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**BREEDING SUCCESS AND FEEDING ECOLOGY OF LITTLE TERN
(Sternula albifrons) IN RIA FORMOSA, ALGARVE**

Dissertação de Mestrado em Biologia,
orientada pelo Professor Doutor Jaime Albino Ramos e pelo Doutor Vítor Hugo Paiva,
apresentada ao Departamento Ciências da Vida da Universidade de Coimbra.

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Breeding Success and Feeding Ecology of Little Tern (*Sternula albifrons*) in Ria Formosa, Algarve.

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia, realizada sob a orientação científica do Professor Doutor Jaime Albino Ramos (Universidade de Coimbra) e do Doutor Vítor Hugo Paiva (Universidade de Coimbra).

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Abstract

Seabirds are top predators, and can be used as sentinels of changes in marine environments. Estuarine small seabirds such as Little Terns (*Sternula albifrons*) are particularly sensitive to alterations in prey availability because they need to feed regularly and closer to their breeding sites. Changes in prey availability influence the seabirds' breeding parameters and success, and other factors such as breeding habitat type, human pressure and presence of predators have a strong influence on breeding success. In this study we analysed the influence of the selection of natural (sandy beaches) and man-made (salt-pans) habitats on reproductive parameters and breeding success of Little Terns breeding in Ria Formosa, Algarve, Portugal. We identified the diet of Little Tern during 2013, 2014 and 2015 breeding seasons, comparing the identification of otoliths and the identification of scales, both found in pellets dropped in the colonies. Also the predators of Little Tern clutches were identified based on footprints and photographs. Finally, we tested the possible relations between a large scale climatic index (the North Atlantic Oscillation index, NAO) and Little Tern diet, breeding parameters (e.g. clutch size, egg size) and breeding success.

Overall, breeding parameters were not influenced by habitat type or colony location. Breeding success was influenced by colony location but not by habitat type, suggesting that the characteristics of the breeding site are more important than habitat type explaining breeding success. The diet of Little Tern was dominated by sand-smelts (*Atherina* spp.) during all breeding seasons according with both identification methods, reflecting the opportunistic feeding behaviour of Little Tern, because this is the most common fish species in Ria Formosa. There were significant differences in secondary prey items between the two identification methods. In general more prey items were identified through otoliths than scales (n = 12, n = 4 respectively), suggesting that otoliths were more resistant to degradation probably due to its high density. However, sardine (*Sardina pilchardus*) scales were also well preserved and thus easily identified when compared to otoliths, suggesting that this prey is underestimated when only otoliths are identified. On the other hand, the second most important prey in Little Terns' diet, Gobies (*Pomatoschistus* spp.), were only identified based in otoliths due to its high density.

Predators of Little Terns' clutches were identified with this work, such as Stone-curlews (*Burhinus oedicephalus*), Cats (*Felis catus*) and Dogs (*Canis familiaris*), suggesting that the breeding success may be affected by the distance of the colonies to urban areas. Our study also suggests that climatic variability (depicted by the NAO index) have an influence on Little Terns' breeding parameters and diet. Years of higher NAO index values were related with a small size of sand-smelts (the main prey of Little Terns), leading to a lower mean egg volume. A higher percentage of sand-smelts in the diet of Little Terns was related with a larger clutch size, and a higher mass of sand-smelts in the diet was related with an increase in egg length, which should be related with a higher amount of young females in active breeding. Overall, our study shows that the environmental variability strongly influenced the Little Terns' breeding and feeding parameters, which can be used as indicators of changes in coastal and estuarine marine environments.

Key words: Breeding parameters, otoliths and scales, predators, North Atlantic Oscillation index (NAO), Little Tern (*Sternula albifrons*).

Resumo

As aves marinhas são predadores de topo que podem ser utilizadas como sentinelas de alterações no ambiente marinho. As aves marinhas estuarinas de pequenas dimensões, como é o caso da Chilreta (*Sternula albifrons*), são particularmente sensíveis a alterações na disponibilidade de presas uma vez que necessitam de procurar alimento regularmente e na proximidade das áreas de nidificação. Alterações na disponibilidade de presas influenciam os parâmetros reprodutores e o sucesso reprodutor das aves marinhas. Também outros fatores como o tipo de habitat disponível para reprodução, a pressão humana e a presença de predadores têm uma elevada influência no sucesso reprodutor. Neste estudo, analisámos a influência da seleção de habitat, natural (praias) e artificial (salinas), nos parâmetros reprodutores e sucesso reprodutor da população reprodutora de Chilreta na Ria Formosa, Algarve, Portugal. Identificámos a dieta da Chilreta durante as épocas reprodutoras de 2013, 2014 e 2015, comparando a identificação de otólitos com a identificação de escamas, ambos presentes nas egagrópilas encontradas nas colónias. Foram também identificados os predadores das colónias de Chilreta com base em pegadas e em fotografias. Por fim, testámos as possíveis relações entre um índice climático de larga escala (índice de Oscilação do Atlântico Norte, NAO), a dieta da Chilreta, os parâmetros reprodutores (e.g. tamanho da postura, tamanho dos ovos) e o sucesso reprodutor.

Não se verificou influência do tipo de habitat e da localização da colónia nos parâmetros reprodutores, contudo o sucesso reprodutor foi influenciado pela localização da colónia apesar de também não ter sofrido influência do tipo de habitat, sugerindo que as características individuais de cada colónia são mais importantes para o sucesso reprodutor do que o tipo de habitat. A dieta da Chilreta foi dominada por peixe-rei (*Atherina* spp.) durante as três épocas reprodutoras de acordo com os dois métodos de identificação, refletindo o comportamento oportunista da Chilreta, uma vez que este é o peixe mais abundante na Ria Formosa. Verificaram-se diferenças ao nível das presas secundárias entre os dois métodos de identificação. No geral, foram identificadas mais presas através dos otólitos do que das escamas ($n = 12$, $n = 4$ respetivamente), sugerindo que os otólitos são mais resistentes à degradação provavelmente devido à sua

elevada densidade. Contudo, as escamas de sardinha (*Sardina pilchardus*) apresentaram um melhor estado de conservação e, por isso, foram mais facilmente identificadas quando comparadas com os seus otólitos, sugerindo que esta presa é subestimada quando apenas os otólitos são identificados. Por outro lado, a segunda presa mais importante na dieta da Chilreta, os cabozes (*Pomatoschistus* spp.), foi detetada apenas com base nos seus otólitos, devido à sua elevada densidade.

Neste trabalho foram identificados predadores das posturas de Chilreta, tais como, o Alcaravão (*Burhinus oedicnemus*), Gatos (*Felis catus*) e Cães (*Canis familiaris*), indicando que o sucesso reprodutor pode ser afetado pela proximidade das colónias a áreas urbanas. Este estudo sugere também que a variabilidade climática (descrita pelo índice NAO) influencia os parâmetros reprodutores e a dieta da Chilreta. Anos com valores do índice de NAO mais elevados estão relacionados com um menor crescimento do peixe-rei (presa principal da Chilreta), levando a uma diminuição no volume médio dos ovos. Uma maior percentagem de peixe-rei na dieta leva a um aumento do tamanho das posturas, e uma maior biomassa de peixe-rei na dieta está relacionada com um maior comprimento dos ovos, o que pode estar relacionado com um maior número de fêmeas jovens a reproduzirem-se. O nosso estudo revela que a variabilidade ambiental influencia fortemente os parâmetros reprodutores e alimentares da Chilreta, o que pode ser usado como indicador de alterações nos ambientes marinhos costeiros e estuarinos.

Palavras-chave: Parâmetros reprodutores, otólitos e escamas, índice de Oscilação do Atlântico Norte (NAO), Chilreta (*Sternula albifrons*).

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1. INTRODUCTION



1.1. Estuarine Seabirds as Bioindicators

Seabirds live in marine habitats such as coastal areas, islands, estuaries and wetlands (Shreiber and Burger 2002). Seabird populations are affected by factors that influence the availability of food resources, quality of breeding habitat, parasites, diseases and predation (Weimerskirch 2002). Most seabird species are top predators, and may be used as sentinels of variations in marine environments. They can be indicators of environmental changes, giving information about temporal changes in prey location and availability, in relation to variation in oceanographic conditions that causes changes in marine food chains (Sydeman *et al.* 2001). To guarantee breeding success, the breeding season is timed with the period of higher food availability (Frederiksen *et al.* 2004). Seabirds are particularly important to identify changes in prey populations and environmental conditions (Ramos *et al.* 2013), since some aspects of their biology reflect changes in food availability (Cairns 1988). This has been shown for both procellariiform species that forage in large oceanic areas, and species such as terns that forage in coastal areas. Previous work showed that seabirds can give early indications of fluctuations in fish stocks and oceanographic conditions (Frederiksen *et al.* 2004). Some authors also studied how seabirds' diets can predict recruitment of fish species (Cairns 1992; Velarde *et al.* 2004; Velarde *et al.* 2013), because seabirds feed on some commercially important fish (Einoder 2009).

Oceanographic conditions are related with atmospheric phenomena, reflected in large scale climatic indices, of which the North Atlantic Oscillation Index (NAO) is one of the best known (Hurrell *et al.* 2003). NAO refers to the change in atmospheric pressure at the sea level between Iceland and Azores, and it influences changes in temperature, salinity and vertical water mixing, which affect the marine ecosystems in the North Atlantic Ocean (Hurrell and Deser 2010). NAO is useful to evaluate the effects of weather and oceanographic phenomena as wind speed, upwelling, ocean currents strength and sea surface temperature (SST) (Hurrell *et al.* 2003). During the positive phase of NAO, westerly winds become stronger and move northwards, leading to an increase of precipitation and sea surface temperature in the northern Europe, and the opposite occurs for southern Europe. During the negative phase of NAO the storm moves to the south of Europe leading to an increase of sea surface temperature, wind speed, and

vertical water mixing in the Iberian Peninsula (Pinto and Raible 2012). Sea surface temperatures above the mean are related with changes in distribution of marine species from low trophic levels resulting in changes in prey productivity and availability (Peck *et al.* 2004). Previous work showed that NAO index can affect the occurrence of fish and characteristics as size and age of maturity of fish populations (Borges *et al.* 2003; Jonsson and Jonsson 2004). Oceanographic conditions related with NAO, such as sea surface temperature, influence the spawning of marine fishes (Coombs *et al.* 2006). This change in oceanographic conditions can deeply affect the populations of marine high trophic level consumers (Sandvik *et al.* 2008) causing changes in breeding success motivated by changes in the availability of prey (Stenseth *et al.* 2003). However, some limitations can be found when breeding success is used to study climate and oceanographic conditions, because other factors such as predation and human pressure can also influence breeding success (Leseberg *et al.* 2000; Beale and Monaghan 2004; Catry *et al.* 2004; Ramos *et al.* 2013). Nevertheless, other parameters related with reproduction, such as time of breeding and clutch size can be used instead, since they are less influenced by predation, but still highly influenced by climatic and oceanographic conditions. Previous studies showed that NAO influences seabird populations in northern Europe (Frederiksen *et al.* 2004; Votier *et al.* 2005; Wanless *et al.* 2005); and it was also shown to influence Little Tern, *Sternula albifrons* breeding and foraging parameters in Ria Formosa, Algarve, Portugal (Ramos *et al.* 2013). Little Tern is an estuarine seabird that breeds in areas with high food availability, such as coastal lagoons and low water depth areas (Paiva *et al.* 2008), usually in sandy beaches and sand bars that are relative free from predators (Medeiros *et al.* 2012). They are highly sensitive to changes in prey distribution and availability because to breed successfully they need to obtain food closer to their colonies (mostly up to 5 Km offshore, Ramos *et al.* 2013). Therefore, oceanographic changes can influence prey availability during the breeding season leading to variation in timing of breeding and in the number of breeding pairs. Marine climatic conditions, represented by NAO and SST, influence Little Terns' diet in Ria Formosa: when NAO is negative the reproduction occurs earlier, and during years with lower SST the availability of prey for Little Terns during the breeding season increases (Ramos *et al.* 2013).

1.2. Breeding Ecology of Estuarine Seabirds

Many factors contribute to seabird reproductive success, such as food availability, predation, competition, quality of breeding habitat and disease (Becker *et al.* 2007). Prey availability is thus important to understand temporal and spatial variations in seabird reproductive parameters (Hamer *et al.* 2002). Seabirds are very sensitive to food availability, and may refrain from breeding or breed later during years of low food availability. This is particularly evident for small seabirds such as terns, as they need to feed frequently within close distance of their breeding sites (Ramos *et al.* 2002).

A high quality of habitat to reproduce is also an important factor for seabirds in general and for birds that breed in coastal areas in particular. Coastal terns breed in coastal lagoon areas, sandy beaches and estuaries, but such natural habitats have been destroyed or deeply modified by man. Tourism is one of the most important factors that contribute to habitat modification, because it is related with urbanization and human recreation in coastal areas, which will reduce breeding success and contribute to the decline of species that breed in coastal areas (Yasué and Dearden 2006). Also, changes in vegetation cover affect breeding success because chick usually hide on vegetation, and will be easily exposed to predators when vegetation is removed (Yasué and Dearden 2006). Human activities near the colonies lead to an increase of predation due to the introduction of new predators as rats and dogs (Catry *et al.* 2004). Urbanization and recreation also decrease beach length, thereby increasing nest flooding risk during high tide (Yasué and Dearden 2006; Medeiros *et al.* 2012).

Anthropogenic pressure in coastal areas has contributed to strong declines in the distribution and size of Little Tern and Kentish Plover (*Charadrius alexandrinus*) populations (Norte and Ramos 2004; Catry *et al.* 2004). As a consequence of alteration and destruction of natural habitats, these two species move to alternative breeding habitats including man-made habitats, as salt-pans and fish-farms. Such change in habitats will expose birds to new factors, affecting their reproduction, survival and conservation (Catry *et al.* 2004). In southern Europe, the most important alternative habitat for estuarine birds is salt-pans (Fonseca *et al.* 2004). These are used by Little Tern as alternative breeding habitat, however moving to salt-pans does not represent always a solution because they can be reconverted for aquaculture, thereby increasing

the human presence and clutches' destruction in the colonies (Catry *et al.* 2004). Also the fast growth of vegetation in salt-pans can cause their abandonment by Little Terns, since they prefer to breed in habitats with vegetation cover under 50% (Lopes *et al.* 2015). Natural habitats such as coastal beaches are relatively open and available sparse vegetation is used by chicks to hide. Excessive rain in salt-pans may also lead to nest flooding causing the destruction of entire colonies (Catry *et al.* 2004).

1.3. Feeding Ecology of Estuarine Seabirds

Seabirds are higher trophic level consumers in marine ecosystems (Catry *et al.* 2006), they are relatively generalist predators (Crains 1988) and are able to explore marine food resources in several ways (Shealer 2002). They feed mainly on small fishes, crustaceans (Crains 1988), cephalopods and molluscs (Shealer 2002). Most marine birds forage during daylight due to visibility and prey availability (Shealer 2002). Changes in food availability affect seabird reproductive parameters (Monaghan *et al.* 1989). This is particularly noticeable for estuarine little seabirds such as terns, because they have less energy stored and spend most part of the time foraging (Pearson 1968). Estuarine seabirds forage opportunistically in estuaries, coastal lagoons and adjacent sea (Catry *et al.* 2006) in transparent low depth waters (Paiva *et al.* 2008).

Estuarine seabirds breed in coastal areas and forage near shore (Hamer *et al.* 2002). During the breeding season estuarine seabirds such as Little Terns forage in a small area around the colonies because they need to return to the nests regularly to incubate the eggs or feed the chicks (Shealer 2002) so they need to obtain all necessary food around the colony to ensure successful breeding (Ramos *et al.* 2013). Virtually all prey fed to chicks is obtained by the male; the female only leave the nest during short periods to feed for herself. In some cases, when there is a large amount of available food, the female helps the male feeding the chicks (Davies 1981). Environmental factors such as strong winds, rain, and low visibility in water column can difficult the capture of enough prey and limit chick growth (Paiva *et al.* 2006a).

Overall, terns (*Sterna* spp. and *Sternula* spp.) feed opportunistically in small pelagic fish, crustacean and insects, and change the diet according with the availability of the different food types (Nisbet *et al.* 2002; Catry *et al.* 2006; Alfaro *et al.* 2011). The

energetic value of prey is an important factor for adults and chicks, and when high energetic prey is scarce chick growth may be limited (Paiva *et al.* 2006b). Crustaceans have low energetic value and its consumption in certain areas is related with their high availability (Catry *et al.* 2006). In addition to prey availability and energetic content, prey type and size are important factors for chick growth, not only for Little Tern (Norman 1992) but also for other terns as Common Tern (*Sterna hirundo*) (Bugoni and Vooren 2004), Roseate Tern (*Sterna dougallii*) and Sandwich Tern (*Thalasseus sandvicensis*) (Shealer 1998). Chick feeding frequency is related with prey energetic value: parents feed chicks more often when prey has a low energetic content. The size of prey delivered to chicks increases with chick age (Paiva *et al.* 2006a).

1.4. Methods to Assess Tern Diet

Diet studies are important to understand the feeding ecology of seabirds and their relationships with marine ecosystems (Duffy and Jackson 1986). All methods used to assess the diet of seabirds have some limitations because they do not allow a full identification of the diet (Duffy and Jackson 1986; Barrett *et al.* 2007). Studies to determine the diet of terns are based on different methods, such as observations of prey delivered to chicks or mates (Bogliani *et al.* 1992; Bogliani *et al.* 1994; Ramos *et al.* 1998a; Taylor and Roe 2004; Paiva *et al.* 2006a; Paiva *et al.* 2006b; Paiva *et al.* 2008), identification of prey items dropped in colonies (Atwood and Kelly 1984; Bogliani *et al.* 1994; Ramos *et al.* 1998a; Ramos 2000; Paiva *et al.* 2006a; Paiva *et al.* 2006b; Paiva *et al.* 2008), or identification of hard parts of the prey present in pellets or faeces collected in colonies (McGinnis and Emslie 2001; Catry *et al.* 2006; Alfaro *et al.* 2011; Ramos *et al.* 2013). All these methods have some limitations, because they do not allow a full identification of the diet, leading to the underestimation or overestimation of certain prey in the diet, in detriment to other prey. The type of prey delivered to tern chicks and mates may show daily and seasonal variations in relation to tide, chick age or changes in prey availability (Safina *et al.* 1990; Ramos *et al.* 1998a; Paiva *et al.* 2006a). Therefore, to determine the diet based on prey deliveries, observations should be spread out throughout the day and season (Ramos *et al.* 1998a), which is time consuming. It is also necessary to be in an adequate location within the colony, so as not to miss prey

deliveries. Observations can lead to the overestimation of prey with peculiar shapes and colours (González-Solís *et al.* 1997) that are easier to identify. Also the identification of the diet based on prey dropped by chicks near nests (Bogliani *et al.* 1994; Ramos *et al.* 1998a) has some limitations, most notably the overestimation of prey that are difficult for the chicks to handle, and therefore rejected (Ramos *et al.* 1998a). Additionally, parents might be feeding their chicks with specific diet items, in response to their development requirements, but feeding themselves on a distinct diet composition (Catry *et al.* 2006).

The determination of diet composition, based on hard parts (mostly otoliths, structures made of calcium carbonate and other inorganic salts in the inner ear of fishes ,Tuset *et al.* 2008) found in pellets, presents several advantages, in particular the fact that a large sample size may be collected in a non-invasive way and over a relatively long time period. In addition, undigested parts reflect what the birds have ingested, thus avoiding the problem of rejected prey, and are an appropriate method to evaluate the diet of adult birds (Barrett *et al.* 2007). However, the identification of prey present in pellets based only on otoliths is also prone to biased results associated with differences in hardness, size, shape and digestibility of the hard parts of different prey (Duffy and Jackson 1986). Moreover, very small otoliths may not appear in the pellets, but in excrements (Barrett *et al.* 2007). Therefore, all hard parts found in pellets, such as fish vertebrae, should be identified, which is commonly used for large seabirds such as shearwaters (Alonso *et al.* 2013), gulls (Kubetzki and Garthe 2003; Pedro *et al.* 2014) or large terns (Ramos *et al.* 1998b). However, small seabirds such as Little Terns *Sternula albifrons* feed on very small fish items (< 10 cm; Paiva *et al.* 2006a; Paiva *et al.* 2006b), from which identifiable vertebrae are not present in the pellets. Prey remains found in faeces are more eroded than those found in pellets, with only small otoliths passing through the intestine (Barret *et al.* 2007). The Little Tern is the smallest tern species and feeds mostly on small fish (Catry *et al.* 2006), therefore, dietary analysis using faeces may not be the most suitable alternative. Fish scales are very abundant in pellets of small seabird species such as Little Terns, but, surprisingly they have rarely been used in the determination of tern diet. To our best knowledge only Naves and Vooren (2006) used scales in the evaluation of the diet of Black Skimmers (*Rynchops niger*). Scales have,

however, been used to assess the diet of other larger seabird species (e.g. genus *Phalacrocorax* spp., Johnson *et al.* 2001; Alarcón *et al.* 2012).

Previous studies reconstructed the diet of Little Terns based on otolith analysis and prey dropped near nests (Bogliani *et al.* 1994; Catry *et al.* 2006; Paiva *et al.* 2006a; Paiva *et al.* 2006b; Ramos *et al.* 2013). Despite the fact that a great array of fish species can be readily identified through their otoliths, some species are difficult to detect through this method. This is the case in Clupeiform fish, from which otoliths are frequently broken or highly degraded in pellets (Bugoni and Vooren 2004; Naves and Vooren 2006). However, in a previous study, scales were effective at identifying Clupeiform fishes (Naves and Vooren 2006). In the Algarve, Portugal, where a long-term study on the diet of Little Terns is ongoing (e.g. Ramos *et al.* 2013), the diet of adult birds has been reconstructed based on the identification of otoliths in pellets (Catry *et al.* 2006). Given the fact that diet of Little Terns may be a useful indicator of changes in estuarine and coastal ecosystems (Ramos *et al.* 2013), it is important to assess whether the use of scales will add relevant information on the diet composition of the species.

1.5. Breeding and Feeding Ecology of Little Tern in Ria Formosa

Little Terns arrive from their wintering quarters in April, incubation period occurs in May-June and chick feeding occurs in May-July. In Ria Formosa, the main factors that influence Little Terns breeding success are human pressure and predation (Medeiros *et al.* 2007). Previous work identified Dogs (*Canis familiaris*), Stone-curlews (*Burhinus oedichnemus*), Gulls (*Larus spp.*) and Rats (*Rattus norvegicus*) as Little Tern predators (Catry *et al.* 2004) in Ria Formosa. Urbanization in barrier islands increased the human presence near the Little Tern colonies, leading to total or partial abandonment of some nesting sites (Catry *et al.* 2004). Ria Formosa is the only location in Portugal where Little Terns breed in both natural (sandy beaches) and alternative (salt-pans) habitats. Salt-pans present the necessary conditions for reproduction and foraging by Little Terns (Catry *et al.* 2004), as they are adjacent to the Lagoon system, and other foraging habitats such as the adjacent sea, salt-pans and artificial channels (Catry *et al.* 2004; Paiva *et al.* 2006a).

Despite the small differences in breeding success between salt-pans and sandy beaches, the Little Tern breeding population is higher on sandy beaches than on salt-pans (Lopes *et al.* 2015). Birds may feel attracted to familiar locations due perhaps to an imprinting process (cognitive memory process; Brooke and Birkhead 1991; Catry *et al.* 2004). Paiva *et al.* (2006a) showed that chicks raised in Ria Formosa sandy beaches have higher growth rates than chicks raised in salt-pans, which may be related with a differential diet composition among the two habitats (Paiva *et al.* 2006a). Despite the movement from natural habitats to alternative habitats, the Portuguese Little Tern breeding population did not decrease in the last 30 years; this shows a quick adaptation to new habitat conditions (Catry *et al.* 2004). More studies are necessary to evaluate the breeding success in sandy beaches and salt-pans to understand the importance of each habitat type for Little Terns' reproduction and conservation. It is necessary to understand how Little Terns' breeding parameters, availability of food and exposition to predators are affected by habitat change. Colony and egg size are indicators of environmental conditions during the laying period, and the size of both increase with food availability (Oro *et al.* 2004). Thus, sandy beaches present better conditions for Little Terns to reproduce, because birds breed early and lay larger eggs on sandy beaches than on salt-pans (Catry *et al.* 2004). This preference to reproduce on sandy beaches can be related with availability of marine prey in the lagoon and adjacent sea, given that marine prey ensure a higher growth rate (Paiva *et al.* 2006b).

The sea adjacent to the lagoon system is hardly used for prey capture, however, the larger foraging flocks were observed at sea, which can be explained by the occurrence of large fish schools in those areas (Paiva *et al.* 2008). The reduced but constant use of the sea for prey capture during different tide cycles suggests that availability of food in the sea is independent from tide. The opposite occurs in the lagoon, where tide influences the use of channels and the main lagoon, affecting the number of birds foraging due changes in prey availability. Thus, tide has probably an important role in explaining the choice of foraging areas by Little Terns (Paiva *et al.* 2008), however it has no influence in prey delivered to chicks (Paiva *et al.* 2006a). Salt-pans are less important foraging habitats, but present a more constant availability of food resources and prey capture is not affected by environmental factors (Paiva *et al.*

2008). Salt-pans are mostly used during periods with strong winds or low visibility, as these reduce prey capture success in the lagoon.

Previous studies identified Little Terns' diet based on otolith identification found on pellets and also based on prey dropped near nests (Catry *et al.* 2006; Ramos *et al.* 2013). Those studies showed that the most important prey in the diet of Little Terns were: *Atherina* spp., *Sardina pilchardus*, *Pomatoschistus* spp., *Fundulus* spp., *Belone belone* and crustaceans (Paiva *et al.* 2006a; Paiva *et al.* 2006b; Catry *et al.* 2006; Ramos *et al.* 2013). There were differences between adult diet and chick diet (Paiva *et al.* 2006a), as chick diet was more diversified than that of adults (Catry *et al.* 2006). *Atherina* spp. is the most abundant prey in Ria Formosa lagoon system; when the tide changes between high and low, they move in the water column and are more available to Little Terns. Crustaceans are present at higher numbers in the salt-pans (Paiva *et al.* 2006a) but possess a low energetic value. Plus, chicks may have difficulties to swallow them; however they are important in periods when more energetic prey is unavailable (Paiva *et al.* 2006a). *Sardina pilchardus* is important for chick growth, and energetic models show that when *S. pilchardus* is removed from the diet chick growth is affected (Paiva *et al.* 2006b). Little Terns feed also on *Fundulus* spp., an exotic euryhaline fish species that occurs only in salt-pans and adjacent channels (Meyer *et al.* 2005).

We studied in detail the breeding and foraging ecology of Little Terns in Ria Formosa, Algarve, in 2015, and compared our data with published information for previous years, in order to provide a more complete picture of the breeding and diet characteristics of Little Terns. The following aspects were studied: a) breeding success in both natural and alternative habitats, using the traditional method of following the outcome of nests during the breeding season and also calculating the probability of nest success based on the Mayfield Method (Mayfield 1961), b) reproductive parameters (egg volume, clutch size and breeding success) were described for sandy beaches and salt-pans, c) nest predators were identified from the observation of predation marks and predator footprints near nests, and also using cameras, d) diet was assessed, based not only on otolith identification, as done in previous studies, but also on fish scales identification, to compare the two identification methods and obtain a more reliable

picture of the diet, and e) the relationship between NAO index and annual variations in the Little Tern breeding parameters and diet.

2. METHODS



2.1. Study Species

Little Tern *Sternula albifrons* (Fig. 1) is a migratory colonial seabird of the Laridae family (Cramp 1985). It is the smallest of the terns, it has long narrow wings, long forked tail and yellow pointed beak (Svensson 2014). Little Terns breed in all continents except in the Antarctic (Cramp 1985). They breed in small groups and some pairs nest solitary (Cramp 1985). Breeding season occurs during summer (Svensson 2014), in coastal or estuarine open areas, mostly in sandy beaches. They can also breed in artificial habitats as salt-pans and fish-farms. They feed near the colonies (within 5 km), mostly in entrance channels, adjacent sea, salt-pans, aquacultures and in low depth coastal lagoons (Catry *et al.* 2006; Paiva *et al.* 2008). They capture prey in shallow water, only a few centimeters deep. Both adults and chicks feed in small fish and invertebrates, mainly insects and crustaceans (Davies 1981). Little Terns' main prey in Portugal are sand-smelts (*Atherina* spp.) and gobies (*Pomatoschistus* spp., Catry *et al.* 2006).



Figure 1. Little Tern incubating at Deserta island, Ria Formosa, Algarve, Portugal.

Little Tern main colonies in Europe are located in Italy, Spain, United Kingdom, and Portugal (Cabral *et al.* 2005). Most of Little Tern European populations winter in western Africa (Meirinho *et al.* 2014). The most important colonies in Portugal are located in Aveiro, Tejo estuary, Sado estuary, Santo André Lagoon, Alvor estuary, Ria Formosa and Castro Marim (Catry *et al.* 2004). Ria Formosa is the only place in Portugal where Little Tern breeds in natural (sandy beaches) and alternative (salt-pans) habitats (Catry *et al.* 2004), and is the most important area for breeding Little Terns in Portugal

since the XX century (Catry *et al.* 2004). Little Tern is considered a *Vulnerable Species* in Portuguese Red Data Book (Cabral *et al.* 2005).

2.2. Study Area

Ria Formosa Natural Park (Fig. 2) is located in southern Portugal, Algarve, and covers approximately 18400 ha. The park is composed by peninsulas, islands, wetlands, salt-pans, intertidal areas, channels and islets (Marcelo and Fonseca 1998). It is a complex lagoon system limited by two peninsulas, Ancão and Cacela, and five barrier islands, Barreta or Deserta, Culatra, Armona, Tavira and Cabanas, from west to east. The barrier islands are separated by six inlets, Ancão, Faro-Olhão, Armona, Fuzeta, Tavira and Lacém (Ceia *et al.* 2010).



Figure 2. Ria Formosa lagoon system. Pa, Pb and Pc represent Little Tern colonies located on sandy beaches. S1, S2, S3 and S4 represent Little Tern colonies located on salt-pans. The colors reflect the level of human disturbance, (red – high level of human pressure, yellow- medium level of human pressure, green- low level of human pressure). Image from Google Earth accessed on 19-02-2016.

The morphodynamics of the Ria Formosa lagoon system vary according to marine currents (Pilkey *et al.* 1989). Inlets present a migration cycle from west to east until they reach a limit point. Then the cycle restarts with the appearance of a new inlet closer to the place of the initial one (Weinholtz 1978). Armona inlet is an exception to this cyclic

process because it has been in the same position during the last centuries, however its length has decreased. Faro-Olhão and Tavira are artificial inlets, stabilized with the construction of jetties (Ceia *et al.* 2010). Ria Formosa is a Natural Park since 1987, and it is important for activities such as tourism, ecotourism, aquaculture, fisheries, Nature conservation, effluent discharges, navigation, and salt extraction (Ceia *et al.* 2010). Ria Formosa is a Ramsar site¹, for being an important Wetland area. It is also classified as a communitarian important wetland for seabirds under the Birds Directive; and it is considered a communitarian important natural habitat for wild fauna and flora under the Habitats Directive.² Ria Formosa is ecologically important as a stopover site for migratory birds (Newton and Mudge 2003), and is the most important breeding location for Little Terns in Portugal (Catry *et al.* 2004).

2.3. Breeding Parameters

Following previous studies (Catry *et al.* 2004; Medeiros *et al.* 2012; Ramos *et al.* 2013), Ria Formosa sandy beaches and salt-pans were surveyed to find Little Tern colonies taking into account the location of colonies in previous years and the movement of birds. First we counted the nests to obtain the annual censuses of Little Terns. To study breeding parameters we marked nests in colonies situated in salt-pans and sandy beaches (Fig. 3). Salt-pan colonies were: (S1) Olhão, 5 de Outubro avenue, (S2) Quinta de Marim (headquarters of Ria Formosa Natural Park) also located in Olhão, (S3) Arraial Ferreira Neto and (S4) Vale Caranguejo, both in Santa Luzia, Tavira. Two of the sandy beach colonies were located in Deserta island, (Pa) in a concession beach closer to the restaurant exposed to high human pressure, (Pb) far from the port and hardly attended by people, and (Pc) the colony located in Faro beach (Fig. 2).

¹ www.ramsar.org accessed on 22-12-2015.

² www.cm-faro.pt accessed on 22-12-2015.

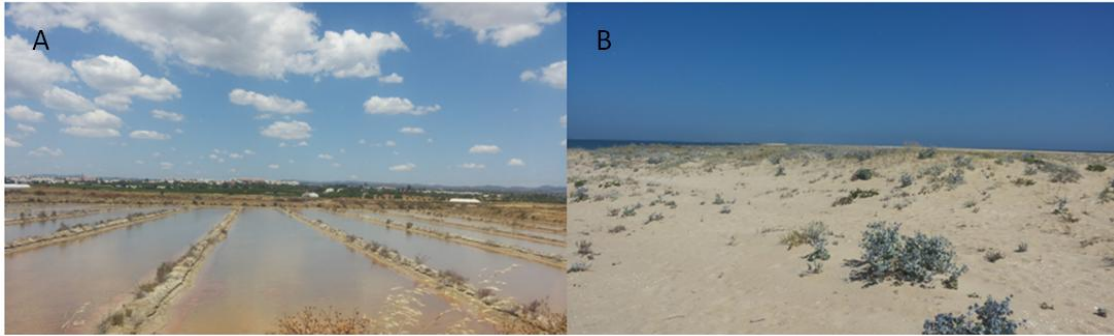


Figure 3. Little Tern colonies of (A) Arraial Ferreira Neto (S3; salt-pan) and (B) Faro beach (Pc; sandy beach).

Nests were numbered with wooden tongue depressors placed about 10-20 cm from the nest (Fig. 4) and eggs were lightly marked with a charcoal pencil. The breadth and length of eggs were measured with calipers, and the volume was calculated with the formula used by Catry *et al.* (2004) (Eggs volume= $K \times L \times B^2$, K is a constant, L is the length and B is the breadth).

Nesting success was estimated based on the traditional method, i.e. observations of the nests and notes taken during visits to colonies made every 3-5 days. We considered nests with success as those: a) when hatched eggs were observed and/or small chicks were found in the nest, and b) when the time needed for hatching was complete and no predation marks were found in or around the nest. We also used the Mayfield method to calculate the probability of nests success based in the exposition time (E) and in the number of unsuccessful nests (I). We calculated the daily unsuccessfully time ($TI=I/E$), then the daily survival rate ($TS=1-TI$) and finally success probability ($PEN=TS^n$, n is the mean number of days for eggs to hatch, 21 days according to Catry *et al.* 2004). To calculate nesting success we excluded nests that were partially predated before the clutch was complete (10%).



Figure 4. Little Tern nests marked with wooden tongue depressors.

During the visits to the colonies we placed cameras close to the nests (Fig. 5) to identify nest predators. The cameras were programmed to take photographs when movement was detected. Thus, we could identify predator based on images obtained with the cameras, and complemented the information obtained with the observation of predation marks. The cameras were placed in both habitats (sandy beaches and salt-pans) during short periods of time (2-3 days). We placed and took the cameras out during the usual visits to the colonies, to minimize disturbance. The use of these devices can lead to limitations in correct identification of nest failure, because cameras are relatively large structures placed near the nests, and may attract or repel predators (Sutherland *et al.* 2004). However, when possible, the cameras were camouflaged by vegetation and placed as far as possible from the nests. Previous work (Sanders and Maloney 2002; Schaefer 2004; Robinson *et al.* 2005) obtained positive results using cameras to take photographs or making videos of bird predators.



Figure 5. Cameras placed near the nests to monitor the visit of potential predators.

2.4. Diet

During visits to the colonies to follow nesting success, we collected pellets regurgitated by Little Terns near the nests (Fig. 6). We also collected prey dropped in the nests' surroundings (Fig. 6), which were placed in paper envelopes and then used to prepare a scale reference collection.

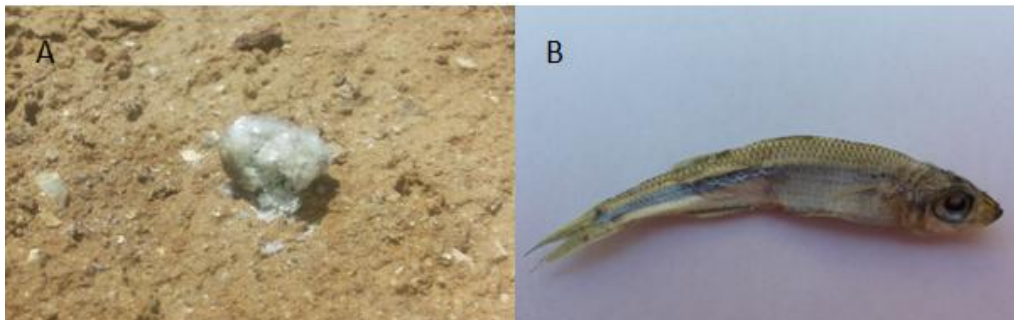


Figure 6. Photographs of (A) a pellet and (B) a prey item *Atherina* spp. found near Little Tern nests.

At the laboratory the otoliths and scales were separate from the pellets. We identified separately otoliths and scales to the lowest taxonomic level possible. The occurrence of insects and crustaceans in the samples was also registered. Otoliths were identify based on book guides (Assis 2004; Tuset *et al.* 2008) and compared with otoliths from previous work present in the laboratory. Scales were identified based on our reference collection made with scales from prey dropped near nests and other specimens from Ria Formosa lagoon system. The length of *Atherina* spp. well preserved

otoliths was measured using an electronic caliper. Then, the mass of sand-smelts was calculated using the formula: *Atherina* spp. Mass = $0,6726x^2 - 0,8113x + 0,2766$ (g), x is the length of the otoliths (according with Ramos *et al.* 2013, this equation was established by J. Martins from University of Algarve, made with fresh specimens caught in Ria Formosa).

2.5. Data Analysis

We compared the frequency of occurrence for each prey in Little Terns' diet at salt-pans according with both methods in different breeding seasons (2013, 2014 and 2015). We used a NMDS (non-metric multidimensional scaling) to obtain a graphical distribution of the parameters (prey, method and year) influencing diet identification of Little Terns. The influence of year, method (scales and otoliths) and interaction year*method in the assessment of diet composition were tested using a permutational ANOVA, through the '*adonis*' function of the '*vegan*' package (Oksanen *et al.*, 2013) within the R-environment (R Core Team 2015). We also used a GLM to test the influence of identification method in the diet at sandy beaches in 2015.

We calculated the nesting success according with two methods: (1) the traditional method, which is based in the observation of the colonies and (2) the Mayfield method (1961) which is based in the exposition time of the nests, and We compared the results obtained.

We used Generalized Linear Models (GLM) to test the effect of colony location and habitat type on reproduction parameters, using both the traditional method and Mayfield method (the response variable was the number of exposition days). Salt-pan colonies were, (S1) Olhão salt-pan, (S2) Quinta de Marim salt-pan, (S3) Arraial Ferreira Neto salt-pan, (S4) Vale Caranguejo salt-pan and beach colonies were (Pa) and (Pb) both in Deserta island beach and (Pc) Faro beach. In habitat variable we distinguish between beach and salt-pan. The reproductive parameters were: (1) egg volume, with a normal distribution (2) clutch size, with poisson distribution (3) nesting success with a binominal distribution (0-1) and (4) exposition time, with normal distribution. Tests were corrected for possible overdispersion of the data.

To complement results obtained from previous years (since 2002), we calculated for salt-pans in 2015, (1) the number of breeding pairs, (2) percentage of clutches initiated between 1 and 15 May, (3) mean clutch size (No. of eggs per clutch), (4) mean egg length (mm), (5) mean egg breadth (mm), (6) mean egg volume (cm³), (7) percentage of *Atherina* spp. in pellets and (8) mean mass of *Atherina* spp. consumed (g). We also obtained the values of North Atlantic Oscillation index for April, May and the mean of both months (matching the egg formation and laying seasons), and the mean for winter months, from December until March, to relate with availability of prey that spawn during the winter (Ramos *et al.* 2013). All mean values are presented with standard deviation. Variables (1) to (8) were correlated using values from 2002 to 2015 to evaluate the relationship between variables. A Linear regression was performed between the variables where correlations were verified. Data from 2002 until 2012 was obtained from Ramos *et al.* (2013), data from 2013 and 2014 was obtained from Lopes (2015) and data from 2015 was obtained during this study. The monthly values for NAO index since 2002 to 2015 are available on the internet in ftp://ftp.cpc.ncep.noaa.gov/wd52dg/data/indices/nao_index.tim. Except the diet analysis, all the statistical analysis was carried out with STATISTICA 7 software.

3. RESULTS



3.1. Breeding Parameters of Little Tern in Ria Formosa

Nesting success calculated by the traditional method of colony monitoring was similar to that computed by the Mayfield method (Table 1). Nevertheless, the Mayfield method estimated a higher nesting success for colonies S1 and S3 when compared to the traditional method (Table 2). Habitat type (sandy beach, salt-pan) did not influence significantly egg volume and clutch size, but nesting success was almost significantly higher on sandy beaches (0.59 ± 0.32) than on salt-pans (0.49 ± 0.46 , Table 1 and 3). However, colony location had a significant influence on nesting success (Table 3). In comparison with the colony located in Deserta island away from the port (colony Pb; reference colony), colonies S2 and S4 (located on salt-pans) had a significantly higher nesting success, while colonies S1 and S3 (also on salt-pans) had a significantly lower nesting success. The beach colony Pa did not differ significantly from the reference colony Pb (Table 4, Fig. 7).

Table 1. Little Terns breeding parameters (clutch size and egg volume) and nesting success (mean \pm SD), using the traditional method of marking nests and following them every 3-5 days, and using the Mayfield method (Mayfield 1961), for both breeding habitats (sandy beaches and salt-pans) in Ria Formosa. Nesting success (0,1) refers to the mean number of successful nests.

	Salt-pans	Beaches
N	116	127
Clutch size (No. eggs nest ⁻¹)	2.28 ± 0.65	2.29 ± 0.68
Egg volume (cm ³)	8.36 ± 0.56	8.35 ± 0.49
Nesting success (0,1)	0.49 ± 0.46	0.59 ± 0.32
Mayfield success (PEN)	0.56 ± 0.38	0.60 ± 0.29

Table 2. Nesting success using the traditional method (nest monitoring every 3-5 days) and the Mayfield Method (Mayfield 1961) for the different Little Tern breeding colonies in Ria Formosa (salt-pans colonies located in (S1) Olhão, (S2) Quinta de Marim, (S3) Arraial Ferreira Neto and (S4) Vale Caranguejo, and sandy beach colonies located in (Pa) Deserta island, near to the port and (Pb) Deserta island, away from the port).

Colony	Salt-pans				Sandy Beaches	
	S1	S2	S3	S4	Pa	Pb
Success (traditional method)	0.12	0.95	0.06	0.82	0.36	0.82
Mayfield Success	0.26	0.95	0.21	0.82	0.40	0.80

Table 3. Generalized linear models (GLM) to estimate the influence of habitat (sandy beach, salt-pan) and colony (salt-pans colonies located in (S1) Olhão, (S2) Quinta de Marim, (S3) Arraial Ferreira Neto, (S4) Vale Caranguejo and sandy beach colonies located in (Pa) Deserta island near to the port, (Pb) Deserta Island away from the port and (Pc) Faro) on Little Tern's breeding parameters (1) clutch size, (2) egg volume and (3) nesting success. Significant values are shown in bold.

Parameters	DF	Habitat			Colony	
		Wald X^2	P	DF	Wald X^2	P
Clutch size (No. eggs nest ⁻¹)	1	0.12	0.73	6	1.8	0.94
Egg volume (cm ³)	1	0.40	0.53	5	8.4	0.14
Nesting success (0,1)	1	3.65	0.06	5	104.7	< 0.001

In comparison with the colony located in Deserta island away from the port (colony Pb; reference colony), colonies S2 and S4 (located on salt-pans) had a significantly higher exposition time, while colony S3 (also on salt-pans) had a significantly lower exposition time. The sandy beach colony Pa and the salt-pan colony S1 did not differ significantly from the reference colony (Table 4, Fig. 8). S2 was the colony with higher exposition time (Fig. 8). Overall, there were no significant differences on the exposition time (Mayfield method) between habitats ($p = 0.38$, $X^2 = 0,76$).

Table 4. Estimated values ($\beta \pm SE$, standard error) and associated probability from Generalized Linear Models for the nesting success and exposition time (based on the Mayfield method) for the different colonies (salt-pans colonies located in (S1) Olhão, (S2) Quinta de Marim, (S3) Arraial Ferreira Neto and (S4) Vale Caranguejo and sandy beach colony located in (Pa) Deserta Island near to the port) comparing with the reference colony located in Deserta island away from the port (Pb). Significant values are presented in bold.

Colony	Nesting success		Exposition time	
	$\beta \pm SE$	Probability	$\beta \pm SE$	Probability
Olhão salt-pan (S1)	-2.07 \pm 0.52	< 0.001	-0.12 \pm 0.07	0.08
Quinta de Marim salt-pan (S2)	2.78 \pm 0.66	< 0.001	0.24 \pm 0.05	<0.001
Arraial Ferreira Neto salt-pan (S3)	-2.92 \pm 0.90	<0.001	-0.21 \pm 0.09	0.03
Vale Caranguejo salt-pan (S4)	1.44 \pm 0.49	0.003	0.14 \pm 0.06	0.02
Deserta Island sandy beach near the port (Pa)	-0.64 \pm 0.45	0.155	-0.11 \pm 0.08	0.17

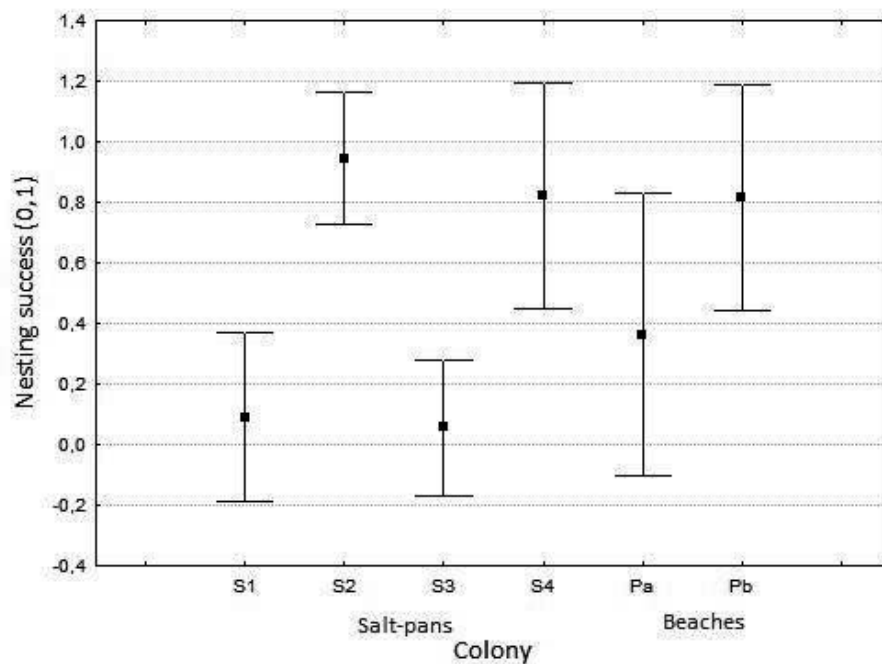


Figure 7. Little Tern nesting success (mean \pm SD) for the different colonies (salt-pans colonies located in (S1) Olhão, (S2) Quinta de Marim, (S3) Arraial Ferreira Neto and (S4) Vale Caranguejo and on sandy beaches located in (Pa) Deserta Island near to the port and (Pb) Deserta Island away from the port).

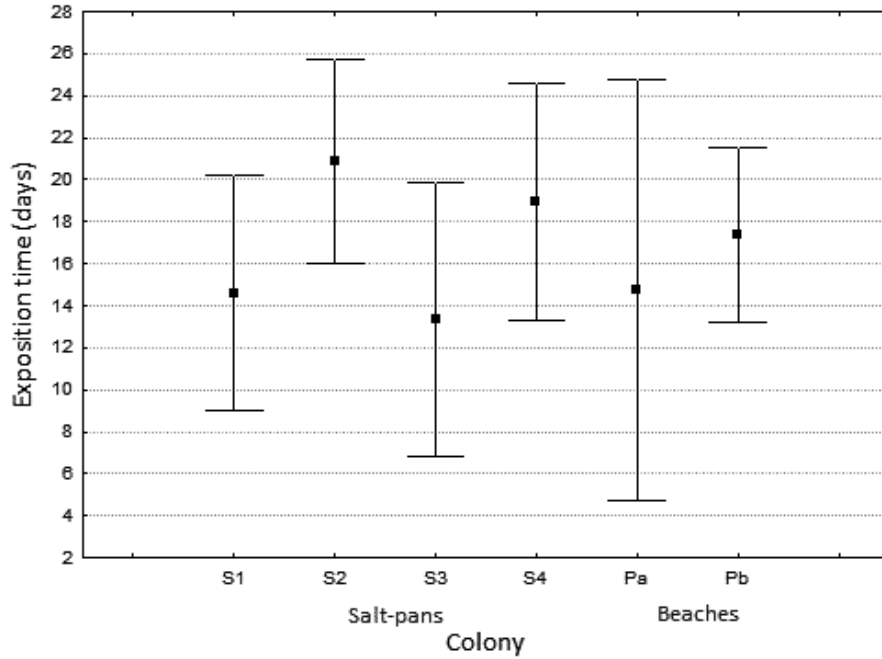


Figure 8. Exposition time (mean \pm SD) for the different Little Tern breeding colonies in Ria Formosa (salt-pans colonies located in (S1) Olhão, (S2) Quinta de Marim, (S3) Arraial Ferreira Neto and (S4) Vale Caranguejo and on sandy beaches located in (Pa) Deserta island, near to the port and (Pb) Deserta island, away from the port).

3.2. Little Tern Diet in Ria Formosa

The diet of Little Terns was dominated by sand-smelts (*Atherina* spp.) in all breeding seasons, using both scales and otoliths. The second most important prey type identified, based on otoliths, was gobies (*Pomatoschistus* spp.), but this prey was not identified when using scales (Fig. 9). It was possible to identify a larger number of prey species based on otoliths ($n = 12$) than in scales ($n = 4$; Fig. 9). Also, *Liza* spp. occurred more in the otoliths of 2015, than in either otoliths or scales of 2013 and 2014 (Fig. 9).

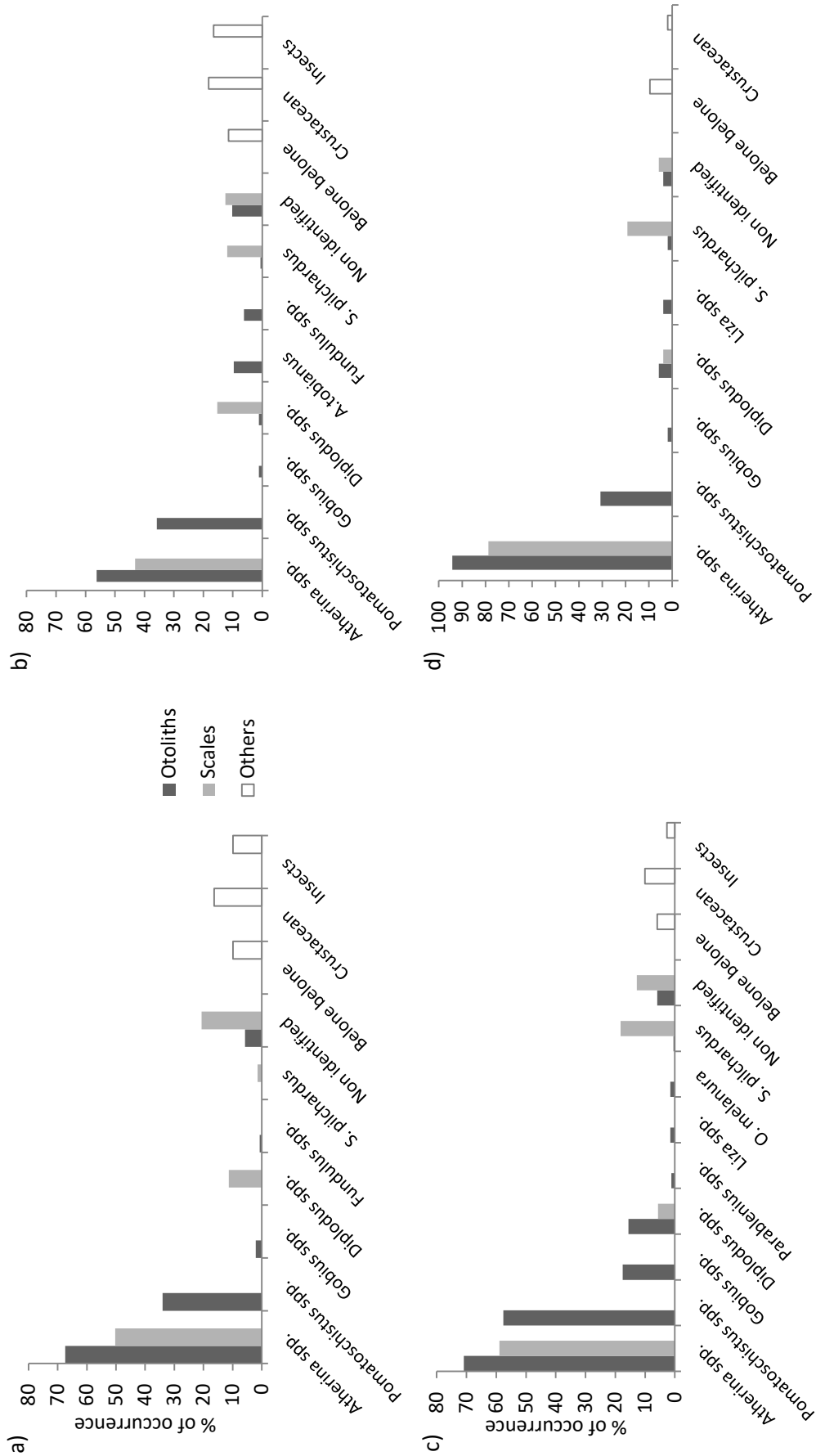


Figure 9. Comparison between both identification methods of Little Terns' diet (i.e. otoliths and scales identification) in a) salt-pans in 2013, b) salt-pans in 2014, c) salt-pans in 2015, d) sandy beaches in 2015. In salt-pans of 2015 *Microchirus spp.*, *Amodytes tobianus* and *Fundulus spp.* occurred <0,5% and thus are not shown in the plot.

The diet of Little Terns in salt-pans varied considerably among years (ADONIS, Pseudo- $F_{1,1445} = 8.5$, $P < 0.01$), with the identification method (ADONIS, Pseudo- $F_{1,1445} = 12.8$, $P < 0.001$) and there was an interaction year*identification method (ADONIS, Pseudo- $F_{1,1445} = 5.5$, $P = 0.02$). Main differences were related with the lower occurrence of *Sardina pilchardus* identified through scales in 2013, when compared to otoliths in 2014-2015. The NMDS analysis shows that sand-smelts had a high importance in the diet according with both otoliths and scales (Fig. 10). Sardines were detected mainly by scale identification and with significantly higher frequencies in 2014 and 2015 than in 2013 (Fig. 9 and 10), and sea breams were also detected at a higher frequency using scales, except in 2015 when their occurrence was higher using otoliths (Fig. 9). Fig. 10 also shows that otolith data for 2015 was separated from the rest of the data, based on prey items detected only in 2015 (*Liza* spp., *Oblada melanura*, *Parablennius* spp., *Microchirus* spp. Fig. 9). We Also found differences between the two identification methods for both sardines and sea breams in Little Terns' diet in sandy beaches during 2015 (Table 5).

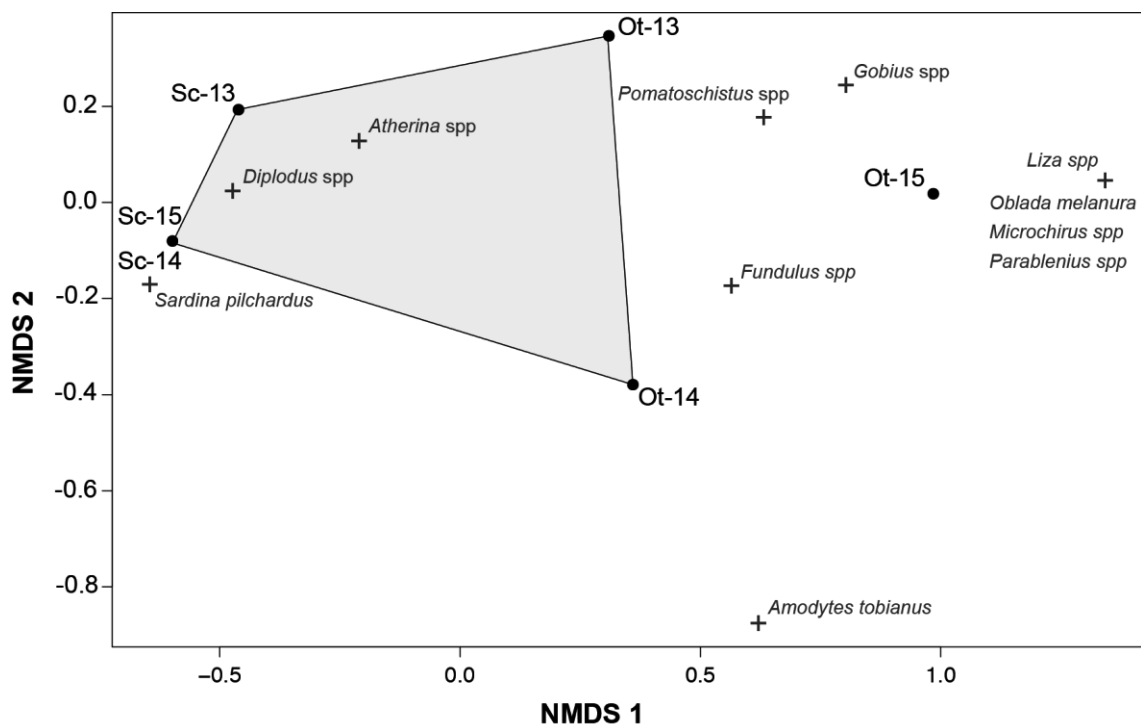


Figure 10. Two-dimensional non-metric multidimensional scaling (NMDS) ordination plot of prey from Little Terns' diet on salt-pans across different identification methods (Sc – scales and Ot – otoliths) and study years (2013, 2014, 2015). The convex hull connects the vertices of the points made by the method-year combination. Stress value = 0.16.

Table 5. Differences in frequency of occurrence of Sand-smelts (*Atherina* spp.), Sardines (*Sardina pilchardus*) and Sea-breems (*Diplodus* spp.) between identification methods (scales and otoliths identification) for beach colonies in 2015 breeding season. Significant values are presented in bold.

Prey	Df	Wald χ^2	P
<i>Atherina</i> spp.	1	40.57	0.65
<i>Sardina pilchardus</i>	1	5.35	<0.05
<i>Diplodus</i> spp.	1	4.56	<0.05

3.3. Little Tern Predators in Ria Formosa

We identified Little Tern predators in both breeding habitats (sandy beaches and salt-pans) during the 2015 breeding season through the footprints found around the nests, and also using photographs taken by the cameras placed near the nests (Table 6). Based on footprints we identified the Stone-curlew (*Burhinus oedicanus*), and the Dog (*Canis familiaris*) (Fig. 11) as Little Tern nests predators. Based on photographs taken by the cameras we identified the Cat (*Felis catus*), the Fox (*Vulpes vulpes*), the Eurasian magpie (*Pica pica*), the Western marsh-harrier (*Circus aeruginosus*), and also the Stone-curlew (*Burhinus oedicanus*) (Fig. 12).

Table 6. Little Terns (*Sternula albifrons*) egg predators during the 2015 breeding season for the two habitat types, sandy beach and salt-pans, of Ria Formosa identified based on marks left by predators, and on photographs taken by cameras placed near nests.

Habitat	Predator	Identification Method
Sandy Beach	Stone-curlew (<i>Burhinus oedichnemus</i>)	Footprints and photographs
Sandy Beach	Cat (<i>Felis catus</i>)	Photographs
Salt-pans	Eurasian magpie (<i>Pica pica</i>)	Photographs
Salt-pans	Western marsh-harrier (<i>Circus aeruginosus</i>)	Photographs
Salt-pans	Dog (<i>Canis familiaris</i>)	Footprints
Salt-pans	Fox (<i>Vulpes vulpes</i>)	Photographs

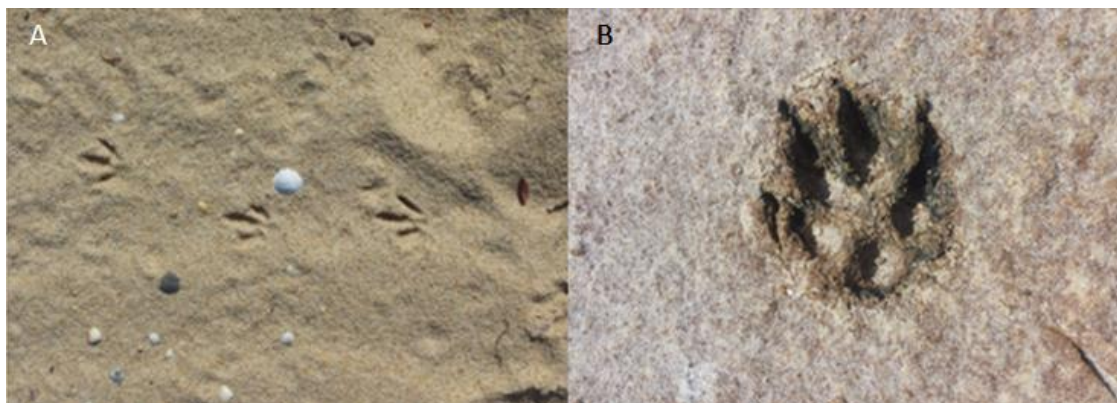


Figure 11. Predator footprints found near Little Tern (*Sternula albifrons*) nests during the 2015 breeding season in Ria Formosa. A- Stone-curlew (*Burhinus oedichnemus*) footprint in the colony located in Deserta island sandy beach away from the port (Pb). B- Dog (*Canis familiaris*) footprint in the colony located in Olhão salt-pan (S1).

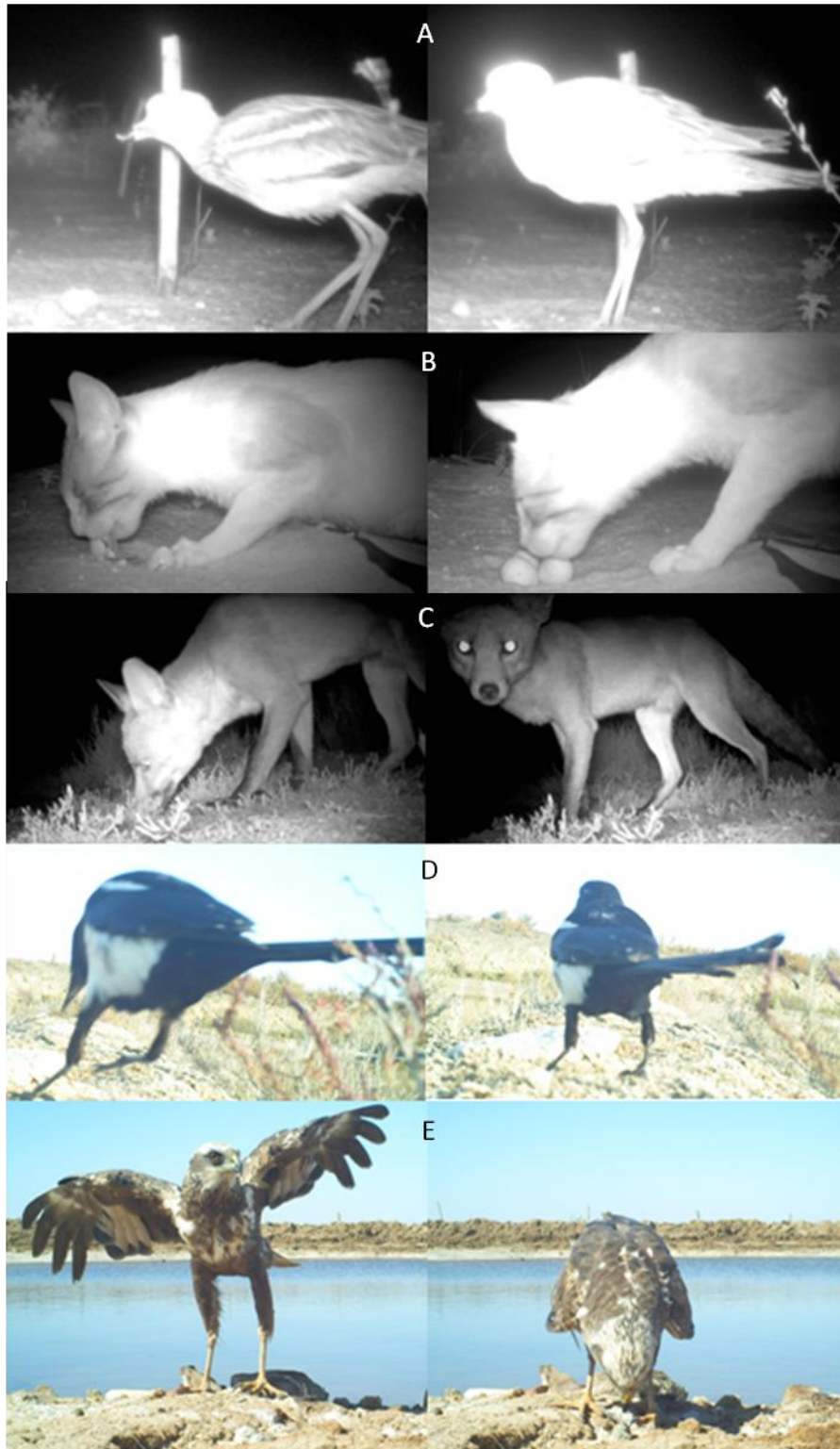


Figure 12. Little Tern (*Sternula albifrons*) nest predators captured by cameras placed near nests during the 2015 breeding season in Ria Formosa. A- Stone-curlew (*Burhinus oedicnemus*); B- Cat (*Felis catus*); C- Fox (*Vulpes vulpes*); D- Eurasian magpie (*Pica pica*); E- Western marsh-harrier (*Circus aeruginosus*).

3.4. The Influence of the North Atlantic Oscillation (NAO) Index and Little Tern's Diet on its Population Numbers and Breeding Parameters

There was a higher number of Little Tern breeding pairs in Ria Formosa salt-pans in 2015 (N = 116), comparing with previous years since 2002 (Table 7). The same pattern was registered for the percentage of clutches initiated between 1 and 15 of May, as the value obtained in 2015 was the second highest since 2002 (Table 7). The larger egg volume was obtained in 2007 while the lower value was obtained in 2011. In 2005 there was a higher percentage of *Atherina* spp. in the pellets, and in 2009 a higher mean mass of *Atherina* spp. was registered using the otoliths found in pellets. A lower percentage and mean mass of *Atherina* spp. were both obtained for 2012 (Table 7).

Table 7. Population numbers (N, breeding pairs), breeding parameters (% of clutches initiated between 1-15 May, clutch size, egg length, breath and volume), % of sand-smelts in the diet, and mean mass of sand-smelts in the diet for Little Terns (*Sternula albifrons*) breeding in salt-pans in 2002-2015. The winter NAO index for this period is also presented. NA—not available. Values are mean \pm SD.

year	N pairs	Clutches initiated between 1-15 May(%)	Clutch size (eggs /clutch)	Eggs Length (mm)	Eggs Breadth (mm)	Eggs volume (cm ³)	% of <i>Atherina</i> spp. In pellets	Mean Mass <i>Atherina</i> spp. (g)	NAO Apr/ May	NAO Winter (Dec-Mar)
2002	53	6	1.87 \pm 0.63	31.66 \pm 1.16	22.98 \pm 0.53	8.14 \pm 0.53	61.6	NA	0.495	0.065
2003	97	16.5	2.68 \pm 0.52	32.06 \pm 0.99	23.48 \pm 0.47	8.61 \pm 0.46	84.8	NA	-0.14	-0.2725
2004	70	7.9	2.2 \pm 0.63	32.08 \pm 1.16	23.40 \pm 0.59	8.56 \pm 0.57	44.8	NA	0.67	-0.07
2005	47	5.8	2.47 \pm 0.66	32.50 \pm 2.68	23.39 \pm 0.59	8.66 \pm 0.92	86.1	NA	-0.79	-0.135
2006	28	s.d.	2.66 \pm 0.45	31.90 \pm 0.99	23.39 \pm 0.41	8.49 \pm 0.36	79.4	NA	0.095	-0.575
2007	32	s.d.	2.61 \pm 0.48	32.04 \pm 0.78	23.6 \pm 0.48	8.68 \pm 0.38	66.8	3.54 \pm 0.84	0.35	0.2575
2008	31	47.5	2.48 \pm 0.63	31.90 \pm 0.79	23.39 \pm 0.54	8.50 \pm 0.46	83.7	3.72 \pm 0.81	-1.43	0.205
2009	35	0	2.34 \pm 0.69	32.08 \pm 1.21	23.55 \pm 0.58	8.66 \pm 0.59	80.5	3.77 \pm 0.87	0.625	-0.265
2010	29	10.3	2.83 \pm 0.38	31.67 \pm 1.05	23.58 \pm 0.44	8.57 \pm 0.47	77.8	3.40 \pm 1.06	-1.13	-1.925
2011	35	2.9	2.37 \pm 0.65	31.10 \pm 0.93	22.96 \pm 0.58	7.99 \pm 0.55	65.2	2.40 \pm 1.68	1.27	-0.685
2012	25	2.2	2.32 \pm 0.85	31.25 \pm 1.09	23.39 \pm 0.62	8.33 \pm 0.54	37.6	1.55 \pm 1.10	-0.21	1.0175
2013	33	0	2.15 \pm 0.76	31.73 \pm 1.08	23.68 \pm 0.56	8.66 \pm 0.53	67.4	2.81 \pm 1.44	0.59	-0.7725
2014	20	2.9	2.00 \pm 0.79	31.42 \pm 1.04	23.46 \pm 0.64	8.42 \pm 0.52	56.3	3.18 \pm 1.36	-0.305	0.5325
2015	116	24.4	2.28 \pm 0.65	31.59 \pm 1.11	23.45 \pm 0.62	8.36 \pm 0.56	70.9	3.13 \pm 1.30	0.415	1.3425

There was a significant negative correlation between the volume of Little Tern eggs and the April NAO index ($r = -0.68$; $p = 0.008$; $n = 14$; Fig. 13), and significant positive correlations between mean clutch size and percentage of *Atherina* spp. ($r = 0.55$; $p = 0.042$; $n = 14$; Fig. 14), and between egg length and the mean mass of *Atherina* spp. in Little Terns' diet ($r = 0.81$; $p = 0.008$; $n = 9$; Fig. 15).

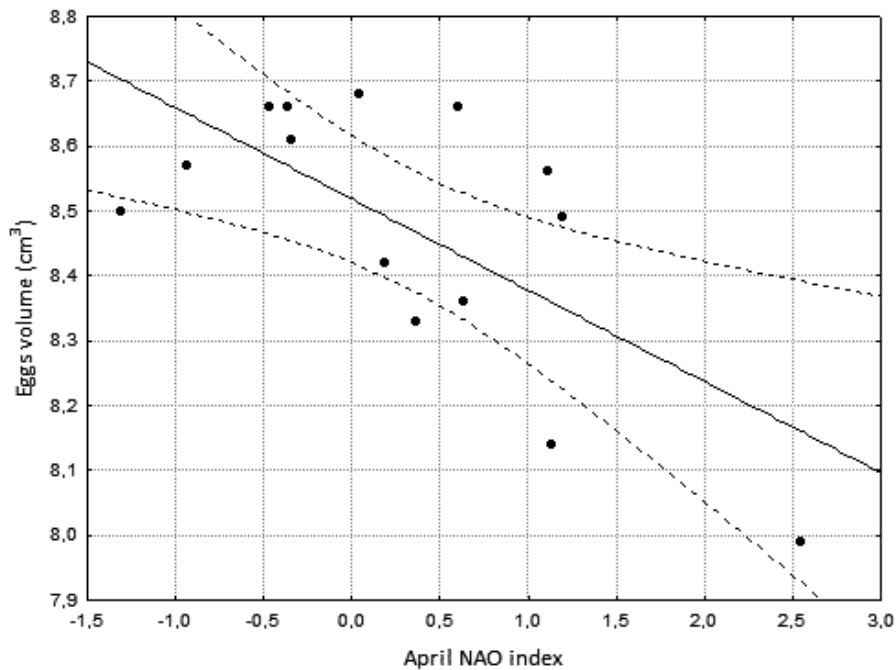


Figure 13. Relationship between Little Tern (*Sternula albifrons*) egg volume in Ria Formosa salt-pans and the April North Atlantic Oscillation (NAO) Index between 2002 and 2015. ($r = -0.68$; $p = 0.008$; $n = 14$; $y = 8.52 - 0.14x$).

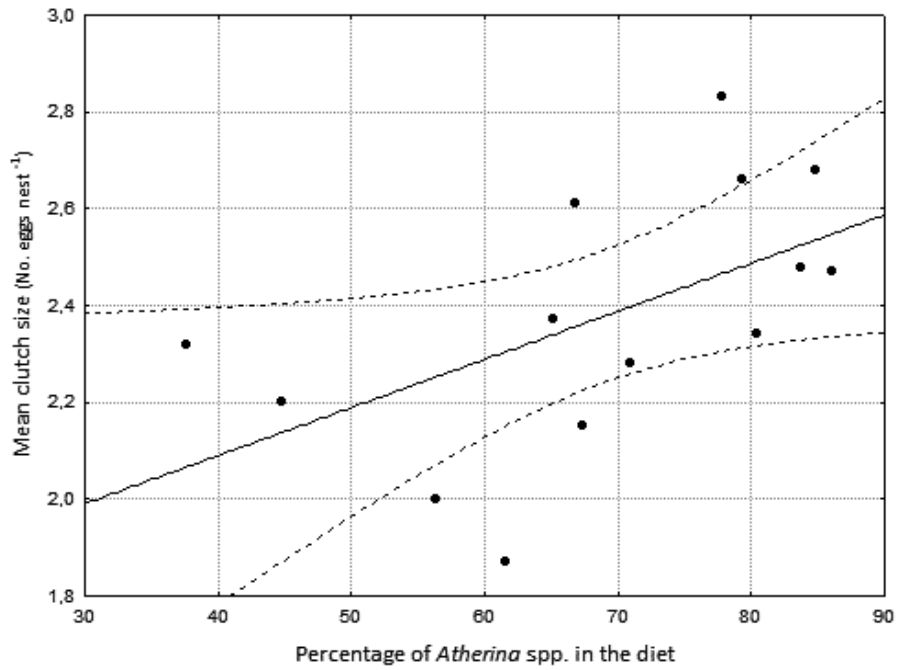


Figure 14. Relationship between mean clutch size (No. eggs nest⁻¹) and percentage of sand-smelts (*Atherina* spp.) in the diet of Little Terns (*Sternula albifrons*). ($r = 0.55$; $p = 0.042$; $n = 14$; $y = 1.69+0.01x$).

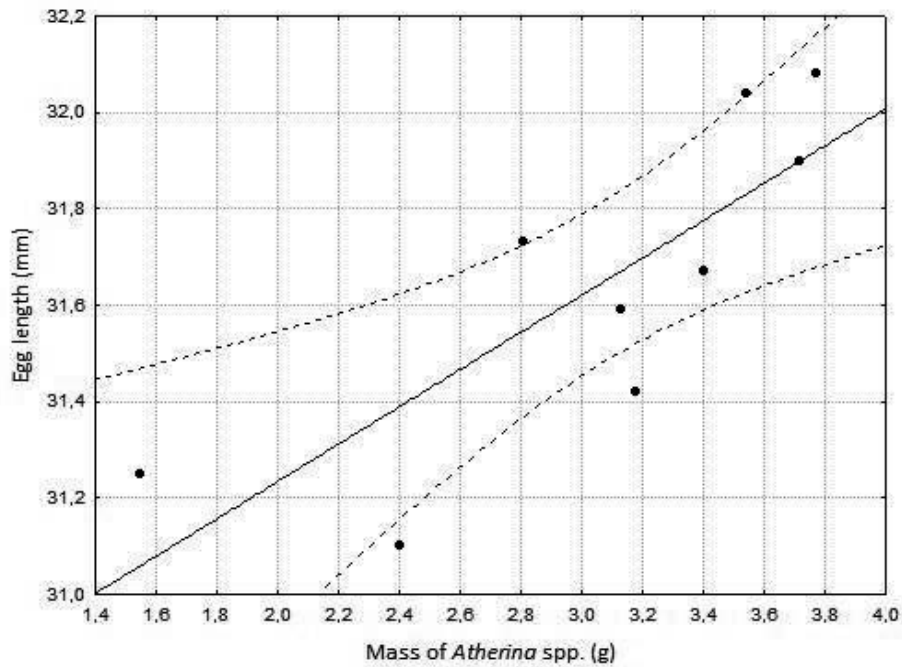


Figure 15. Relationship between (*Sternula albifrons*) egg length (mm) of Little Terns and the mean mass of sand-smelts (*Atherina* spp.) in their diet ($r = 0.81$; $p = 0.008$; $n = 9$; $y = 30.46+0.39x$).

4. DISCUSSION



4.1. Breeding Parameters of Little Tern in Ria Formosa

Our results suggest that there were no differences in breeding success between salt-pans and sandy-beach colonies in 2015. However, the comparison between the two habitats showed an almost significant difference, with higher values for the sandy-beaches. Safina *et al.* (1989) found higher breeding success of Common Tern (*Sterna hirundo*) breeding in beaches when compared to salt-pans, in Long island, New York. However, the differences were mainly found among the different colonies. Significant differences between habitats were also not detected by Catry *et al.* (2004) studying Little Terns in Ria Formosa, and by Norte and Ramos (2004) studying Kentish Plover (*Charadrius alexandrinus*, a species which also breeds in both sandy beaches and salt-pans) in 4 colonies of the Portuguese coast. This shows the resilience and adaptation of birds to breed in new habitats such as the salt-pans. In fact, the Little Tern population in Portugal has not declined in the last 30 years, though the breeding success is influenced by the location of colonies (Catry *et al.* 2004). In both habitats there were colonies with high and low breeding success, which can be explained by the different characteristics of each colony, particularly the accessibility of the site by predators, since habitat *per se* can be less relevant in explaining breeding success than the characteristics of each site (Norte and Ramos 2004). Breeding colonies with low breeding success are likely associated with negative impacts, such as high human disturbance and predation. We obtained the highest and lowest value of breeding success in salt-pan colonies (i.e. colonies S2 and S3, respectively) showing that a more regular breeding success occurs in the natural habitat (sandy beaches). This is contrary to what was reported by Catry *et al.* (2004), since they found both highest and lowest breeding success on the sandy beach colonies, and a more homogeneous breeding success among salt-pan colonies. It is important to notice that in salt-pans we had breeding success values closer to zero, revealing that some breeding locations chosen by Little Terns were exposed to very poor conditions. This was probably related with predation, because this is the main cause of clutch loss in Ria Formosa (Catry *et al.* 2004, this study). Salt-pans have a design which facilitates the detection of nests by predators due to the linearity of the paths (Rocha *et al.* 2016). All these differences between the results obtained by Catry *et al.* (2004) and our study are related with different location of the target colonies between studies.

Moreover, this might also be related with variability of the environmental conditions previously and presently (e.g. inter-annual differences in human pressure, predation and weather conditions, Catry *et al.* 2004).

There were almost no differences between the traditional and the Mayfield method to calculate the breeding success for each colony, similar to the results obtained by Norte and Ramos (2004) for Kentish plovers. Klett and Johnson (1982) obtained differences in the breeding success values between the two methods, however the highest and lowest values of breeding success were similar for both methods as verified in our study. Our study suggests that there were no differences in exposition time between the two habitats, which was expected because no differences were obtained in breeding success too. However we obtained large differences between the colonies. The exposition time obtained was related with breeding success as expected, the highest value of exposition time was obtained for the same colony with the highest breeding success (colony S2), and the same was obtained for the colony with the lowest exposition time (colony S3). The nests that were active a longer period were more likely to hatch, which occurred in colony S2 where a very low number of nests were predated. This was similar to what was reported by Norte and Ramos (2004) for the breeding success of Kentish plovers, with the exception of one colony, where, despite a long exposition time, the hatching success was very low. This is usually related with a stochastic phenomenon, such as a very high tide on sandy beach colonies or the sudden appearance of a predator. In fact, the sudden appearance of a Dog on colony S1, led to a very low hatching success on that colony with a long exposition time.

4.2. Methods to Assess Little Terns' Diet

Overall, more prey species were identified using otoliths than scales, probably due to the fact that otoliths are the densest part of the fish (Pooper and Combs 1982), present a higher resistance to digestion (Treacy and Crawford 1981) and are better preserved in pellets than scales. This was the case of gobies; despite the small size of its otoliths they were the second most important prey according to the identification of otoliths, which was most likely related to the high otolith density of this species. Otoliths of different fish species have different resistance to digestion, and are found in pellets

with different levels of erosion (Hjelset *et al.* 1999). When fish otoliths are too thin, fragile or with outer projections they are more easily degraded by gastric juices during digestion. Such degradation may hamper prey identification, which often happens with otoliths from clupeid fish, such as sardine (Jobling and Breiby 1986). Yet, clupeid fish scales are large and possess a typical silhouette, being easier to identify even when slightly degraded (Patterson *et al.* 2002). The annual differences in the occurrence of sea breams that we detected may be related with the ingestion of different sea bream species by Little Terns in different years. Six different sea bream species inhabit the Ria Formosa lagoon system (*D. annularis*, *D. bellotti*, *D. cervicus*, *D. puntazzo*, *D. sargus* and *D. vulgaris*; Ribeiro *et al.* 2006), but we were unable to identify them to species level using either scales or otoliths, because both were degraded in the pellets.

Published studies of tern diet usually lack specificity about which hard parts were used to identify diet items or refer only to the identification of otoliths (Mauco and Favero 2005; Catry *et al.* 2006; Alfaro *et al.* 2011; Ramos *et al.* 2013). It is essential to know whether fish scales were identified to understand the full scope of the data that is presented. When clupeoid fish or sea breams are detected using otoliths (Catry *et al.* 2006; Mauco and Favero 2004), it is important to also identify the scales of those (and similar) species, to ensure that their occurrence is accurately quantified. This should be very important for small seabird species, because the small fish that they ingest possess very small otoliths, which are more easily degraded and therefore more difficult to identify. We observed this for otoliths with protuberant structures or vertices such as those of gobies and sardines, which were easily eroded by degradation. Our study shows that assessing otoliths only does not provide a complete picture of Little Tern diet because it underestimates important prey such as sardines and sea breams. Future studies should be clear about which hard parts were used for identification. When identification is based on otoliths, it should be complemented with the identification of scales to assure a more reliable picture of the diet.

Previous work has shown that the composition of a seabirds' diet can be used as a bioindicator of prey availability (Einoder 2009). Hence, shifts in seabirds' diet can be seen as an early warning for possible reduction in commercial fish stocks, such as the decline in sardine fisheries at the California current (Velarde *et al.* 2013). In Portugal, one of the worst periods ever for sardine fisheries, one of the most relevant and

lucrative fisheries in Portugal (Borges *et al.* 2003), was 2012-2013, then it improved in the following years (IPMA 2016). Our data shows that sardines occurred much less in the diet of Little Terns in 2013 than in 2014 and 2015. Therefore, the diet of Little Terns might potentially be used as a sentinel of sardine abundance in southern Portugal.

4.3. Little Terns' Diet

Sand-smelts (*Atherina* spp.) were by far the most important prey in Little Tern's diet for 2013, 2014 and 2015 breeding seasons using both identification methods, scales and otoliths. The second most important prey were gobies (*Pomatoschistus* spp.); this was also obtained in previous studies at Ria Formosa since 2002 (Catry *et al.* 2006; Paiva *et al.* 2006a; Ramos *et al.* 2013). These results are probably related with the opportunistic feeding behaviour of Little Terns (Catry *et al.* 2006), because these two important prey are characteristic of estuaries and coastal lagoons (Catry *et al.* 2006), and are the two most abundant fish species in Ria Formosa (Ribeiro *et al.* 2012). Gobies are benthic species with low energetic value (Paiva *et al.* 2006a), and its consumption is probably related with a great availability during calm conditions and at low tide than to a preference (Catry *et al.* 2006). Sand-smelts were also identified as one of the most important prey for terns in other locations such as Italy (Bogliani *et al.* 1992; Bogliani *et al.* 1994) and Namibia (Braby *et al.* 2011).

In our study, sardine was consumed at a higher amount by birds breeding in sandy beaches than in salt-pans (using both scales and otoliths). A similar result was obtained for chick diet by Catry *et al.* (2006), which shows that sardines represent a very important prey for Little Terns breeding in sandy beaches. However, scales were not identified by Catry *et al.* (2006) suggesting that this prey was probably underestimated and it may have been more important in the diet than was shown. Because sardine is one of the most energetic prey in Little Terns' diet (Paiva *et al.* 2006a) it can help explaining the higher number of breeding pairs in sandy beaches (Catry *et al.* 2006). In previous studies *Fundulus* spp. was identified as an important prey in the diet (Catry *et al.* 2006; Ramos *et al.* 2013) probably due to its higher energetic content (Paiva *et al.* 2006a), however it was not to relevant in our study. Its presence in the diet is irregular

since 2002 (Ramos *et al.* 2013), presumably as a reflection of changes in the presence of this fish species in Ria Formosa: this is an euryhaline species that only occurs in salt-pans and adjacent channels, and changes in its occurrence in the diet are probably related with alterations in salinity of salt-pans due to the salt extraction (Paiva *et al.* 2006a).

Terns' diet was not only composed of fish, but crustaceans and insects were also present. The consumption of crustaceans is not related with a preference by terns because crustaceans present a low energetic content (Paiva *et al.* 2006a). Its consumption is probably related with a high availability of this prey in salt-pans (Catry *et al.* 2006). For this reason crustaceans were present in very low values in the Little Terns' diet for sandy beaches in 2015, when compared with salt-pans. Insects have an inconsistent importance in Little Terns diet (Catry *et al.* 2006), and this was also shown for Common Tern (Bugoni and Vooren 2004).

4.4. Little Tern Predators in Ria Formosa

Previous studies already reported the Stone-curlew (*Burhinus oedicephalus*) as the main predator of Little Tern clutches in south Portugal (Catry *et al.* 2004). This was also registered in our study, as we detected it through both photographs and footprints. Also, domestic animals (Dog and Cat) were also identified in previous studies not only as Little Tern clutch predators (Catry *et al.* 2004; Medeiros *et al.* 2007) but also of other tern species (Burger and Gochfeld 1990). The presence of domestic predators can be related with the proximity of the salt-pan colonies with human settlements, leading to the introduction of domestic predators in the colonies (Catry *et al.* 2004). These predators were also identified in our study, the Dog was identified in salt-pans through the footprints near the nests, and the Cat was identified based on photographs on Deserta Island. There was a previous small fishermen community in Deserta Island, and cats were presumably abandoned on the island. Other predators identified such as the Eurasian Magpie and the Western Marsh-Harrier are generalists and opportunistic (Sorace and Gustin 2009), and were previously detected as nest predators of other coastal waterbird species (Valle and Scarton 1999, Kiss 2006). A bird from the same genus, *Circus pygargus* was previously documented as a predator of Little Tern clutches by Catry *et al.* (2004). The fox was also caught by the cameras and it was reported as a

clutch predator of other tern species, *Sternula antillarum* (Massey and Fancher 1989; Burger and Gochfeld 1990).

We found predation marks that were not possible to identify, likely belonging to other predators, such as gulls (*Larus* spp.), brown rats (*Rattus norvegicus*) or Turnstone (*Arenaria interpres*), identified by Catry *et al.* (2004) as predators of Little Tern nests in Ria Formosa. The Peregrine Falcon, which also occurs in coastal Portugal (Lourenço *et al.* 2013), has been reported as predator of tern clutches such as Common Tern, Roseate Tern (Nisbet 1992), Elegant Tern (*Sterna elegans*, Velarde 1993) and Little Tern (O'Connell *et al.* 2014).

4.5. The Influence of The North Atlantic Oscillation (NAO) Index and Little Tern's Diet on its Population Numbers and Breeding Parameters

Our study suggests a negative relation between mean Little Tern egg volume and the April North Atlantic Oscillation (NAO) index between 2002 and 2015. This can be related with a smaller size of sand-smelts during years with higher values of NAO (Ramos *et al.* 2013). Previous work showed that fish size can be affected by climatic conditions and alterations in the NAO index (Jonsson *et al.* 2003). Poor climatic conditions can thus translate into low energetic content per prey, which in turn might diminish the amount of energy available for reproduction, with negative consequences in breeding parameters such as low egg volume. Previous studies reported that for female seabirds a high quality diet during pre-breeding is crucial to form and lay larger eggs (Sorensen *et al.* 2009).

Years with a larger mean clutch size seem to be related with years of higher percentage of *Atherina* spp. in Little Terns' diet, as reported by Ramos *et al.* (2013) for the period 2002-2011. *Atherina* spp. is a species with a high calorific content, when compared to other prey consumed by the species (e.g. shrimp and gobies) (Paiva *et al.* 2006a), leading to a higher amount of energy available for reproduction and likely explaining the previous cause-effect relationship.

Previous studies have shown that female age and experience is related with the size of the eggs laid (Robertson *et al.* 1994; González-Solis and Becker 2004), and younger females lay more narrow and long eggs due to the low elasticity of the oviduct

(Robertson *et al.* 1994). Therefore, during years with higher food availability, a larger number of young females breed or lay larger eggs. Thus, when more *Atherina* spp. is available more young females may breed, leading to an increase in the mean egg length as verified in our study.

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