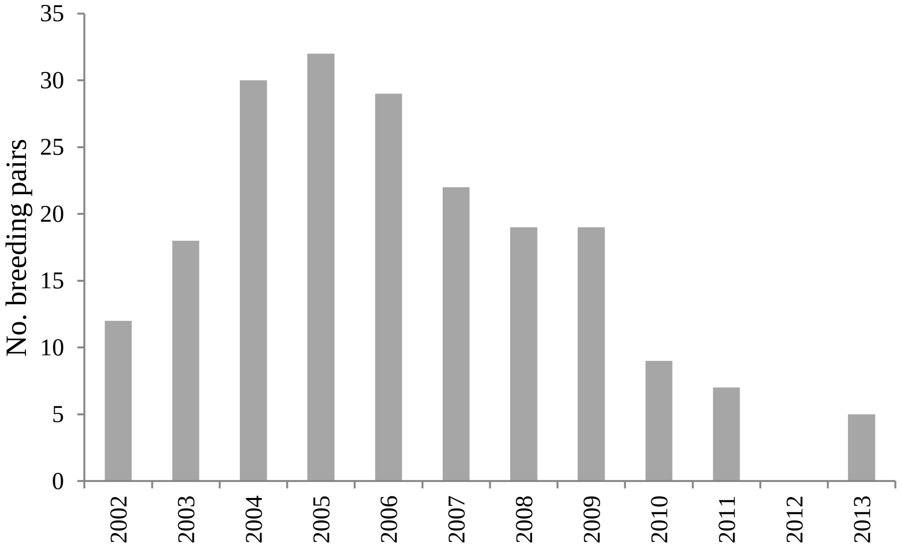

Figure 10 - Number of Little Tern (*Sternula albifrons*) breeding pairs in salinas of Santa Luzia, Ria Formosa (Algarve) between 2002 and 2013.

3.2- Resources availability, breeding parameters and number of breeding pairs

3.2.1- Reproductive parameters of Little Terns nesting in salinas

Breeding variables, such as the timing of breeding, assessed as the percentage of clutches initiated in the period of time from 1 to 15 May, clutch size, evaluated as the number of eggs per clutch and egg sizes of Little Terns nesting in salinas of Ria Formosa were studied in more detail from 2002 to 2014 and are resumed in table III. Despite the lack of data for the years of 2006 and 2007, it is noticeable a considerable variation of the percentage of clutches initiated between 1 and 15 May over the years (Table III). Little Terns laid their eggs in the first fortnight of May for all study years with the exception of 2009 and 2013, however the percentage of clutches initiated in this period was always lower than 17% with the exception of 2008, when 47.5% of the clutches were initiated in the first fortnight of May.

The mean clutch size changed over the study years (Table III), with the minimum value of 1.87 eggs per clutch in 2002 and the maximum value of 2.83 eggs per clutch in 2010. Regarding the egg measurements, the eggs with the smallest mean volume were registered in 2011 (= 7.99 cm^3) followed by 2002 (= 8.14 cm^3; Table III).

Table III - Variation in the reproductive parameters of Little Terns (*Sternula albifrons*) in salinas of Ria Formosa (Algarve) from 2002 to 2014. For each year, the sample size (*N*) refers to the number of completed clutches and values for clutch size and egg measurements are represented by mean ± SD. n.d. means no data. Data from 2002 to 2011 were taken from Ramos et al. (2013).

Year	*N*	Clutches laid 1-15 May (%)	Clutch size (eggs/ clutch)	Egg length (mm)	Egg breadth (mm)	Egg volume (cm3)
2002	53	6	1.87 ± 0.63	31.66 ± 1.16	22.98 ± 0.53	8.14 ± 0.53
2003	97	16.5	2.68 ± 0.52	32.06 ±0.99	23.48 ± 0.47	8.61 ± 0.46
2004	70	7.9	2.2 ± 0.63	32.08 ±1.16	23.40 ± 0.59	8.56 ± 0.57
2005	47	5.8	2.47 ± 0.66	32.50 ± 2.68	23.39 ± 0.59	8.66 ± 0.92
2006	28	n.d.	2.66 ± 0.45	31.90 ± 0.99	23.39 ± 0.41	8.49 ± 0.36
2007	32	n.d.	2.61 ± 0.48	32.04 ± 0.78	23.6 ± 0.48	8.68 ± 0.38
2008	31	47.5	2.48 ± 0.63	31.90 ± 0.79	23.39 ± 0.54	8.50 ± 0.46
2009	35	0	2.34 ± 0.69	32.08 ± 1.21	23.55 ± 0.58	8.66 ± 0.59
2010	29	10.3	2.83 ± 0.38	31.67 ± 1.05	23.58 ± 0.44	8.57 ± 0.47
2011	35	2.9	2.37 ± 0.65	31.10 ± 0.93	22.96 ± 0.58	7.99 ± 0.55
2012	25	2.2	2.32 ± 0.85	31.25 ± 1.09	23.39 ± 0.62	8.33 ± 0.54
2013	33	0	2.15 ± 0.76	31.73 ± 1.08	23.68 ± 0.56	8.66 ± 0.53
2014	20	2.9	2 ± 0.79	31.42 ± 1.04	23.46 ± 0.64	8.42 ± 0.52

3.2.2- Diet of Little Terns nesting in salinas

Regarding Little Tern diet in Ria Formosa, it was dominated by sand-smelts (*Atherina* spp., probably *Atherina presbyter* which is the most abundant fish species in the water column of Ria Formosa lagoon system, Ribeiro et al., 2008) and gobies (*Pomatoschistus* spp.) (Table IV). Pelagic prey such as garfish (*Belone belone*) occurred in less than 12% of the pellets and sardine (*Sardina pilchardus*) occurred in less than 3% of the pellets with the exception of 2005 (Table IV). In 2014 a new species appeared in the Little Terns' diet, *Ammodytes tobianus*.

Table IV - Annual variation in the diet of adult Little Terns (*Sternula albifrons*) in salinas of Ria Formosa (Algarve). Data are in % of occurrence of each prey species in the diet. The sample size (= no. of pellets) for each year is indicated in parenthesis. Data from 2002 to 2011 were taken from Ramos et al. (2013).

Prey species	2002 (87)	2003 (130)	2004 (130)	2005 (351)	2006 (97)	2007 (220)	2008 (141)	2009 (113)	2010 (113)	2011 (46)	2012 (133)	2013 (141)	2014 (176)
Atherina spp.	61.6	84.8	44.8	86.1	79.4	66.8	83.7	80.5	77.8	65.2	37.6	67.4	56.3
Pomatoschistus spp.	34.2	38.1	63.2	17.1	36.1	50.9	38.3	28.3	35.8	32.6	31.6	34.0	35.8
Fundulus spp.	6.8	5.7	9.2	0	1.0	5.0	0	0.9	0	10.9	9.0	0.7	6.25
Gobius spp. [a]	4.1	0.9	8.0	5.4	7.2	24.1	14.2	8.0	9.9	8.7	2.3	2.1	1.1
Belone belone	12.3	10.5	5.7	0	4.1	0	0.7	1.8	0	2.2	5.3	9.9	11.4
Diplodus spp.	5.5	3.8	2.3	8.6	2.1	4.1	8.5	13.3	11.1	8.7	0.8	0	1.1
Sardina pilchardus	0	0	0	8.6	1.0	2.7	0	0.9	0	0	1.5	0	0.6
Liza spp.	0	0	0	0	0	2.3	0	0	0	10.9	0	0	0
Ammodytes tobianus	0	0	0	0	0	0	0	0	0	0	0	0	9.7
Insects	0.1	0.1	0	3.6	9.3	2.7	9.9	5.3	8.6	10.9	15.8	9.9	16.5
Crustacea	17.8	9.5	54.1	10.0	11.3	0.5	2.1	0.9	3.7	26.1	14.3	16.3	18.2
Other prey [b]	0	0	0	0.4	0	1.4	5.0	1.8	1.2	10.9	0	0.7	0
Not identified	41.1	24.8	21.8	45.7	19.6	27.3	27.7	21.2	3.7	41.3	9.8	5.7	10.2

[a] Include *Lesueurigobius friesii* (3.7% in 2010) and *Parablennius* spp. (0.9% in 2007, 1.42% in 2008).
[b] Include *Bothus* spp., *Engraulis encrasicolus*, *Microchirus boscanion*, *Mullus surmuletus*, *Symphodus* spp., *Spondyliosoma* spp., *Serranus* spp., *Oblada melanura*.

In general, the frequency of occurrence of insects has been increasing since 2004 and the consumption of crustaceans, after reaching a peak in 2004, remained at relatively low frequencies of occurrence (below 11%) until 2011, when the consumption of crustaceans increased until it reached about 26% (Table IV). It seems that in years with lower consumption of sand-smelts birds ingested more gobies, crustaceans and other unidentified prey (Fig. 11), and this was particularly noticeable in 2004.

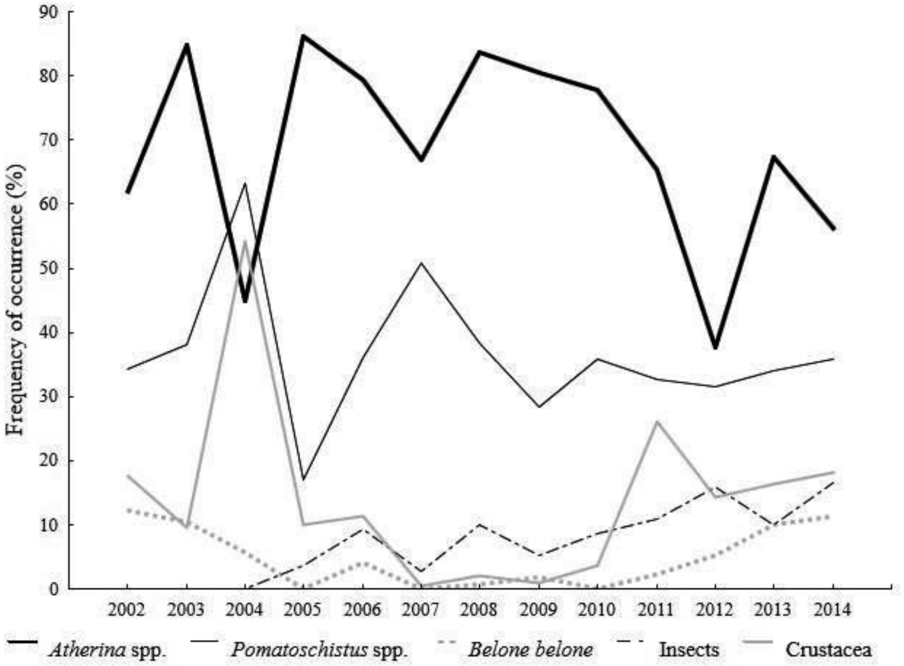

Figure 11 - Frequency of occurrence of *Atherina* spp., *Pomatoschistus* spp., *Belone belone*, insects and crustaceans in the diet of Little Tern in Ria Formosa (Algarve) from 2002 to 2014.

The estimated mean mass of *Atherina* spp. ingested by adult Little Terns on the several study years is present on table V. This parameter varied significantly among years ($F_{7,1195} = 56.83$, $p < 0.001$) and the post-hoc Tukey test showed that in 2012 the mean mass of sand-smelts was significantly lower than in the other years, followed by 2011 and 2013.

Table V – Estimated mean mass (g) of sand-smelts (*Atherina* spp.) ingested by adult Little Terns (*Sternula albifrons*) in Ria Formosa (Algarve) from 2007 to 2014. Results are presented in mean ± SD. The *N* represents the number of *Atherina* spp. otoliths measured in each year. Data from 2007 to 2011 were taken from Ramos et al., 2013.

Year	*N*	*Atherina* spp. mass ± SD (g)
2007	248	3.54 ± 0.84
2008	270	3.72 ± 0.81
2009	207	3.77 ± 0.87
2010	61	3.40 ± 1.06
2011	23	2.40 ± 1.68
2012	90	1.55 ± 1.10
2013	188	2.81 ± 1.44
2014	116	3.18 ± 1.36

3.2.3- Census data, environmental variables, breeding parameters and diet

From 1979 to 2014, oscillations in the total number of breeding pairs seem to be related with the variations in the NAO index, which is shown in the figure 12. Figure 12 relates the number of Little Tern breeding pairs with the mean NAO index, measured between Azores and Iceland for April and May and, by looking at that figure, it seems to exist an inverse trend between these variables, particularly evident from 2002 to 2014, when there are more census data available (Fig. 12).

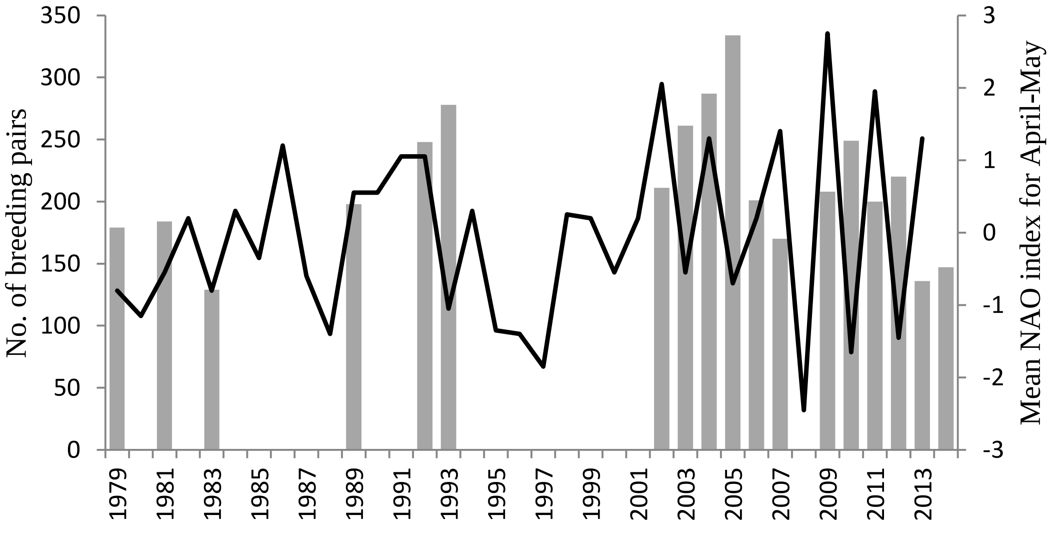

Figure 12 - Census data of Little Tern (*Sternula albifrons*) breeding pairs in Ria Formosa (Algarve) in both breeding habitats (sandy beaches and salinas, grey bars) in relation to the NAO index over the years (black line), measured between Azores and Iceland. NAO index is represented by the mean of April and May values.

In fact, from 1979 to 2014, correlations showed a significant negative correlation between the number of pairs nesting on sandy beaches and the NAO index for May (r = -0.55, p = 0.02, n = 17, fig. 13). However, this correlation was not significant for April.

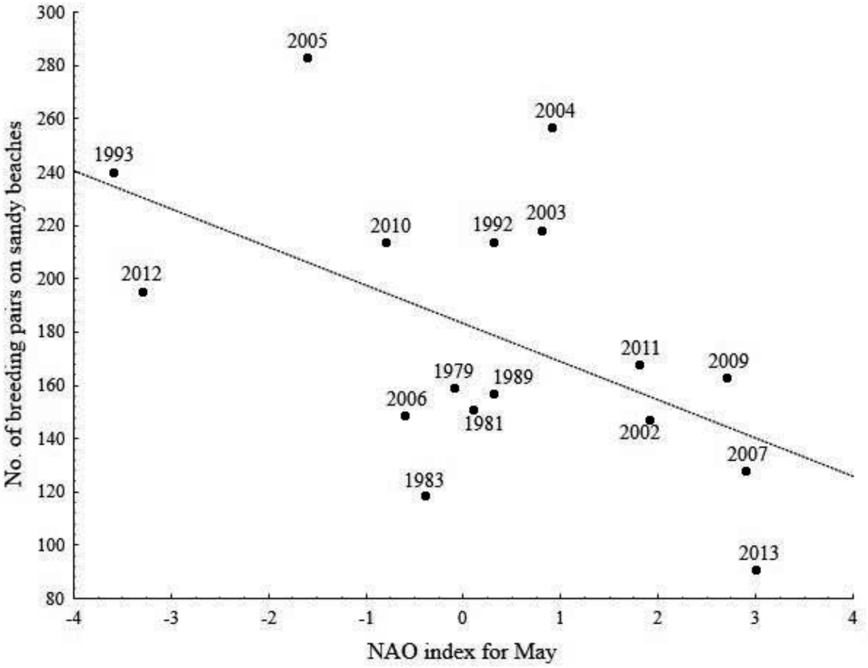

Figure 13 - Relationship between the number of Little Tern (*Sternula albifrons*) breeding pairs on sandy beaches of Ria Formosa (Algarve) and the NAO index (measured between Azores and Iceland) for May of the years 1979, 1981, 1983, 1989, 1992, 1993, 2002-2007 and 2009-2013. (r = -0.54, p = 0.03, n = 17).

The total and yearly number of breeding pairs (nesting in salinas and on sandy beaches) since 1979 was also negatively correlated with the NAO index in May, although only approaching significance (r = -0.43, p = 0.08, n = 17). SST values did not show an important relation with the census data, neither in salinas nor on sandy beaches. From 2002 to 2014 a negative relation was found between the total number of breeding pairs nesting in both breeding habitats and the NAO index in May (r = -0.57; p = 0.07; n = 11), but a strongest relation was found between the number of breeding pairs on sandy beaches and the NAO index in May (r = -0.62, p = 0.04, n = 11). Once again, these correlations were not significant with the NAO index in April and SST did not show a significant relation with the number of breeding pairs.

The NAO index, measured both between Gibraltar and Iceland, and between Azores and Iceland, had significant correlations with the percentage of clutches initiated between 1 and 15 of May (r = -0.73, p = 0.02, n = 10 for the Gibraltar / Iceland NAO index; r = -0.68, p = 0.03, n = 10 for the Azores / Iceland NAO index, fig. 14). In positive NAO index years, the percentage of clutches initiated between 1 and 15 May is lower than that on negative NAO index. With negative values of the NAO index, the percentage of clutches initiated between 1 and 15 May is higher and so, Little Terns breed earlier than in years with positive NAO index values.

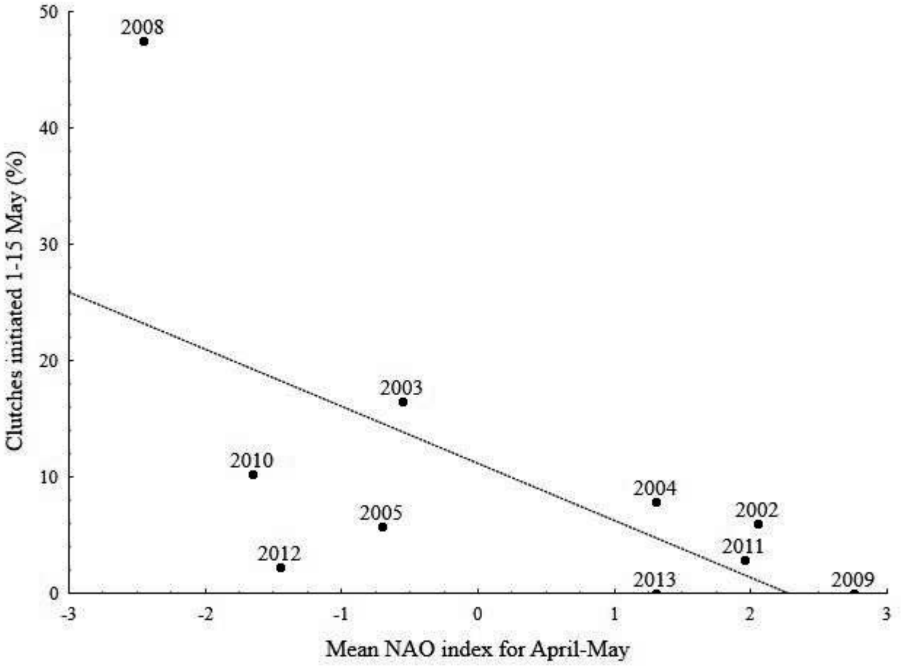

Figure 14 - Relationship between Little Tern (*Sternula albifrons*) timing of breeding (assessed as the % of clutches initiated between 1 and 15 of May) in salinas of Ria Formosa (Algarve) and the mean NAO index measured between Azores and Iceland, for April-May of 2002-2005 and 2008-2013 (r = -0.63, p = 0.05, n = 10).

Apart from the timing of breeding, the NAO index also influenced the clutch size. The NAO index for April and the mean for April/May, measured between Azores and Iceland was negatively correlated with the clutch size (r = -0.59, p = 0.04, n = 12; r = -0.53, p = 0.08, n = 12, respectively; fig. 15).

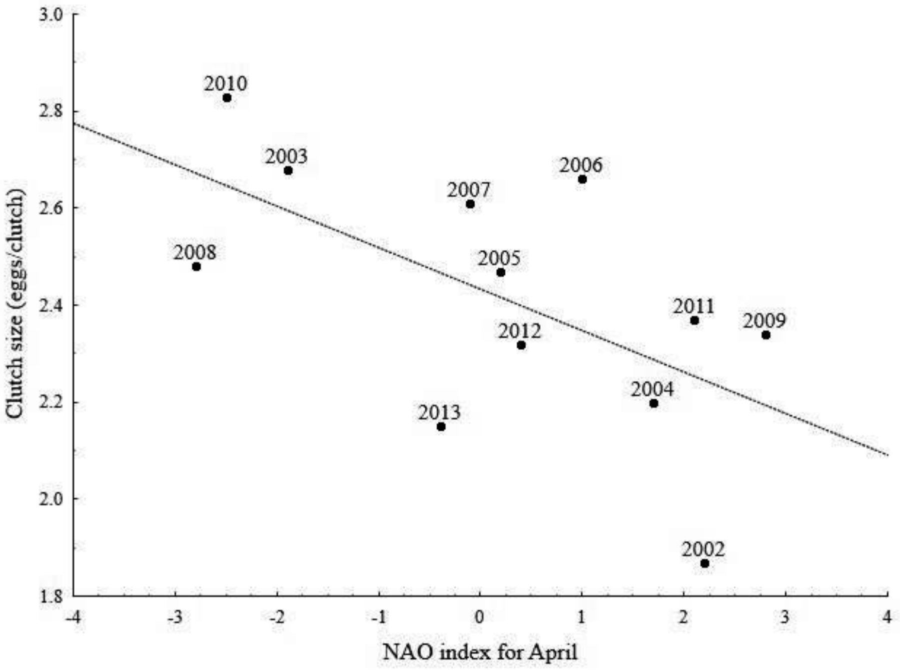

Figure 15 - Relationship between Little Tern (*Sternula albifrons*) clutch size (assessed as the number of eggs per clutch) in salinas of Ria Formosa (Algarve) and the NAO index measured between Azores and Iceland, for April of 2002-2013 (r = -0.60, p = 0.04, n = 12).

Little Tern's clutch size was positively correlated with the percentage of sand-smelts in their diet (r = 0.56, p = 0.05, n = 13, fig. 16). In years with more *Atherina* spp. in their diet, Little Terns lay more eggs per clutch. Although in a much less significant way, the percentage of *Atherina* spp. in the diet also influenced positively the egg volume (r = 0.40; p = 0.17; n = 13). The *Atherina* spp. estimated mean mass was positively related with the volume of the eggs although this relation was not significant (r = 0.58; p = 0.14; n = 8).

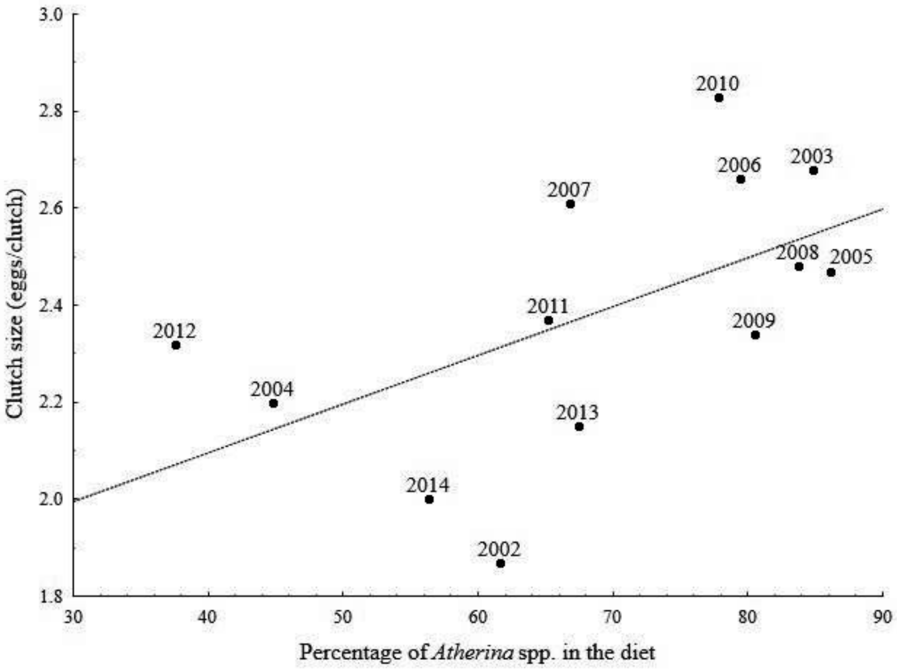

Figure 16 - Relationship between Little Tern (*Sternula albifrons*) clutch size (number of eggs per clutch) in salinas of Ria Formosa (Algarve) and the percentage of occurrence of sand-smelts (*Atherina* spp.) in the diet of adults in May-June of 2002-2014 (r = 0.56, p = 0.05, n = 13).

The number of breeding pairs in salinas was positively related with the percentage of *Atherina* spp. in Little Terns diet (r = 0.64, p = 0.03, n = 12, fig 17).

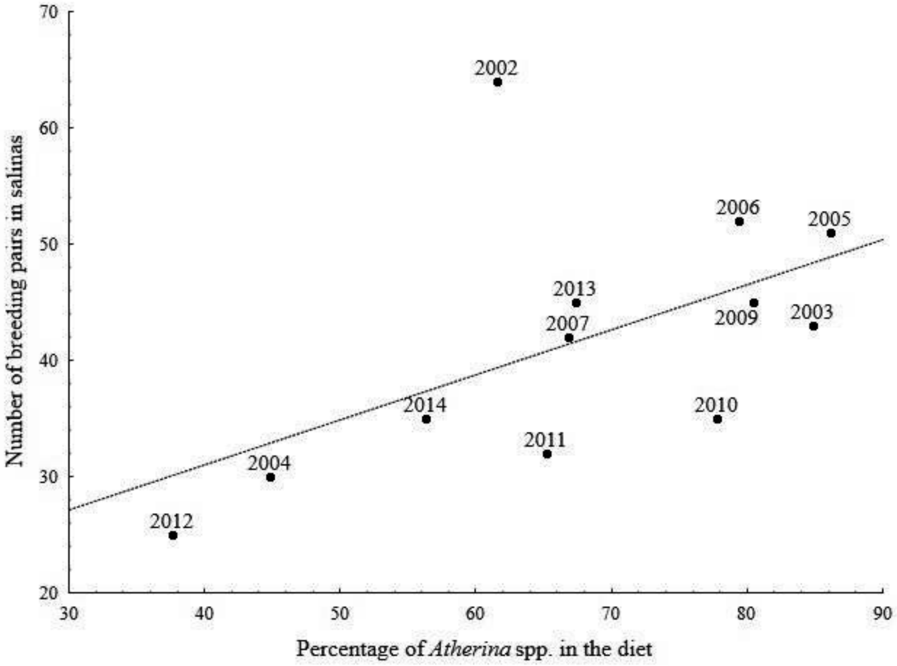

Figure 17 - Relationship between the number of Little Tern (*Sternula albifrons*) breeding pairs in salinas of Ria Formosa (Algarve) and the percentage of occurrence of *Atherina* spp. in the diet of adults in May-June since 2002 (r = 0.55, p = 0.06, n = 12).

Chapter 4 – Discussion

4.1- Vegetation cover and the number of breeding pairs

Little Tern populations are vulnerable to habitat change, disturbance and predation, and their conservation requires active habitat protection and site management (Fasola and Canova, 1996; Catry et al., 2004; Medeiros et al., 2007). Habitat loss is one of the major threats to Little Tern (Kotliar and Burger, 1986). The total number of Little Tern breeding pairs in Ria Formosa seems to be decreasing especially on sandy beaches and particularly since 2005. Strong declines in the number of breeding birds occurred from 2005 to 2006 and from 2012 to 2013 on sandy beaches, but this decline was not accompanied by reductions in the number of birds nesting in salinas, where the percentage of breeding birds increased considerably in the relation to the total in these years. On sandy beaches, breeding Little Terns are subject to the sedimentary and vegetation dynamics of the system. The Ria Formosa barrier island system is very dynamic, characterized by migration and/or opening of new inlets, closure of others and also by changes in shape and extent of the islands (Dias et al., 2004; Ceia et al., 2010). The variations in the number of breeding pairs and the relocation of the colonies could be an adaptation to the system dynamics.

The number of Little Tern breeding pairs nesting in salinas was always smaller than those nesting on sandy beaches. Despite having a lower number of breeding pairs, salinas had a significant percentage of individuals nesting in this habitat in relation to the total number of breeding pairs. In all years with available census data from 1979 to 2014, with the exception of 1983, the number of breeding pairs nesting in salinas was higher than 10% of the total and, in 2002 and 2013 that value reached 30% of all breeding Little Terns. Salinas, thus, constitute an important alternative breeding habitat. Breeding in salinas is reported for Little Tern in Spain and Italy (Fasola, 1986; Purroy, 1997), but not as important as it is in Portugal (Catry et al., 2004).

The comparison of vegetation cover between salinas and sandy beaches in 2013 shows that vegetation cover on sandy beaches is much smaller than that in salinas. It is also noticeable that sandy beaches with a smaller vegetation cover had a higher number of breeding pairs. In salinas, despite the vegetation cover was always higher than that on sandy beaches, the same pattern is observed, i.e. Little Terns do no nest in highly vegetated areas. Little Terns avoid nesting in highly vegetated areas to avoid predation on eggs and chicks, because a higher vegetation cover provides shelter for predators. Vegetation can also reduce the ability of terns to manoeuver and, among other reasons, effectively escape predators (Gochfeld, 1983; Kotliar and Burger, 1986; Fasola and Canova, 1991).

In salinas, however, Little Terns nest in areas with a higher percentage of vegetation cover and Mirra (2004) observed that in this habitat birds appeared to prefer corridors with a slightly higher percentage of vegetation than those with complete absence of vegetation. This can be an adaptation to nest in this habitat because vegetation can also be an important shelter for chicks against poor weather conditions (heat and rain) and avian predators (Davies, 1981), as chicks cannot move through the water of the tanks from one corridor to another. Although salinas are less subjected to human disturbance than sandy beaches, predation by mammals such as dogs (*Canis familiaris*) and foxes (*Vulpes vulpes*), and by birds such as turnstones (*Arenaria interpres*), birds of prey and crows (*Corvus corax*), is likely to be much higher, and nesting within some vegetation is likely to provide some protection against these predators. In both breeding habitats there is an active choice by birds for nesting in areas with a small percentage of vegetation cover over the complete absence of vegetation, such as that registered by Goutner (1990) in Evros Delta, Greece (average 15% of vegetation) and by Medeiros et al. (2012) in Ria Formosa (less than 30%). For the Little Tern sibling species, Least Tern (*Sterna antillarum*), Gochfeld (1983) registered a vegetative cover of ± 10%.

In fact, our study shows that for each studied sandy beach, there was a strong negative relationship between the percentage of vegetation cover and the number of breeding pairs, after controlling for the effect of climatic variation. This means that, on a local scale, vegetation cover is an important variable in explaining the variation in the size of the Little Tern breeding population. Therefore, a main problem for breeding Little Terns is the natural growth of vegetation that leads birds to abandon the colony site such as our colony site of Santa Luzia (see also Medeiros et al., 2007) and that registered by Kotliar and Burger (1984; 1986) for Least Terns. These habitats with low vegetative cover, preferred by Little Tern to nest, may be considered as ephemeral because of vegetation succession and, in fact, this species adopts a fugitive strategy, moving readily from one site to another (Gochfeld, 1983), because, as our regression model predicted, when the vegetation cover is too high (e.g. 90%), the percentage of the total of breeding pairs nesting on sandy beaches is close to 0%. For salinas, the same pattern was observed for the salinas of Santa Luzia: the site was abandoned when the vegetation cover was unsustainable for Little Terns to breed, but birds returned immediately following vegetation removal.

4.2- Annual variations in diet

Data from 2012, 2013 and 2014 added to previous existent data from 2002 to 2011 confirm that Little Tern diet is dominated by sand-smelts (*Atherina* spp.) followed by gobies (*Pomatoschistus* spp.), although in 2012 the frequency of occurrence of *Atherina* spp. was lower than in the other years. *Atherina* spp. is a pelagic fish and *Pomatoschistus* spp. is a bottom-dwelling fish, both characteristic from coastal lagoons and estuarine waters (Sobral and Gomes, 1997). These two fish species, beyond being the most important in Little Tern diet (Catry et al., 2006; Paiva et al., 2006a; Paiva et al., 2006b), are also the two most abundant fish genera in Ria Formosa (Ribeiro et al., 2008), highlighting the opportunistic feeding character of Little Terns (Catry et al., 2006). The predominance of two or three fish species in terns' diet was already been registered by other authors: Brenninkmeijer et al. (2002); Granadeiro et al. (2002); Bugoni and Vooren (2004). The low frequencies of occurrence or absence of *Fundulus* spp. in Little Terns diet in some years (e.g. 2005, 2008, 2010, 2013) is probably attributed to the fact that this species is euryhaline and was never collected in the lagoon habitat Ria Formosa lagoon (Ribeiro et al., 2008), occurring only in salinas and adjacent channels. Modifications in the salinity of salinas, typical in the processes of semi-industrial salt-extraction, could be a reason for the decline in the frequency of occurrence of this species in Little Tern diet (Paiva et al., 2006b). The consumption of bottom-dwelling fishes (*Pomatoschistus* spp.) can be explained by the fact that Little Terns usually forage in very shallow waters and at a higher rate during low tide (Paiva et al., 2006a), which are conditions that increase the availability of these fishes for foraging birds (Paiva et al., 2008). The consumption of crustaceans by Little Tern was variable along the years (Cramp, 1985; Catry et al., 2006). As this prey has a low energetic value, its consumption reflects a greater availability rather than a true preference (Catry et al., 2006). Insects have an inconsistent importance in terns' diet, as showed by Granadeiro et al. (2002) and Bugoni and Vooren (2004) for Common Terns (*Sterna hirundo*). Years with higher consumption of insects can be due to poor conditions for Little Tern to forage in the sea or in the lagoon or due to the lower abundance of fish species. In 2014 a new species (*Ammodytes tobianus*) was found in the diet of Little Tern adults from the identification of otoliths. However, this species was already registered as dropped prey items around Little Tern nests by Paiva et al. (2006b), meaning that it may occur occasionally in the diet of Little Terns. From 2010 to 2014 it seems that the frequency of occurrence of insects, crustaceans as well as the pelagic fish *Belone belone* has been increasing. Differences in Little Terns' diet over the years may have been driven by annual changes in consumption of *Atherina* spp. and *Pomatoschistus* spp. which, in turn, may be a result of annual differences in the availability of these prey species in Portuguese coastal estuaries and lagoons. Catry et al. (2006) suggested that, in fact, Little Tern diet can be a reliable indicator of the abundance of these fish species at Ria Formosa.

4.3- Resources availability, breeding parameters and number of breeding pairs

The addiction of the years 2012, 2013 and 2014 to the previously available data shows that Little Tern breeding parameters had important annual variations from 2002 to 2014, particularly in clutch size and the percentage of clutches initiated between 1 and 15 May. Comparing with other studies, Hong et al. (1998) registered clutch sizes of 2.48 eggs per clutch in 1995 and 2.35 in 1996, for Little Terns nesting on an islet in Republic of Korea. Oro et al. (2004a) registered clutch sizes of 2.35 eggs per clutch in 1996 and 2.36 in 1997 in the Ebro Delta, Spain. In relation to egg measurements, Hong et al. (1998) recorded a mean egg volume of 9.24 cm^3 in 1995 and 8.76 cm^3 in 1996, and Oro et al. (2004a) recorded egg volumes of 8.81 cm^3 in 1996 and 9.58 cm^3 in 1997. It is important to note that these studies were carried out in Little Terns natural habitats, whereas our study was made in salinas.

In relation to the timing of breeding, Little Terns show from 2002 to 2014 a substantial variation in the percentage of clutches initiated between 1 and 15 May. In all study years, with the exception of 2009 and 2013, birds always initiated laying in the first fortnight of May, and 2008 was an exception where 47.5% of the clutches were initiated before 15 May. The timing of breeding is related to seasonal nesting patterns; birds that failed early in the season and birds nesting for the first time (inexperienced or lower quality breeders) tend to nest later in the season (Hong et al., 1998; Medeiros et al., 2007) and, thus, they initiate their clutches after 15 of May. A similar pattern was reported for Least Terns, in California (Massey and Atwood, 1981), and for Roseate Terns (*Sterna dougallii*) in northeastern United States (Burger et al., 1996) where the first wave of nesting began in the first or second week in May and the second wave began in mid-June. The second wave of nesting consisted mostly of birds nesting for the first time and re-nesting by birds that failed the first breeding attempt. The timing of breeding is, thus, related with birds' productivity. These nesting patterns lead to seasonal declines in the number of nests initiated, clutch size, egg size, hatching success and fledgling success, because a nest initiated later in the breeding season is progressively less likely to succeed than a nest initiated earlier in the season (Burger et al., 1996; Medeiros et al., 2007). Productivity is, therefore, higher among the earliest nesting birds which lead to a strong selection for earlier breeding. However, selection for even earlier breeding does not occur presumably because of higher predation rates on early nests (Burger et al., 1996) and because of environmental conditions and consequent lower food resources early in the season (Perrins, 1966).

This study shows a relation between the North Atlantic Oscillation index and (1) Little Tern breeding population size and (2) two breeding parameters (clutch size and timing of breeding). The NAO index is known to influence ecological dynamics in the marine system by affecting individual, population and community levels (Hurrell and Deser, 2010) and the responses to this environmental variable are related to changes in population dynamics, abundance and spatial distribution (Ottersen et al., 2001). Years with positive NAO index are characterized by an intensified "Azores High" and deeper "Iceland low" pressure values, which is associated with warmer and wetter conditions over Northern Europe and dryer conditions over the Southern Europe. A positive NAO phase is also characterized by increased precipitation and sea-surface temperature (SST) in northern Europe and opposite conditions in Southern Europe (Stenseth et al., 2003; Pinto and Raible, 2012). With negative values of NAO index, the opposite pattern is expected: the "Azores High" is weaker and the "Iceland Low" is shallower, which results in warmer and wetter conditions with increased precipitation and SST over the Southern Europe. A negative NAO phase is also responsible to increase the wind speed and the vertical water mixing along the Iberian Peninsula (Stenseth et al., 2003; Pinto and Raible, 2012).

Reproduction is a process that requires a burst of energy, so, breeding at the right time is crucial for seabirds to ensure that the energy demands of reproduction coincide with peak food availability. The NAO index, for instance, can be seen as an environmental cue used by seabirds to predict the seasonal peak of food supply and adjust their timing of breeding accordingly (Frederiksen et al., 2004). It is also important for seabirds to start the breeding season in a good body condition which depends on food supply that, therefore, depends on climatic conditions (Reed et al., 2006).

In the present study there was a negative correlation between the NAO index and the clutch size, and the link between these two variables should be food availability (Bolton et al., 1992). Food availability is, in this case, expressed by the percentage of *Atherina* spp. in Little Tern diet because these birds consume the most available prey in Ria Formosa lagoon system, which is *Atherina* spp. (Catry et al., 2006). In fact, our data also show a positive correlation between the percentage of sand-smelts in birds' diet and clutch size. To produce eggs, birds need to obtain good food resources because the quality of the eggs formed may affect the survival of the chicks (Durant et al., 2004). Our data is consistent with this affirmation given the positive correlation between the percentage of *Atherina* spp. in Little Terns diet and their egg volume. There was also a highly significant correlation between the spring NAO index (April-May) and the timing of breeding. Most of the birds initiated their clutches later in the season (after 15 May) when the NAO index values were positive. Frederiksen et al. (2004) showed a significant correlation between first egg dates and NAO index values for black legged kittiwakes (*Rissa tridactyla*) and common guillemots (*Uria aalge*) in the Isle of May, North Atlantic. Møller et al. (2006) found a relationship between the NAO index for May and laying dates assessed by the mean ringing date of young for Artic Terns (*Sterna paradisaea*) nesting on Denmark. Also in the North Atlantic, the same pattern was observed by Wanless et al. (2009) for Atlantic Puffin (*Fratercula arctica*) and Razorbill (*Alca torda*). The NAO, a large-scale climatic phenomenon, can influence the arrival and settlement of terns on the breeding grounds (Wanless et al., 2009). Little Terns are migratory seabirds and conditions on the wintering grounds or during the spring migration may be important in determining the timing of their breeding.

Most of the studies that relate environmental variables, mainly the NAO index, with breeding parameters are for costal seabirds breeding in the North Sea and demonstrate that these seabirds show a delay in breeding following a low winter or early spring NAO index (Frederiksen et al., 2004; Reed et al., 2006; Wanless et al., 2009). Our study shows the opposite for Little Terns breeding in Ria Formosa (Algarve). Winter and early spring with negative values of the NAO index could be characterized by poor foraging conditions in the North Sea however the effects of the NAO are different in our study area that is further south than their study areas (Sandvik et al., 2008; Ramos et al., 2013). This study shows a negative relation between the number of breeding Little Terns nesting on sandy beaches and the NAO index for May which means that years with a negative NAO index had less breeding pairs nesting in this habitat. Drinkwater et al. (2003) used 44 years of data on seabirds breeding in the Wadden Sea, Northeastern Atlantic, and found significant correlations between the NAO and the breeding numbers of some species of seabirds, including fulmars, herring gulls, lesser black-backed gulls, common gulls, black headed gulls, kittiwakes, sandwich terns, guillemots and razorbills. However, this is a relationship found in the North Atlantic where the effects of the NAO are different from those in our study area. The links between this environmental variable and number of nesting pairs should be the food supply and the fitness components. By having a relation with the availability of food (Ramos et al., 2013) and with fitness components such as timing of breeding and clutch sizes, in the end, the NAO index has a relation with the number of breeding pairs breeding in Ria Formosa.

In this study, two versions of the North Atlantic Oscillation were used and that measured between the Azores and Iceland showed more significant relationships and with more variables than the NAO version measured between Gibraltar and Iceland. This can be due to the fact that the NAO measured between Azores and Iceland covers a more stormy and turbulent area (Jianping and Wang, 2003).

4.4- Conservation implications and measures

This study shows that general environmental conditions, as captured by the NAO index, influence the number of Little Tern breeding pairs. In general this study shows a relation between negative NAO conditions and (1) earlier breeding, (2) larger clutch size, and, consequently (3) higher number of breeding pairs. Diet also has an important relation with breeding parameters (clutch and egg sizes) and, as a consequence, with the number of breeding pairs. Environmental variables affect breeding parameters not only directly but also through changes in diet (Drinkwater et al., 2003). Due to the fact that climatic variation (the NAO index in this case) did not show a significant relationship with the number of breeding pairs nesting in each sandy beach colony, we suggest that on a local scale, the percentage of vegetation cover is more important in controlling the variation in the Little Tern breeding population size. Following the same rationale, in a more regional scale the food resources, determined by the climatic conditions and the NAO index, should play an important role in the number of Little Tern breeding pairs.

A negative relationship between the percentage of vegetation cover and the number of breeding pairs on each colony throughout the years, suggests that an important protective measure to preserve Little Tern breeding habitat on a local scale should be the removal of part of the vegetation cover. According to the fitted regression model, in order to have, for instance, 30% of the breeding pairs nesting on sandy beaches for a given year, the percentage of vegetation cover should not be higher than ~30%. This procedure was further supported by the results of the habitat management experiment preformed in Santa Luzia. There, Little Terns reacted immediately to the vegetation removal action performed just some months prior to incubation, by returning to that area and breeding there on that same year. However, it is also important to control other variables that reduce birds' breeding success as the possible flooding, human disturbance and predation.

On sandy beaches, breeding Little Terns are subject to high levels of anthropogenic pressure. Human activities related to tourism on sandy beaches, such as the use of the beaches for general recreation and by off-road vehicles, lead to the destruction, degradation and disturbance of many breeding sites (Gochfeld, 1983; Catry et al., 2004). These anthropogenic changes lead to variations in habitat use patterns and may force birds to nest in alternative habitats, which requires plasticity and poses ecological and evolutionary challenges to the individual Little Terns (Erwin et al., 1981; Medeiros et al., 2012). Protection of Little Terns on sandy beaches by access restriction, signposts, wardening to prevent human disturbance around the colonies, as well as predation control can be effective actions for increasing nesting success and conservation of breeding Little Terns and other species such as plovers (Burger, 1984; Burgess and Hirons, 1992; Fasola and Canova, 1996; Medeiros et al., 2007; Medeiros et al., 2012).

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