Afonso Duarte dos Reis Rocha

LIVING IN HUMAN CREATED HABITATS:
THE ECOLOGY AND CONSERVATION OF WADERS ON SALINAS

Tese de doutoramento em Biociências, ramo de especialização em Ecologia, orientada pelo Professor Doutor Jaime Albino Ramos
e co-orientada pelo Professor Doutor José António Masero Osório, apresentada à Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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Living in human created habitats: the ecology and conservation of waders on salinas

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Doctoral thesis in Biosciences (Scientific area of
Ecology) presented to the University of Coimbra, supervised
by Prof. Dr. Jaime Albino Ramos and cosupervised by Prof.
Dr. José António Masero Osório

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Chapter II

Rocha A., Masero J. & Ramos J. The effects of management suspension on roosting waterbirds. Submitted for publication.

Chapter III

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Chapter IV

Rocha A., Silva R., Villegas A., Sánchez-Guzmán J., Ramos J. & Masero J. Morphological, physiological and behavioural responses to salinity during growth: an experiment with black-winged stilt chicks. Submitted for publication.

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Contents

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Summary

Intertidal wetlands are among the most productive habitats and support large number of waterbirds worldwide, however they are also one the most threatened habitats on earth. The loss of intertidal habitats is considered a key factor to explain the current decline of the majority of wader populations. At a global scale, anthropogenic supratidal habitats such as coastal salinas can function as buffer zones against the loss of natural coastal habitats. When the intertidal flats are no longer available as the tide advances, waders are forced to move to high-tide roosts, where they must remain until the receding tide exposes their feeding grounds once again. During the high-tide period waders congregate in dense flocks at salinas and spend most of their time sleeping, preening or feeding. Coastal salinas are man-made habitats exploited for the extraction of salt, by solar and wind evaporation of seawater in a series of shallow interconnected evaporation ponds varying in size, water depth and separated by dykes. Unlike natural wetlands, the water level and salinity in salinas are stable and predictable as a result of management. The management of water level and saltmarsh overgrowth in the ponds and dry areas from salinas are particularly important in determining their use by waders. The different salinities and fluvial dynamics in salinas originate a particularly high benthic invertebrate prey density for waders. During migratory periods, waders can opportunistically exploit this food source and rapidly rebuild their fat stores and pursue their journey. The un-vegetated linear paths between ponds are used also by ground-nesting waterbird species to breed, but their linear structure may facilitate the detection of nests by predators. This negative effect may, however, be counterbalanced by the advantages of breeding in mixed colonies. After hatching, chicks of waders feed by themselves along the ponds of salinas and are limited to hypersaline waters prior to fledging. However birds raised in saline environments can present physiologic and behavioural adaptations to cope with high salinity.

This thesis evaluates the advantages and disadvantages of salinas as breeding and foraging habitats for shorebirds, specifically detailing actions that can be implemented to enhance the value of salinas for waders populations. In Chapter I we assess the potential role of supply ponds from traditional salinas as a complementary foraging area for waders during autumn migration. We evaluated the density of waders during the entire tidal cycle

and the feeding behaviour for a subset of species, following an experimental drawdown. The high density and biomass of benthic invertebrates during the flooded period decreased rapidly after sediment exposure due to sediment drying followed by a high predation rate. We show the first evidence that supply ponds after dewater can support large numbers of migratory foraging waders during a short time window. Dunlin *Calidris alpina*, ringed plover *Charadrius hiaticula*, sanderling *Calidris alba* and little stint *Calidris minuta* using our experimental pond exceeded 10 % of the Tagus population. Substrate dryness was the major factor affecting the benthic invertebrate community rather than bird predation. The dominance of the polychaete worm *Capitella capitata* seems to attract large numbers of ringed plovers, and explains also their high food intake rate, which allows birds to rapidly reach their daily energy requirements. Some wader species could reach their daily biomass needs and gain mass foraging only or mostly on the dewater pond. In Chapter II we benefited from the suspension of management actions on a high-tide roost over one year, and used bird survey data before and after this event to analyze the effect of management suspension on waterbird populations. We grouped waterbird species by their leg length and predicted that small waders would be more affected by the lack of management than large waders. The effects on bird density were investigated trough a before-after control-impact design. We demonstrate that the suspension of management actions in high-tide roosts, even for a relatively short period of time, carried large negative effects especially for small and medium wader populations, which showed a significant reduction in winter (50% and 38% respectively) and in the migratory periods. Our results indicate that the densities of the several groups of bird recovered differently: large waders reached the initial densities after two seasons, medium waders after three and six seasons (autumn and winter, respectively) and small waders and spring migratory medium shorebirds did not recover the initial densities before the end of the study period. In Chapter III we evaluated the importance and the risks of breeding in salinas using kentish plover *Charadrius alexandrinus* as a model species. We analyzed the role of nest-site characteristics, predation and nesting proximity to breeding species with aggressive antipredator behaviour on kentish plover breeding success. Our results show that salinas are an important alternative breeding habitat for kentish plover, as their nesting success was similar or even higher than in natural habitats. Kentish plover selected nest-sites on the edges of the paths, however nest-site characteristics were not correlated with nesting success. Predation was the main cause of nest loss in saltpans

(42%), mostly by carrion crows *Corvus corone* and red foxes *Vulpes vulpes*. Kentish plover show a linear increase in nesting success as their breeding season increasingly overlapped with that of the black-winged stilt *Himantopus himantopus*. We found that breeding close to semi-colonial species with marked anti-predator mobbing behaviour significantly increased nesting success in a more timid and non-colonial shorebird species such as the kentish plover. In Chapter IV we focus in understanding the physiological and behavioural mechanisms of waterbirds to cope with salinity. We compared the basal metabolic rate (BMR), body mass, growth rate, plasma ions, hematocrit, heat shock proteins, and behavioural responses of captive-reared black-winged stilt chicks in fresh, saline and hypersaline water treatments. Additionally, we compared BMR and the saltgland mass in wild fledglings from freshwater and hypersaline wetlands. Our results showed that wild and captive-reared fledglings did not differ in BMR, and the mass of saltglands of wild fledglings from freshwater and hipersaline habitats were similar. All physiological and morphological variables did not differ among treatments. However, head-shaking behaviour was different according to the source of salinity with chicks significantly increasing their head-shaking rate as salinity increased. Our results support the key role of behavioural adjustments in avoiding salt stress during growth. In Chapter V we describe a non-lethal biopsy technique to collect samples of visible adipose tissue from the furcular region in small and medium-sized bird species. The technique was evaluated in terms of processing time, feasibility in the field, and bird health. Biopsy procedures involved making a skin incision 2-3 mm long to collect 2-16 mg of adipose tissue. The incisions took 10-15 days to heal completely and had no apparent short-term negative effects in the suit of physiological parameters. The body mass of dunlin and hybrid passerine *Carduelis carduelis* × *Serinus canaria* was similar before and after biopsy. We recommend this non-lethal biopsy to sample adipose tissue as an alternative to lethal methods in wild and captive bird studies. Taken altogether, this research highlights the need to protect and manage coastal salinas, and promote the maintenance of a network of high quality roosts for the protection of wader populations during their annual cycle.

Key words: anthropogenic habitats, water management, intake rate, nest success, salt stress, ecophysiology, fat stores.

Resumo

As zonas húmidas intertidais são habitats altamente produtivos e suportam milhares de aves aquáticas em todo o mundo, contudo são também um dos habitats mais ameaçados. A perda de habitats intertidais é considerada um dos principais factores que contribui para o actual declínio da maioria das populações de aves aquáticas. Globalmente, habitats artificiais supratidais como as salinas costeiras podem funcionar como zonas tampão contra a perda de habitats costeiros naturais. Quando as zonas intertidais deixam de estar disponíveis devido à subida da maré, as aves são impelidas a voar para refúgios supratidais, onde permanecem até os locais de alimentação intertidais voltarem a ficar acessíveis. Durante o período de preia-mar as aves limícolas concentram-se nas salinas em bandos densos, passando a maior parte desse período em repouso, limpeza da plumagem ou em alimentação. As salinas costeiras são habitats antropogénicos explorados para produção de sal. A evaporação da água salgada ocorre pela acção do vento e calor ao longo de um conjunto de tanques interligados, com diferentes tamanhos e profundidades. Ao contrário das zonas húmidas, a gestão dos níveis de água permite que a profundidade da água e a salinidade nos tanques das salinas sejam estáveis e previsíveis. A gestão adequada dos níveis de água, bem como o controlo da expansão da vegetação halófita e ruderal nos tanques e nas zonas não alagadas são particularmente importantes para que as aves limícolas ocorram nas salinas. As diferentes salinidades e hidrodinamismo nos tanques das salinas permitem o desenvolvimento de elevadas densidades de determinados invertebrados bentónicos que constituem um importante recurso alimentar para as aves limícolas. Durante os períodos migratórios as aves limícolas podem explorar oportunisticamente este recurso alimentar e rapidamente armazenar reservas energéticas para prosseguir a sua viagem. A reduzida cobertura vegetal nos cômoros que delimitam os tanques das salinas permite a nidificação de algumas espécies de aves aquáticas, contudo a linearidade destas estruturas pode facilitar a detecção dos ninhos por predadores. Esta desvantagem pode contudo ser compensada pela mais-valia de os cômoros permitirem a nidificação de diferentes espécies de aves aquáticas em colónias mistas. Depois de eclodirem, as crias das aves limícolas alimentam-se de forma autónoma e acompanham os progenitores ao longo dos tanques das salinas mantendo-se restritas às águas salinas até serem voadoras. Estas crias devem possuir

adaptações fisiológicas ou comportamentais que lhes permitem lidar com a elevada salinidade do habitat.

Esta tese avaliou as vantagens e desvantagens das salinas como habitat de alimentação e de nidificação para aves aquáticas, focando em particular as acções de gestão que podem ser tomadas para incrementar o valor das salinas para as aves costeiras. No capítulo I avaliámos as potencialidades dos viveiros de salinas tradicionais como zona de alimentação complementar para aves limícolas durante o período de migração outonal. Estudámos a densidade de aves ao longo do ciclo de maré e o comportamento alimentar num subgrupo de espécies após a drenagem completa da água de um tanque experimental. A elevada densidade e biomassa de invertebrados bentónicos registadas durante o período de inundação do tanque, decresceu rapidamente após a exposição do sedimento devido à desidratação do sedimento seguida da elevada taxa de predação. Demonstrámos, pela primeira vez, que os viveiros das salinas depois de drenados podem acolher elevados números de aves limícolas em alimentação por um período temporal reduzido. O tanque suportou mais de 10% das populações de pilrito-comum *Calidris alpina*, borrelho-grande-de-coleira *Charadrius hiaticula*, pilrito-das-praias *Calidris alba* e pilrito-pequeno *Calidris minuta* que ocorreram no estuário do Tejo. Comparativamente com a taxa de predação exercida pelas aves a desidratação do sedimento foi o factor mais preponderante para o declínio da comunidade bentónica no tanque. A predominância do poliqueta *Capitella capitata* pareceu ser responsável pela atracção de um elevado número de borrelhos-grande-coleira e ainda pela sua elevada taxa de alimentação, permitindo que a espécie assegurasse rapidamente as necessidades energéticas diárias. Algumas espécies de aves limícolas conseguiram ainda garantir as suas necessidades diárias de biomassa e ganhar peso alimentando-se exclusivamente ou maioritariamente no tanque experimental. No capítulo II aproveitámos o facto das acções de gestão num refúgio de preia-mar terem sido suspensas por um período de um ano e, de termos acesso a 10 anos de censos de aves aquáticas nesse refúgio, para analisar os efeitos desta suspensão nas populações de aves aquáticas. As aves aquáticas foram agrupadas mediante o comprimento do tarso, e colocou-se a hipótese de que as aves limícolas de menor dimensão seriam mais afectadas pela falta de gestão do que as aves limícolas maiores. O efeito na densidade das aves foi estudado através do desenho experimental pré-pós-controlo-impacte. Demonstrámos que a suspensão das acções de gestão no refúgio de preia-mar, mesmo por um curto espaço de tempo, acarretaram um elevado efeito negativo para as populações de aves limícolas nomeadamente as de pequena e média dimensão, registando-se uma redução significativa durante o inverno (50% e 38%, respectivamente) e períodos migratórios. Os nossos resultados demonstraram que a densidade dos diferentes grupos de aves recuperou de forma diferenciada: as aves limícolas de maior dimensão atingiram a densidade inicial após dois anos, e as aves limícolas de dimensão média após três a seis anos (no outono e inverno, respectivamente). As aves limícolas de pequena e média dimensão (na primavera) não recuperaram a densidade inicial durante o período em estudo. No capítulo III avaliámos os benefícios e os riscos da nidificação de aves limícolas nas salinas utilizando como espécie modelo o borrelho-de-coleira-interrompida *Charadrius alexandrinus*. Estudámos o papel que as características do local de nidificação, a predação e a nidificação na proximidade de espécies reprodutoras com comportamento anti-predatório agressivo, têm no sucesso reprodutor. Os nossos resultados demostram que as salinas são importantes locais alternativos para a nidificação do borrelho-de-coleira-interrompida, pois o seu sucesso reprodutor foi semelhante ou mesmo superior ao registado em habitats naturais. Os borrelhos seleccionaram os limites dos cômoros como local para a construção dos ninhos, contudo as características destes locais não estão correlacionadas como o sucesso reprodutor. Nas salinas a predação foi o principal factor para destruição das posturas (42%), tendo sido identificados a gralha *Corvus corone* e a raposa *Vulpes vulpes* como os principais predadores. Os borrelhos evidenciaram um crescimento linear do sucesso reprodutor quando o seu período reprodutor se sobrepôs ao dos pernilongos *Himantopus himantopus*. Demonstrámos que nidificando na proximidade de espécies semicoloniais com comportamento anti-predatório agressivo o sucesso reprodutor de aves limícolas tímidas e não coloniais como o borrelho-de-coleira-interrompida aumentou significativamente. No capítulo IV pretendemos conhecer os mecanismos fisiológicos e comportamentais que as aves aquáticas utilizam como resposta à salinidade. Comparámos a taxa metabólica basal (BMR), o peso, a taxa de crescimento, a concentração de iões no plasma, o hematócrito, as proteínas de stress e as respostas comportamentais das crias de pernilongo criadas em cativeiro e com acesso exclusivo a água doce, salina ou hipersalina. Adicionalmente, comparámos as BMR e o peso seco das glândulas do sal em crias selvagens capturadas em habitats de água doce e água hipersalina. Os nossos resultados demonstraram que crias selvagens e crias criadas em cativeiro não diferiram nos valores da BMR, e o peso das glândulas do sal de crias selvagens de habitats de

água doce e água hipersalina foi semelhante. Todas as variáveis fisiológicas e morfológicas não diferiram entre as diferentes salinidades. Contudo, a taxa de movimentos da cabeça foi diferente consoante a salinidade da água, e as crias aumentaram significativamente os movimentos da cabeça com o aumento da salinidade. Os nossos resultados evidenciam o papel preponderante do ajuste comportamental para evitar o stress salino durante o crescimento das crias. No capítulo V descrevemos uma técnica de biópsia não letal para a recolha de amostras de tecido adiposo na região fúrcular de aves de pequeno e médio porte. A técnica foi avaliada em termos de tempo de processamento, da sua praticabilidade no campo e na saúde das aves. A biópsia envolveu a realização de uma incisão com 2-3mm de comprimento para a recolha de 2-16mg de tecido adiposo. As incisões demoraram 10-15 dias a sarar por completo e não evidenciaram efeitos negativos a curto prazo nos parâmetros fisiológicos. O peso dos pilritos-comuns e dos passeriformes híbridos *Carduelis carduelis* × *Serinus canaria* foi semelhante antes e após a biópsia. Recomendamos esta técnica não letal para a amostragem de tecido adiposo em alternativa aos métodos letais em estudos com aves selvagens e aves de cativeiro. No seu conjunto os resultados desta tese evidenciam a necessidade da protecção e a gestão das salinas costeiras, bem como a manutenção de uma rede de refúgios de qualidade para a protecção das aves limícolas durante o seu ciclo anual.

Palavras-chave: habitats artificiais, gestão da água, taxa de alimentação, sucesso reprodutor, stress salino, ecofisiologia, reservas de gordura.

General Introduction

Eleven years ago I first became interested in waders and since them have spent countless hours watching and studying them along the Tagus estuary and occasionally in other geographic regions. Over that period I've often asked myself "why do waders fascinate me so much" especially during the catpture sessions when I'm up to my knees in mud and freezing in the cold winter's night. The best answer I've come up with is because they are such free spirits living in some of the most beautiful places on earth; moving endlessly to feed and roost following the tide rhythms, and undertaking the longest migratory flights in the animal kingdom.

Waders and coastal habitats

Waders, also commonly named as “shorebirds”, because most of them are associated with coastal wetlands, are one of the most diverse and widespread group of birds. Waders belong to the order *Charadriiformes* which is a large and diverse group, covering 508 populations of 211 species in eleven families (Wetlands International 2002). Other than in Antarctica, waders occur on nearly every shoreline of the world, as well as in many other biotopes from the plateaus of the Andes and the African Rift Valley lakes to the estuaries and the tundra of the northern hemisphere. The majority of the species eat small invertebrates picked out from the mud or exposed substrate, and different morphologies of their bills enable several species to feed in the same habitat without direct competition for food.

Waders are known for their extraordinary long distance migrations. Many species travel more than 5,000 km in their annual circuit (Johnson *et al.* 1989, Williams and Williams 1990). Some species undertake the longest non-stop migratory flights known in the animal kingdom, frequently surpassing 10,000 km (Gill *et al.* 2009; Battley *et al.* 2012). Every spring, massive numbers of waders travel from coastal wetlands spread across the globe to breed in the Arctic during the boreal summer, and return to their wintering grounds at lower latitudes. They optimize the use of habitats that become available in summer for breeding at higher latitudes, taking advantage of a long daylight period, plentiful food resources for the chicks and fewer pathogens and parasites (Mendes *et al.* 2005; Piersma *et al.* 2005). The preparation for long distance flights involves remarkable physiological adaptations. Before migration birds store large amounts of fat, frequently over 30% of their body weight (Piersma & Van Brederode 1990). During migration some species become true flight machines, their muscles and heart increase in size and their liver, stomach and intestine, less important organs during flight, reduce (Piersma & Gill, 1998). During such migrations waders use a network of stopover sites, where birds stay for hours, days, or weeks, resting, moulting and refuelling for upcoming flights. These areas are strategically used in periods of high productivity and allow waders to gain 0.4–5.4 % of their body mass per day (Zwarts *et al.* 1990a). Some of the most important stopover sites are in coastal areas such as the Wadden Sea in Europe, Delaware Bay in North America, the Yellow Sea in Asia and the Banc d'Arguin in Africa, and these areas appear to control much of the rest of the annual cycle and survival of these waders (e.g. Ens *et al.* 1990, Piersma 1994, van de Kam *et al.* 2004). For instant, during northward migration at least two million waders of 36 species (Barter 2002) stop at the Yellow Sea, the most important staging area on the East Asian/Australasian Flyway, to feed on abundant polychaetes and bivalves (Ahn, Kang & Choi 1995; Choi *et al.* 2010).

The entire range of a migratory bird species through which it moves on an annual basis from the breeding grounds to non-breeding areas, including stopover sites as well as the area within which the birds migrate is called flyway. The migrations of waders can broadly be grouped into eight flyways: the East Atlantic flyway, the Mediterranean/Black Sea flyway, the West Asia/Africa flyway, the Central Asia/Indian sub-continent flyway, the East Asia/Australasia Flyway, and three flyways in the Americas and the Neotropics (Fig. 1). The East Atlantic flyway is one of the largest and the best studied. Approximately 15 million waders travel along this migratory route linking the northern breeding grounds between east Canada and mid-northern Siberia to the wintering areas in Western Europe

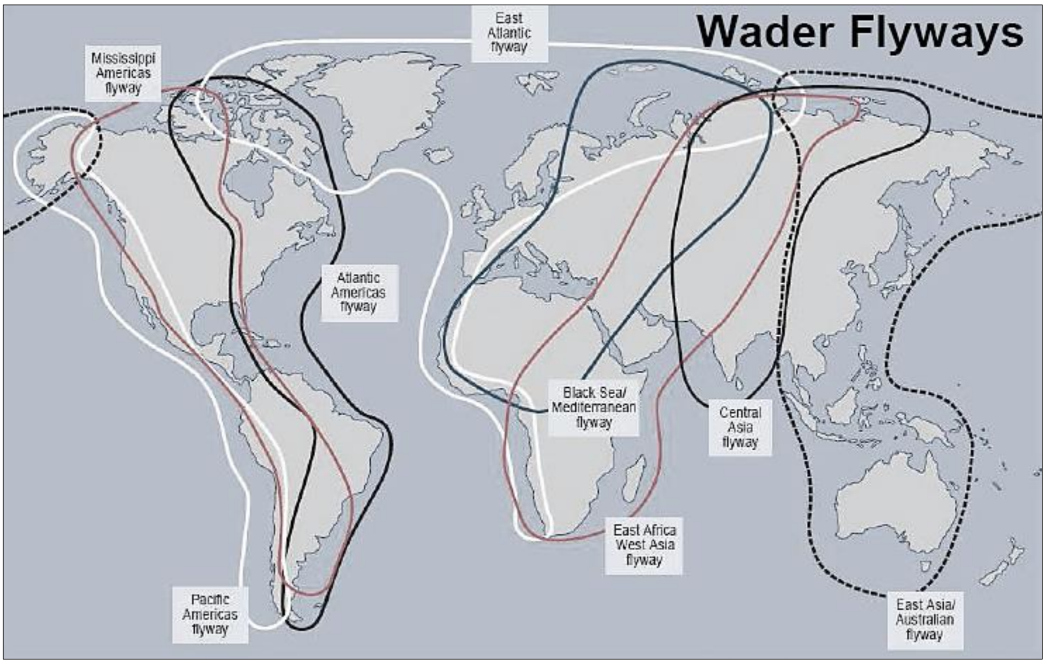

Figure 1. The eight flyways of waders. Adapted from (Boere & Stroud 2006).

and Africa (Stroud *et al.* 2004). The major wintering area for waders in the East Atlantic flyway is the Banc d’Arguin (Mauritania, West-Africa), which covers an area of over 10,000 km^2 of shallow inshore waters bordering the Sahara (Wolff *et al.* 1993). During the winter months over two million waders exploit the abundance and species richness of molluscs (Smit & Piersma 1989; Ens *et al.* 1990; Zwarts *et al.* 1990a, 1997; Hodgson 2010; Ahmedou Salem *et al.* 2014). In southwest Europe the Tagus estuary, with 320 km^2 (Fig. 2) is the second most important wetland for waders in Iberia (Delany *et al.* 2009), holding an unquantified number of migratory waders and regularly 50000 waders during the winter (Moreira 1995). The estuary justified its international importance (under Ramsar Convention) by supporting more than 1% of wintering populations of pied avocet *Recurvirostra avosetta*, black-tailed godwit *Limosa limosa*, grey plover *Pluvialis squatarola*, dunlin *Calidris alpina*, ringed plover *Charadrius hiaticula*, bar-tailed godwit *Limosa lapponica*, and of the breeding population of black-winged stilt *Himantopus himantopus* (Delany *et al.* 2009). Moreover, the rice-fields in the northern part of the estuary could hold 40% of the western European population of black-tailed godwit during the spring migration period (Lourenço *et al.* 2010).

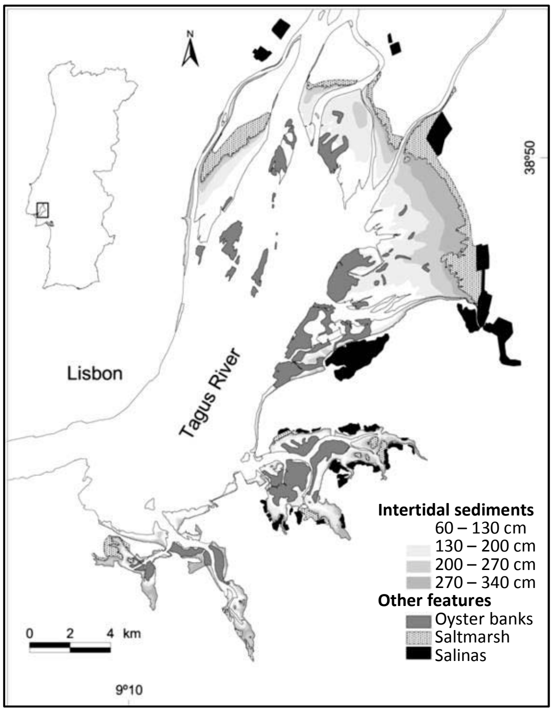

Figure 2. Main features of Tagus estuary intertidal areas and near shore areas. Intertidal sediments were classified according to the bathymetry (Santos 2009).

The Tagus estuary is characterized by extensive mudflats that cover most of the 97 km^2 of intertidal areas (Moreira 1995), and large areas of saltmarsh along the northern margin. At supratidal margins there are considerable areas of abandoned or converted saltpans. The right margin of the river is strongly humanized, particularly in the Lisbon region, but also at the southern part of the left margin. This mesotidal estuary presents primary semi-diurnal tides and an extreme range changing from 0.9 m in neap tide to 4.1 m in spring tide (Dias, Valentim & Sousa 2013).

During the non-breeding season most waders congregate to forage on coastal wetlands of temperate and tropical regions, where they live according to tidal rhythm. During low-tides, when intertidal flat areas are exposed, waders feed essentially on macrobenthic invertebrates such as polychaetes, bivalves and gastropods buried in the sediments. These feeding grounds become unavailable as the tide rises and birds are forced to find refuge in supra-tidal habitats.

On the feeding grounds, the distribution of waders is largely determined by the distribution of food, the substrate type and the distance from feeding areas to roosting sites (Zwarts & Wanink 1993; Dias *et al.* 2006).

Coastal habitats are also used by waders as breeding grounds but at much lower densities, particularly sandy beaches, coastal saltmarshes, coastal mudflats, rocky outcrops, sand spits and sandbars (e.g. Prater 1976; Davidson 1991; Milsom *et al.* 2002). Exposed sandy beaches comprise approximately three-quarters of the world's shorelines (Bascom 1980) and are important breeding areas for kentish plover *Charadrius alexandrinus* (Pienkowski 1992; Norte & Ramos 2004) in Europe, and along the Pacific coast for snowy plovers (*Charadrius nivosus*; Page and Stenzel 1981; Colwell *et al.* 2005). Other important breeding habitat is the coastal grazing marshes, wet grasslands derived from saltmarshes (Williams & Hall 1987), which support large breeding populations of lapwing *Vanellus vanellus* and redshank *Tringa totanus* (e.g. Smith 1983; Jefferson and Grice 1998; Owen *et al.* 2009).

Around the world, almost half of the wader populations are declining (48%), in contrast to only 16% of the populations that are increasing (Delany *et al.* 2009). The reasons for such widespread decline are diverse, but generally caused by loss or degradation of breeding sites, and notably loss of critical stopover and wintering habitats (Zöckler, Delany & Hagemeijer 2003; Stroud *et al.* 2006). For instance, in the Wadden Sea, the unsustainable levels of industrial shell-fishing have led to the redistribution of birds from the high quality feeding areas (Piersma *et al.* 2001; van de Kam *et al.* 2004), and declines in the biogeographical populations of long-distance migrant waders have occurred and are continuing

(Davidson 2003). Intensification of agriculture is a major driver of change to wetlands (Millennium Ecosystem Assessment 2005) and is a major adverse factor affecting the status of waders not only in western Europe, with a long past of agricultural activities, but also in other regions such as eastern Europe and central Asia (Stroud *et al.* 2006). The increased human pressure on natural coastal areas throughout the world has contributed to marked changes in the structure of coastal ecosystems, leading to strong declines in biodiversity and services provided by these ecosystems (Fraser and Keddy 2005; Assefa *et al.* 2007; Sutherland *et al.* 2012). At a global scale, anthropogenic habitats such as coastal pastures, rice fields, extensive aquaculture ponds and saltpans may play a significant role as buffer areas against the loss of natural coastal habitats for declining wader populations (e.g. Masero 2003; Zwarts 2009; Navedo *et al.* 2014). Moreover traditional saltpans could hold significantly higher abundance and diversity of overwintering waders than other man-made habitats such as aquacultures (Sripanomyom *et al.* 2011).

Coastal saltpans

Coastal solar salinas also called saltpans, salt ponds or saltworks are man-made habitats used to obtain salt by solar evaporation of sea water in a series of shallow interconnected evaporation pans varying in size, water depth and salinity. Unlike natural wetlands, the water level and salinity in coastal saltpans is stable and predictable as a result of management. They are key coastal sites for conserving waterbirds in Europe, Asia, North America, Australia and Africa (e.g. Velasquez 1992; Alcorn and Alcorn 2000; Takekawa *et al.* 2001; Masero 2003; Sripanomyom *et al.* 2011). For instance the saltpans in San Francisco Bay, support large wintering wader populations relative to other important wetlands in the USA during all seasons (Warnock & Takekawa 1995; Page, Stenzel & Kjelmyr 1999; Takekawa *et al.* 2001; Warnock *et al.* 2002). Coastal waders and other waterbirds use saltpans as feeding, roosting and breeding sites (e.g. Tinarelli and Baccetti 1989; Rufino and Neves 1991; Mcculloch and Borello 2000, Masero 2003; Dias *et al.* 2006; Takekawa *et al.* 2006; Sripanomyom *et al.* 2011).

Saltpans occur in large areas in several geographical regions (e.g. shores of

Atlantic Europe, the Black Sea and Mediterranean Sea, Namibia, South Africa, Yellow Sea, San Francisco Bay; Davis and Giordano 1995; Davis 2000), extending, for example over 100,000 ha in the Mediterranean basin, where Spain, Greece, Italy, France and Portugal hold 77% of the Mediterranean saltpans (Sadoul, Walmsley & Charpentier 1998).

The extensive coastline of Portugal exposed to hot and dry winds and summer high temperatures, has always shown favourable conditions for the development of coastal solar salinas (Gonçalves 1971). Salt production through solar evaporation of sea water in Portugal was introduced by the Phoenicians in the 9th century BC, and was intensively exploited during the Roman period (Vieira 1951). By the 17th century salinas had developed in almost all Portuguese estuaries and coastal lagoons (Neves & Rufino 1992). In the beginning of the 1930's, the Tagus estuary was responsible for 48% of the Portuguese salt production (230,000 tons), and in its left margin, the Alcochete salpans was the largest production center (30% of the national production; Lepierre 1936). After 1936, however, the salt industry faced a deep crisis in the Mediterranean region as well as in Portugal due to the development of cold conservation techniques and the appearance of new industrialized salt production processes that could provide higher salt production at lower costs (Paracuellos *et al.* 2002). The decline peaked in the 1980s due to European subsidies to aquaculture, encouraging people to exchange the seasonal salt production activity for an alternative continuous activity, thus an increasing number of salinas were abandoned and converted to fish farms or even rice fields (Vieira & Bio 2011). Nowadays, most Portuguese salinas are abandoned or with low production, mainly due to the lack of workforce and high labour costs, which increased while salt consumption and price declined. Traditional solar salinas are still found along the Portuguese coast, but from the 230 active traditional saltpans in the Tagus estuary (Neves 2005), most were destroyed/abandoned or recently converted to shrimp farms producing atlantic ditch shrimp *Palaemonetes varians* in extensive regime, and only one still produces salt, the Canto salina in the Achochete region. With the decline in the production of artisanal salt, associated environmental, cultural, historic and human values are lost.

Salina compartments

The salt extraction in artisanal solar saltpans involves three types of compartments: supply, evaporation and crystallizer ponds. The supply pond is the largest and deepest of the system (Fig. 3). It communicates with the sea through an inlet channel which supplies seawater to the system during spring tides. The evaporation ponds (B) constitute the largest evaporation surface.

They receive water from the supply pond and supply brine to the crystallizer ponds (C) where the deposition of sodium chloride takes place. Connection between ponds takes place, through openings in the dyke closed with wooden sluices.

Salt production, salinity and water regimes

The salt production in Alcochete region as in Portugal is restricted to the dry season, a period from spring to autumn. After this period, the sluices are opened during spring tides, and the saltpan is flooded except the supply pond that stays drained after fish harvesting in the end of productive period. The saltpan stays flooded until March, and during this non-productive season the salinity in the whole system approaches that of the supply water, i.e. ≤ 35 ‰, while water depths vary per compartment. In this period water depth in the supply, evaporation and crystallizer ponds is approximately few centimetres, 15 - 30 cm and 50 - 60 cm, respectively. The productive season begins with the empting of the ponds followed with specific works on the substrate of the salt ponds, which include e.g. reparation of the separation dykes (paths), and dredging ditches. In June, the

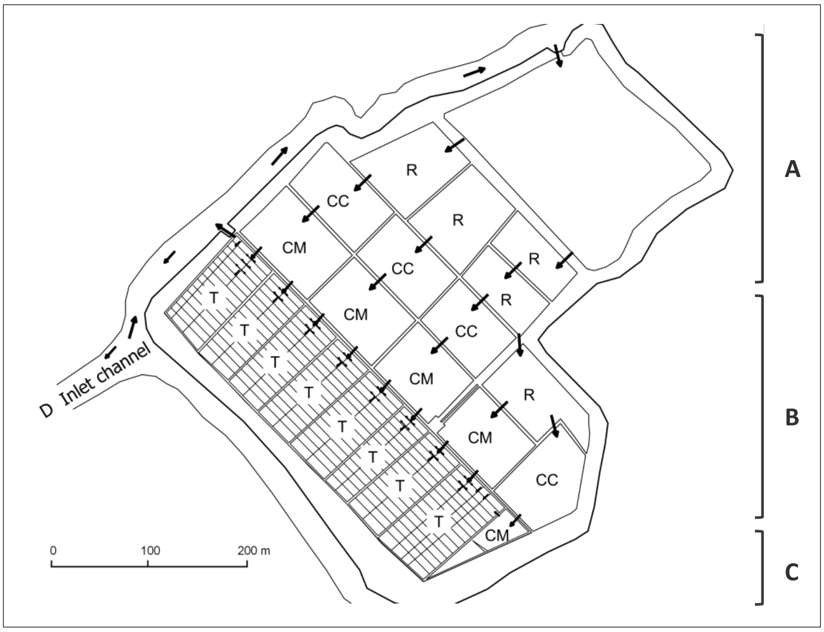

Figure 3. Scheme of Canto salina in 1952 (with local terms for the different pond types: R- reserve, CC – contra-caldeirão, CM - caldeirão de moirar, T - talhos). A: supply ponds; B: evaporation ponds; C: crystallizer ponds; D: inlet channel (the direction of the water flow in the various compartments is indicated by arrows; in the crystallizer section flows are only indicated for some ponds), adapt from Lopes (1954).

ponds are filled again. They are subsequently supplied with new sea water every 15 days, during spring tides. As water flows by gravity, the salt concentration increases by evaporation to near saturation point, and the brine is moved to the crystallization pans, which produces a salinity gradient ranging from 30 to 60‰ in the supply pond to 300‰ in the crystallizers. During the productive season, the water depth in the supply, evaporation and crystallizer ponds is about 80 - 100 cm, 10 - 15 cm, and a few centimetres, respectively.

Food supply for waterbirds

The different salinities and fluvial dynamics in salt ponds originate a particularly high benthic invertebrate prey density for waterbirds (Velasquez 1992; Masero 2003; Takekawa *et al.* 2006). The salinity acts as the major limiting factor of the benthic community (Vieira & Amat 1997; Sánchez-Guzmán *et al.* 2006), and species richness declines along the salinity gradient (Sánchez-Guzmán *et al.* 2006; Evagelopoulos, Spyrakos & Koutsoubas 2007). Along this gradient, the density and biomass of macrobenthic invertebrates are also variable between seasons mainly due to the dominance of one or more species (Evagelopoulos *et al.* 2007). In ponds with low salinity other factors can positively influence the benthic fauna such as the presence of fish (Kneib 1988), the presence of submerged plants providing a substrate for many macroinvertebrates (Wolfram *et al.* 1999) and sediment texture (Vieira & Amat 1997).

The highest density of the macrobenthic community is reached during the non-productive season in all salina's compartments (Vieira & Amat 1997). The supply ponds display the greatest diversity of organisms, biological productivity of organic matter and ecological stability of the entire system (Rodrigues *et al.* 2011), and most abundant groups are gastropods, bivalves and insects (Vieira & Amat 1997). Evaporation pans are dominated by opportunistic deposit feeders such as gastropods (*Hydrobia sp.*), polychaete worms (*Capitela sp.*) and chironomid larvae (*Chironomus sp.*), with high levels of density and biomass in autumn (Evagelopoulos *et al.* 2007, 2008). In evaporation and crystallisation ponds chironomid larvae and brine shrimp *Artemia sp.* are the most abundant benthic invertebrate prey. Chironomid larvae could be highly abundant in spring and autumn (7,023 ± 392 ind^{-1}m^{-2}; Sánchez *et al.* 2006) in traditional and industrial saltpans from south-west Spain or in winter in several Portuguese saltpans (> 5,000 ind^{-1}m^{-2}; Batty 1992; Pedro and Ramos 2009). Brine shrimp can reach very high local densities with up to 32,600 adult ind^{-1}m^{-2} during winter and spring (Masero *et al.* 1999) in saltpans from south-west Spain. Takekawa et al. (2006) found that brine shrimp is the main food supply for migratory waterbirds foraging in evaporation ponds during spring in São Francisco Bay, USA. Other important prey found during spring in Portuguese saltpans is *Ephydridae* larvae (Rufino 1989; Pedro & Ramos 2009).

In the productive season, the benthic community decreases, especially in the evaporating and the crystallizing ponds. During this period the supply pond acts as the biological reservoir for the other ponds and holds the highest zoobenthic density (5,601 ind^{-1}m^{-2}), where the dominant groups are gastropods and bivalves (Vieira & Amat 1997). Specialized and tolerant to salinity biota develops in evaporation and crystallisation ponds, such as gastropods (Vieira & Amat 1997) and mostly brine flies and brine shrimp (Rodrigues *et al.* 2011). In the crystallizers, when salinity reaches values exceeding the tolerance limits of *Artemia* and *Ephydridae* larvae, they die and stay deposited at the bottom in large quantities (Rodrigues *et al.* 2011).

Salinas as roosting and foraging grounds

The shallow water and high biological richness of saltpans attract and support important numbers of waterbirds in many regions, including Europe, Africa, North America, Asia and Australia (Velasquez 1992; Alcorn & Alcorn 2000; Masero & Pérez-Hurtado 2001; Warnock *et al.* 2002; Round 2006; Sripanomyom *et al.* 2011). For instance, in San Francisco Bay, USA, saltpans are critically important for waterbirds as stopover and wintering grounds, harbouring 64,253 to 108,171 waders year^{-1} (Brand *et al.* 2014). When tide rise birds are forced to move to high-tide roosts, usually located in fields, beaches, salt marshes or saltpans (Rehfisch *et al.* 1996; Dias *et al.* 2006b; Rosa *et al.* 2006), where they must remain until the receding tide exposes their feeding grounds again. At neap tides some elevated intertidal areas may remain exposed during high-tide and the birds can also roost there (Rosa *et al.* 2006). During the high-tide period waders congregate in dense flocks at saltpans and spend most of their time sleeping, preening or feeding, but remain vigilant. The large expanses of water in ponds facilitate taking flight and predator avoidance, while the shallow, sheltered paths likely create a favourable microclimate for roosting (Warnock & Takekawa 1996).

Roost-site selection and roost fidelity may be depend on several factors and vary among waders' species and ages (Rehfisch *et al.* 1996; Luís, Goss-Custard & Moreira 2001). One important factors is the distance between the favoured roosts and the feeding grounds, or between alternative roosts, which is directly related with energy expenditure (Rogers *et al.* 2006a; van Gils *et al.* 2006). Therefore the distance to a favoured high-tide roost can limit the access of waders to foraging areas when the flights to and from the roosts involve an energetic expenditure that is relevant for the birds (Luís *et al.* 2001; Rogers 2003). Piersma *et al.* (1993) estimated that the costs of these movements in red knot *Calidris canutus* from Dutch Wadden Sea corresponds to about 10% of the daily energy spent by the species. For instance, in the Tagus estuary, the density of wintering dunlin in foraging areas declined significantly with distance to the nearest roost, and fewer than 20% individuals forage more than 5 km away from their roosts (Dias *et al.* 2006b). Safety is other factor, which is related with the risk of predator attack during routine movements (Cresswell 1994; Rogers *et al.* 2006a), but also in the roosts and perhaps in combination with human disturbance (Nisbet 2000; Rosa *et al.* 2006). Thermal stress, induced either by cold or windy weather, leads to an increment of maintenance costs (Wiersma & Piersma 1994). Therefore, it is advantageous for waders to reuse roosts that are undisturbed rather than taking the risk of searching for new roost, and birds frequently move among a few roosts within an estuary (Rehfisch *et al.* 1996; Pearce-Higgins 2001). Based on roost stability, counts of roosting waders have been used to assess local changes in their abundance (Mitchell, Moser & Kirby 1988) and to evaluate the success of management actions.

The water depth is the key environmental factor controlling the access to the substrate where the benthic invertebrates live (Ntiamoa-Baidu *et al.* 2008), allowing waders to forage in salts ponds (Fig. 4). Unlike intertidal flats, foraging time is not restricted by tidal inundation and so invertebrate prey may be continuously available (Velasquez & Hockey 1992; Weber & Haig 1997). The presence of foraging waders in saltpans is positively correlated with the abundance of prey at accessible water depths (Sánchez, Green & Castellanos 2006c), and birds select the most abundant prey size (Davis & Smith 2001; Pedro & Ramos 2009). Bird predation could decrease prey density by 32-35% (Sánchez, Green & Alejandre 2006a) in a confined area such as a salt pond. Ponds are used by foraging birds throughout the tidal cycle, suggesting preferential foraging even when intertidal flats are exposed (Velasquez 1992; Luís, Goss-Custard & Moreira 2002; Masero 2003; Yasué & Dearden 2009). The unlimited foraging time and food availability in salinas are particularly critical for the daily food intake, and hence fuelling rates of long distance migratory species (Zwarts *et al.* 1990a; Kvist & Lindstrom 2003), which need to store large fuel loads. The foraging and roosting value of saltpans during high tide can therefore limit population size, and contribute significantly for the maintenance of a high density of foraging waders on the intertidal mudflats (Velasquez & Hockey 1992; Masero *et al.* 2000).

Salinas as breeding grounds

Coastal salinas also provide suitable breeding habitats for waterbirds worldwide. For instance in the Pacific

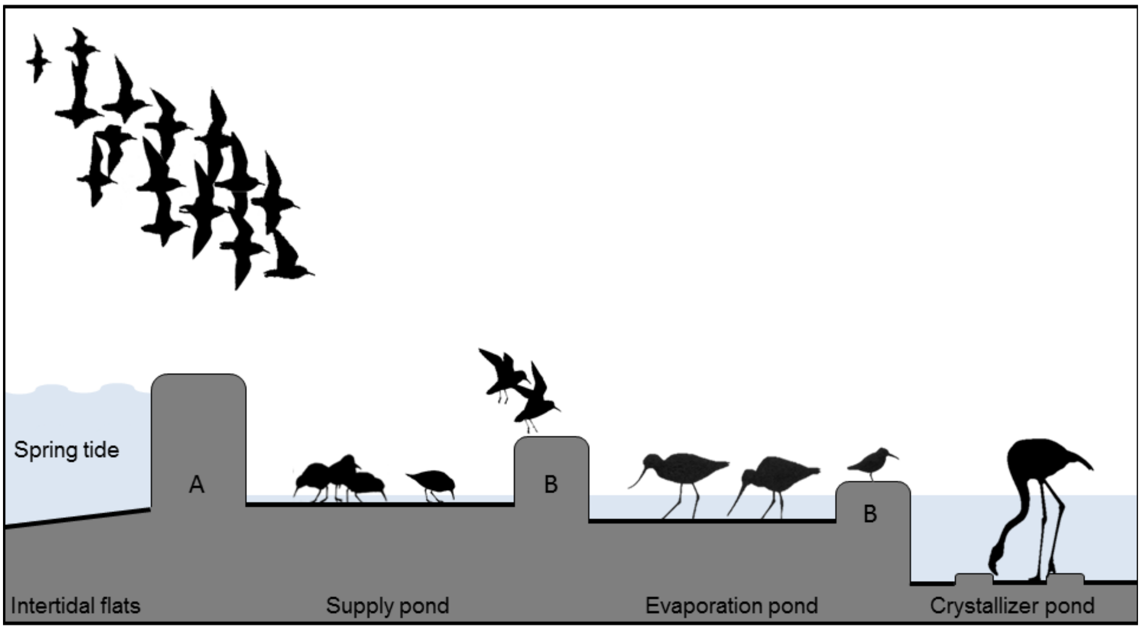

Figure 4. Representation of a salina compartments and limits (A - protection wall, B - path between ponds) during spring tide (4.1 m) outside the salt production period, showing the different water levels (2 cm in supply ponds; 10 cm in evaporation ponds and 50 cm in crystallizer ponds) and their use as foraging and roosting areas by different species of waterbirds.

coast of USA important numbers of snowy plover nest in evaporation ponds (Page & Stenzel 1981). The Makgadikgadi saltpans in Botswana, comprise the most important breeding site for lesser flamingo *Phoenicopterus minor* and greater flamingo *Phoenicopterus roseus* in Africa (Mcculloch & Borello 2000). In South Africa, the second largest colony of Caspian terns *Sterna caspia* is at the Redhouse saltpans (Martin & Randall 1986). In the Mediterranean basin, waterbirds tend to breed in artificial and semi-artificial habitats, especially in salinas, in response to excessive human pressures on their natural habitats (Britton & Johnson 1987; Neves & Rufino 1994; Masero & Pérez-Hurtado 2001). The salina of Giraud on the Mediterranean coast of France is the largest salina in Europe (12,000 ha), and is particularly important to the (Johnson & Cézilly 2007), shelduck *Tadorna tadorna* and for several species of gulls, terns and for kentish plover (Britton & Johnson 1987). In Portuguese salinas four species of waterbirds such as black-winged stilt, pied avocet, kentish plover and little tern *Sternulla albifrons* breed in appreciable numbers (Neves & Rufino 1994; Fonseca, Grade & Fonseca Luis 2005). Black-winged stilts and pied avocets are more abundant in supply ponds, where denser vegetation helps camouflage their nests (Rufino *et al.* 1984). In contrast, kentish plovers and little terns occur more frequently in semi-open areas of the evaporation ponds (Fonseca *et al.* 2005).

In saltpans waterbirds have to cope with a linear landscape formed by the path or dyke network used by workers to move around the ponds, which is significantly different from the landscape of natural coastal wetlands. The dry areas in the saltpans such as the dykes between ponds, islands, pond margins, pond floor and the salina protection wall are used by ground-nesting waterbird species to breed (Fig. 5). Additionally, birds use different ponds to nest depending on water depth (Fonseca *et al.* 2005). The proximity between nest sites and foraging areas, and also the proximity to water, are important to maintain homeostasis for nesting birds during long periods under hot conditions (Maclean 1975; Kalsi & Khera 1986), and influence nest attendance (Amat & Masero 2004). The limited unvegetated areas in salinas with reduced human presence during the breeding season promotes the formation of sparse or dense colonies of ground-nesting waterbirds (Sadoul *et al.* 1998). The increasing density of nesting birds reduces the predation risk (Brown & Brown 1987; Campobello, Sarà & Hare 2012). Moreover, the antipredator benefits for timid species nesting close to species with anti-predator behaviour results often in higher reproductive success than that of conspecifics nesting without such protector species (Campobello & Sealy 2011). For example in southern Europe black-winged stilts breed mainly in active or abandoned salinas (Rufino & Neves 1991; Tinarelli 1991), nesting colonially or semi-colonially (Cramp & Simmons 1983) and mob terrestrial and avian predators near their nesting sites (Gochfeld 1984). This aggressive antipredator behaviour may benefit more timid and solitary species such as kentish plover by nesting in the area defended by these "protective umbrella" species. Overall, several studies reported a similar nesting success of waterbirds in anthropogenic and natural coastal habitats: 1) Yasué *et al.* (2007) found no significant differences in nesting success of Malaysian plover *Charadrius peronii* breeding in sandy beaches and semi-natural saltflats in Thailand, 2) Catry *et al.* (2004) reported a similar nesting success of Little terns breeding in sandy beaches and coastal salinas in Portugal.

The kentish plover is a coastal wader species which breeds traditionally in coastal sandy beaches and inland lakes, lagoons, alkaline grasslands and seasonal watercourses (Cramp & Simmons 1983). In

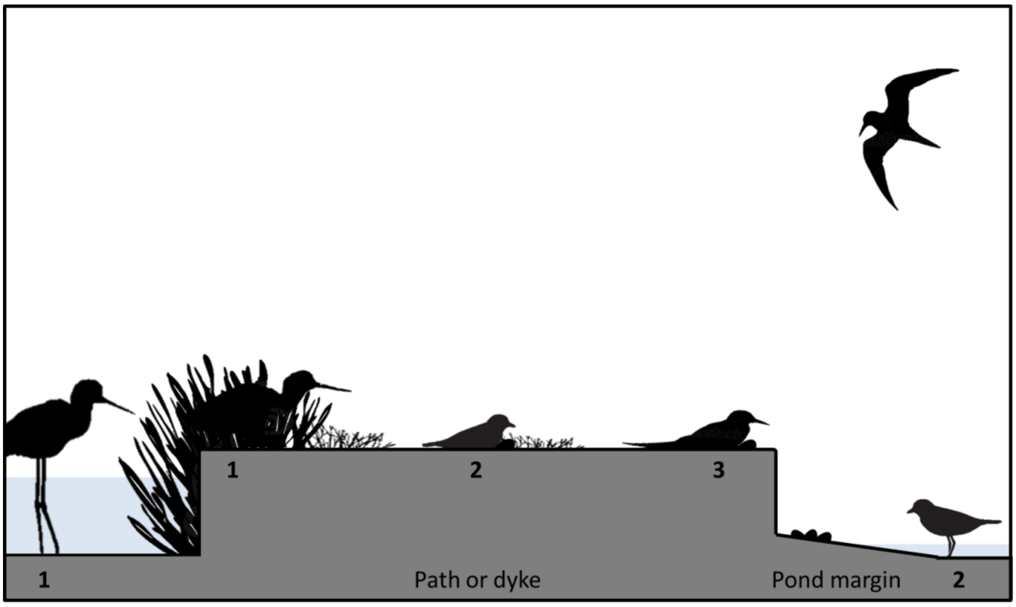

Figure 5. Representation of a salina as breeding ground for waterbirds, showing the main nesting areas: paths between ponds and pond margins. Breeding species such as black-winged stilt (1), kentish plover (2) and little tern (3) select nest sites with a decreasing vegetation cover.

Europe, the breeding populations of this plover species have declined or disappeared in many coastal regions (Schulz & Stock 1993; Domínguez & Vidal 2003), mainly due to the increase of tourism and the destruction of natural habitats (Bauer and Thielcke 1982; Lafferty *et al.* 2006; Delany *et al.* 2009). Today, large numbers of kentish plovers breed in saltpans located in Southern Europe (Rufino 1989; Amat 1993; Pardal 2000) and in other geographical regions worldwide (Page & Stenzel 1981; Yang *et al.* 2011). As in the natural habitats, kentish plover selected nest-site with sparse vegetation (Székely 1990; Page *et al.* 2009a), often nesting on substrates that are similar in colour to their plumage (Marriott 2003), and close to rocks and debris of wood resulting from the maintenance of dyke structures in saltpans. Structures can work as a shelter to predominate winds (Norte & Ramos 2004), vegetation cover near the nest can shelter the chicks after hatching (Lorenzo & González 1993), and both can also help to dissimulate the nest-site, and enable the detection of possible terrestrial predators by incubating birds (Walters 1984). Norte and Ramos (2004) carried out a literature survey and found that the nesting success of kentish plover in saltpans is similar to that of natural sandy beaches of the Portuguese west coast. The most striking aspect of their literature survey was the strong variation in breeding success among sites, irrespectively of the habitat type.

Salinas' constraints for breeding and foraging waders

When waterbirds move to alternative habitats such as salinas, different variables will affect their breeding ecology and could function as ecological traps when reproductive output is lower than in natural habitats. In fact, **predation** is one of the main causes of nest failure in saltpans (Cuervo 2005; Chokri & Selmi 2011). The linearity and limited area of paths and banks of salinas could facilitate the detection of nests by predators, and nest predation may increase with the increasing of nest density (Tinbergen, Impekoven & Franck 1967; Göransson *et al.* 1975; Page, Stenzel & Winkler 1983). Moreover, the proximity of salinas to urban areas constitutes one of the most serious threats to their waterbird communities, primarily owing to increased disturbance and predation by human-related opportunistic predators such as dogs and cats (Burger 1989). Like in natural habitats several species of mammals and birds (Fraga & Amat 1996) were observed predating eggs, or their presence was recorded close to nests in saltpans. For example, the cape grey mongoose *Galerella pulverulenta* was the main predator of Kelp gull *Larus dominicanus* eggs and chicks in saltpans from South Africa (Martin & Randall 1986). Marks from teeth of rats *Rattus spp*. were found in eggshell fragments of avocets, black-winged stilts (Cuervo 2004) and little terns (Catry *et al.* 2004) breeding in saltpans. In saltpans from Eden Landing in USA the nests of snowy plovers are predated mainly by avian predators such as northern harrier *Circus cyaneus*, comon raven *Corvus corax* and California gull *Larus californicus* (Robinson 2008). Other avian predators such as montagu's harrier *Circus pygargus*, turnstone *Arenaria interpres* and gulls were identified from footprints and eggshell fragments around failed nests of little terns (Catry *et al.* 2004). During the non-breeding period, predator disturbance seems to be a determinant factor in the choice of high-tide roost by waders. The presence of avian predators is several times higher in the saltpans than in the intertidal roosts, and saltpans are mostly used when mudflat roosts become unavailable (Rosa *et al.* 2006).

Water depth is the major limiting factor in the selection of different ponds by feeding waterbirds to roost and forage in saltpans. This limitation is directly related with bird size and more specifically to leg length. Small waders such as sandpipers forage in water less than 5cm deep, and up to 15cm in the case of large waders such as godwits (Isola *et al.* 2000; Ntiamoa-Baidu *et al.* 2008; Ma *et al.* 2009). Despite the relatively water level stability, nest flooding is common in saltpans (Pardal 2000) and results from water management during salt production or as a consequence of saltpans abandonment (Múrias *et al.* 2002). Nests located closer to the water surface in islets or pond margins may be subjected to higher flooding rates than those situated on higher dykes (Chokri & Selmi 2011).

The **human activities** in saltpans are essentially restricted to the salt production period, which encompasses the waterbirds' breeding season. The salina structure also contributes negatively to waterbirds breeding success and to their use by non-breeding birds. For instance, bird disturbance and nest stepping by man working in the ponds (Fonseca *et al.* 2005, A. Rocha, personal observations), and the height and verticality of dykes of the evaporation and crystallizer ponds can be deadly traps for unfledged chicks which fall in to these ponds (A. Rocha, personal observations). The abandonment of salt production and the corresponding lack of maintenance works promote the vegetation overgrowth in dykes and ponds, leading to habitat loss and the phasing-out as roosting and breeding grounds (Pardal 2000).

The proximity of saltpans to industrial areas may lead to a high concentration of pollutants such as mercury in the food webs of saltpans (Cotin *et al.* 2011). Relatively high concentrations of this **pollutant** were found in little terns feeding in salinas in Portugal (Tavares *et al.* 2007; Paiva *et al.* 2008), and, in Spain, where the high mercury concentrations may reach such critical values that have a negative influence on breeding success (Cotin *et al.* 2011). Nevertheless the values of mercury in salinas may be lower than those in other important man-made habitats; Tavares *et al.* (2004) found a higher concentration of this pollutant in black-winged stilt chicks reared in rice-fields than in salinas.

Water **salinity** determines the distribution of benthic invertebrates and aquatic animals, and thus influences the use of foraging ponds by waterbirds. Velasquez (1992) found that the highest foraging densities of waterbirds occurred with salinities of 25–70 and 170–220 ‰ at saltpans in South Africa. *Takekawa et al.* (2006) reported that most waterbirds forage with salinities of 81–150 ‰ in evaporation ponds at San Francisco Bay, USA. However, waterbirds may suffer adverse effects when foraging in saltpans (Hannam *et al.* 2003) due to hypersaline conditions that present energy costs for metabolism and chick growth due to salt ingestion. Birds may avoid drinking highly saline water, which causes loss of body mass by dehydration (e.g., Purdue and Haines 1977; Hannam *et al.* 2003), and also avoid roosting in highly saline waters because salts reduce the waterproofing of feathers and thus increases the costs of thermoregulation (Rubega & Robinson 1996).

Salinity

Each year, millions of waterbirds, including waders, leave the breeding grounds (freshwater habitats) and arrive suddenly in marine habitats (non-breeding grounds) and vice versa. Migratory waders have an opportunistic foraging behaviour and they can take advantage of temporary superabundance of food (Piersma 1996). This opportunistic foraging behaviour leads them to move abruptly between salinities, for example between marine water on intertidal mudflats estuaries and hypersaline water on saltpans (Masero *et al.* 2000; Masero 2002). The relatively higher food availability (López *et al.* 2010) and the scarcity of blood parasites in saltpans (Gutiérrez *et al.* 2013) may partly compensate the osmotic and ionic costs due to salt ingestion of foraging in highly saline waters. To deal with osmotic stress induced by high salinity, waders could maintain a low body mass in order to reduce maintenance costs during energy-costly activities (Gutiérrez *et al.* 2011). Despite such capacity, they avoid drinking salty water, since birds cannot maintain water balance through renal excretion alone (Willoughby & Peaker 1979) due to the poor concentrating ability of avian kidneys (Braun 1981, 1999). Instead, waders possess a supraorbital salt gland that allows them to maintain internal salt and water balance (Gutierrez et al 2013), by excreting excess sodium chloride (NaCl) ingested with food and water via the nostrils (Dosch 1997; Sabat 2000). Maintaining a functional salt-gland and excreting excess salt should impose significant physiological costs which, are energetically expensive for birds (Burger & Gochfeld 1984; Nyström & Pehrsson 1988), due to the increased size and metabolic intensity of the organs and tissues involved in salt excretion (Gutierrez *et al.* 2011). Waders may alter several physiological and behavioural parameters in order to cope with salt stress. Their basal metabolic rate (BMR) may reflect the effects and interactions of salinity with the organism itself in terms of the minimal energetic requirements. Gutierrez *et al*. (2012a) showed that BMR was significantly lower in inland adult dunlin than in coastal individuals, presumably because the diet of inland birds is low in salt content, therefore they will not need to invest so much in osmoregulation when compared with coastal birds, which feed mostly on marine invertebrates. Exposure to saline water may cause dehydration, which is reflected in an increased haematocrit level (Kinneary 1993) and also contributes to the loss of immune response (Gutierrez *et al.* 2013), which may ultimately affect individual fitness (Dosch 1997). However waders can present adaptations to cope with high salinity (Mahoney & Jehl 1985). For instance, Purdue and Haines (1977) suggested that snowy plovers at hypersaline lakes in the western USA maintain water balance by not consuming salt water and eating insects with high freshwater contents. Mahoney and Jehl, (1985) found that Wilson's phalaropes *Phalaropus tricolor* and American avocets showed a combination of behavioural and anatomical adaptations to expel adherent salt water from their prey, and thereby largely avoid the problems of salt ingestion in alkaline and hypersaline lakes.

The black-winged stilt inhabits a variety of habitats ranging from inland freshwater, to brackish, saline and hypersaline wetlands (Cramp & Simmons 1983). In southern Europe black-winged stilts breed mainly in active or abandoned salinas (Rufino & Neves 1991; Tinarelli 1991). Black-winged stilt chicks are precocial and feed by themselves in small family groups alongside their parents. Chicks are limited to hypersaline shallow waters and may use several ponds during their growing period, but most of them stay in the same salina until they fledge (Tavares *et al.* 2007). The osmotic and ionic costs induced by salinity, could be unequal in adults and chicks (Bildstein 1993). Chicks may be more susceptible to suffer the effects of salt ingestion because their organism is still developing and they are limited in foraging space. Little is known about the development and function of salt glands in chicks, which may be under-developed at the chick stage (Barnes & Nudds 1991). In addition, there is evidence that the ingestion of high salt loads affect birds by reducing chick growth rates, body mass and dehydration (Johnston & Bildstein 1990). However birds raised in high saline environments can present behavioural adaptations to cope with high salinity, i.e. by selecting and handling prey in such a way as to minimize the ingestion of salt (Sabat 2000).

Fat reserves

Rapid changes in body mass of migratory birds are known since the 19th century (Meissner 1998). Migratory birds gain weight before departure and lose weight during flight. Few species of long distance migratory birds cover the whole distance between the breeding and wintering grounds and vice versa in one flight (Gill *et al.* 2009). Birds must use some refueling sites along their route to replenish energy reserves to complete migration (Warnock & Bishop 1998; Warnock 2010). Pre-migratory body mass increase is due to both fat and protein loads (Meer &

Piersma 1994). Fat is the main source of energy used during flight because its energetic yield is 7-8 times higher than the energetic value of wet protein (Masman & Klaassen 1987). Quality and quantity of fat stored play a significant role in migratory traits such as migration speed (Alerstam & Lindström 1990), flight range (Castro & Myers 1989), fueling rate and stopover duration or diet selection (McWilliams *et al.* 2004). Adequate fat stores are also important for avian reproduction (Esler & Grand 1994) and survival (Morrison, Davidson & Wilson 2007), and the amount of fat stored may also influence predation risk (Cimprich & Moore 2006). Hence, assessing fat depots is often important in bird studies. Visual scoring of subcutaneous fat is non-destructive, relatively easy, and a quick method of assessing fat stores of birds. Sub-cutaneous fat is scored in the interclavicular depression and in the axillary region (*apteria lateralis*) where the upper part of the breast muscle (pectoralis) is visible under thin skin (Owen, Daunt & Wanless 2010). Fatty acids from fat stored can provide valuable information about the physiology, foraging ecology and toxicology of birds. Fatty acids allow the evaluation of spatial and temporal variations in diet and trophic links (Käkelä *et al.* 2005, Iverson *et al.* 2007, Williams and Buck 2010), and quantitative diet estimates from predators and prey (Budge, Iverson & Koopman 2006). Other studies used fatty acids to analyze the bioaccumulation of pesticide residues (Enderson & Berger 1968), plastic chemicals (Tanaka *et al.* 2013) and carotenoid loads, responsible for ornamental and health functions (e.g. Møller *et al.* 2000; Bortolotti *et al.* 2003). For all these reasons, the use of fatty acids from fat stored is growing in ecological and physiological studies. However sampling fatty acids from stored fat involve limited samples of bird carcasses or sacrificed birds. Lethal methods are undesired under many circumstances, particularly when large sample sizes are required, endangered species are involved, or if repeated samples are needed from the same individual. Several investigators (Käkelä *et al.* 2009; Wang, Hollmén & Iverson 2010; Owen *et al.* 2013) have sampled adipose tissue of seabird species by non-destructive biopsy, but only Owen *et al.* (2010) reported details about their biopsy method. The use of non-lethal methods for sampling fatty acids in other group of birds such as waders must firstly be tested for potential negative effects on the individuals that are sampled.

Saltpans can play an important contribution in the study of waterbirds, particularly waders. Both the high abundance of waders in saltpans and the possibility of water level management in specific ponds to attract birds, make saltpans important places to trap waders for several studies. Moreover the proximity of saltpans to urban areas can reduce the logistic cost of studying waders in this man-made habitat.

Outline of the thesis

Coastal saltpans present a high conservation value worldwide. The saltpans from Tagus estuary play an important role as feeding, roosting and breeding habitats for waders that travel along the East Atlantic flyway. My experience in monitoring waterbirds from Samouco saltpans complex, the availability of a long-term data set from breeding and foraging birds, and the access to facilities in these saltpans were preponderant for the preparation of this thesis. The overall objective of this proposal is to understand the main factors and constrains that explain the selection of foraging and breeding sites in saltpans. Our proposal made original contributions in five subjects of current wader ecological research: exploitation of food resources by migratory waders in a drawdown experiment; the effects of management abandonment of high-tide refuges on waterbird populations; breeding ecology of waders in saltpans; the effect of salinity on chick growth of black-winged stilt; and non-lethal biopsy for sampling subcutaneous adipose tissue in small and medium-sized birds. To achieve the proposed goal, the following specific objectives were pursued:

Chapter I – Evaluate the importance of supply ponds from traditional saltpans for waders during autumn migration in a drawdown experiment. Bird abundance and benthic biomass and abundance were recorded over a period of 16 days. The predation pressure by waders was assessed with an exclusion experiment. A subset of four species (dunlin, ringed plover, redshank and black-tailed godwit) were used as model species to analyse density of foraging birds using the salina, and their feeding behaviour as the substrate dried out and the availability of food resources for waders decreased.

Chapter II – Evaluate the effects of management suspension in saltpans used as a high-tide roost by waterbirds, using 10 year of bird counts. The abundance of four waterbirds groups (according to their exposed leg length) were investigated using a before-after-control-impact (BACI) type design during winter, migratory periods and summer. We evaluated the impact a one-year management suspension in bird abundance during the subsequent six years.

Chapter III – Evaluate the nesting success of kentish plover in saltpans over an eight years period. Factors that could influence their nesting success in saltpans were evaluated: nest-site characteristics, predation and the benefits provided by breeding close to species with anti-predator behaviour. We evaluated whether the aggressive antipredator behaviour of breeding black-winged stilts benefit kentish plover nesting success using an artificial egg experiment, and identified nocturnal and diurnal nest predators using movement triggered cameras.

Chapter IV – Evaluate whether salt ingestion can affect the development of black-winged stilt chicks inhabiting saltpans by measuring physiological (chick growth, BMR, concentration of Na^+ and Cl^- ions in plasma and haematocrit) and behavioural (the ability to remove adherent salt water from prey and bill before and after ingestion by head-shaking) variables. These parameters were measured in chicks kept in experimental cages with three different water treatments (0 ‰, 20 ‰, and 60 ‰) during three weeks.

Chapter V – Development of a method to obtain samples of adipose tissue from the furcular region of small and medium-sized birds using captive hybrid songbirds and dunlins. The biopsy method was evaluated in terms of processing time, feasibility of use in the field, and effects on several physiological indices of bird health (body mass, visible fat deposition, wound healing, hematocrit levels, total white blood cell counts, and heterophil: lymphocyte ratios) over 16 days.

Chapter I

Managing ponds of salinas to increase their value as foraging sites for migratory shorebirds

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Abstract

Natural intertidal areas are being lost at alarming rates, which is a threat to estuarine biodiversity. Some anthropogenic supratidal habitats adjacent to intertidal areas may provide foraging grounds for shorebirds and other waterbirds which rely on these intertidal areas for feeding. Coastal saltpans support important numbers of foraging shorebirds worldwide. The low salinity ponds of these saltpans may be used for artisanal fishing, so they are drained regularly to harvest fish, at which time shorebirds may feed opportunistically on the drained ponds. However, detailed information about the drivers of foraging use of these ponds by migrating shorebirds is very scarce. Here, we experimentally drained a supply pond in coastal saltpans of the Tagus estuary, a key stopover site for thousands of shorebirds using the East Atlantic Flyway. We assessed the temporal variation of the food supply and foraging behaviour of shorebirds in the experimental pond, and performed an exclosure experiment to know the impact of shorebird predation in this food supply. The experimental pond (9.1 ha) provided a suitable foraging ground for a large number of migrant shorebirds during 6 - 12 days. We recorded a maximum of 16 shorebird species at high tide, from which dunlins *Calidris alpina*, ringed plovers *Charadrius hiaticula*, little stints *Calidris minuta*, redshanks *Tringa totanus* and sanderlings *Calidris alba* were the most abundant during high and low-tide periods. Total shorebird density rapidly increased after draining to a maximum of 156 birds·ha^{-1}. The abandonment of the experimental pond was directly related to the reduction of food supply due to other factors than shorebird predation. The benthic invertebrate community on the experimental pond was dominated by the polychaete worm *Capitella capitata*. The high density of polychaetes (2,215 ± 329.9 ind·m^{-2}) seems to explain the large percentage (> 50% of the total population using the saltpans) of feeding birds in the pond at both low and high tide periods. Although the food supply decreased over time, shorebirds maintained the same intake rate by increasing searching effort. Dunlins, ringed plovers and redshanks could meet their daily energy requirements and gain mass foraging only or mostly on the pond. Our results show that supply ponds after dewatering might play a significant role as foraging grounds for migratory shorebirds during stopover periods.

Key words: saltpans, shorebirds, benthic invertebrates, foraging behaviour, gross intake rate.

Introduction

Estuaries support large numbers of shorebirds worldwide during the winter and the migratory periods (van de Kam *et al.* 2004). Here, they rely on intertidal flats to forage during low tide and move to supratidal areas to roost as the tide rises. Although intertidal habitats are among the most productive habitats, they are also among the most threatened, with degradation by human development and climate change occurring at record rates (Hoegh-Guldberg & Bruno 2010). The reduction of intertidal foraging areas tends to increase the density of shorebirds on the remaining areas, where they can deplete prey, are more prone to interfere with other birds and are more vulnerable to predators (Durell *et al.* 2000; Gill, Norris & Sutherland 2001; Rosa *et al.* 2006). Globally, many populations of long-distance migratory shorebirds are declining (e.g. Hua *et al.* 2015, Simmons *et al.* 2015), and the loss of intertidal habitats is a key factor in explaining such decline (e.g. IWSG 2003).

Some anthropogenic supratidal habitats adjacent to the intertidal areas can provide foraging habitats for shorebirds and thus contribute to buffer the loss of their natural intertidal habitats (Masero 2003; Dias *et al.* 2014; Navedo *et al.* 2015). Coastal saltpans are anthropogenic supratidal habitats exploited for sea salt. A large number of coastal saltpans lie in strategic positions along the flyways of migratory waterbirds, and they support important numbers of foraging shorebirds and other waterbird groups in Europe, Africa, America, Asia and Australia (e.g. Velasquez 1992; Alcorn and Alcorn 2000; Takekawa *et al.* 2001; Sripanomyom *et al.* 2011; Catry *et al.* 2011). Unlike intertidal mudflats, foraging time in these supratidal environments is not restricted by tidal inundation and they contain predictable and abundant food resources for shorebirds (e.g. Britton and Johnson 1987, Masero *et al.* 1999). In fact, some small-sized shorebirds may prefer to continue foraging in the supratidal ponds even at low tide instead of flying to adjacent intertidal mudflats to forage on the exposed intertidal flats (Luís *et al.* 2002; Masero 2003; Yasué & Dearden 2009; Dias, Granadeiro & Palmeirim 2009).

In this multi-pond ecosystem, artisanal fishing is an activity that has been developed in low salinity ponds, such as supply ponds (Medeiros Rocha *et al.* 2012). These ponds are drained regularly to facilitate fish harvest, at which time shorebirds may feed opportunistically in the benthic macroinvertebrates of harvested ponds. Long-distance migrating shorebirds must deal with the constraints of a narrow window of time to replenish fat and protein reserves on their stopover sites (Drent *et al.* 2003), so opportunistic feeding in harvested ponds at high tide (i.e. when the intertidal foraging grounds are unavailable) may contribute significantly to meet their high energy demands. In fact, in semi-traditional aquaculture farms, the regular drainage of ponds has been proposed as a measurement to provide supplementary habitat for shorebirds (Green *et al.* 2015). However, detailed information about the drivers of shorebird foraging in harvested ponds of coastal saltpans is limited.

The Tagus estuary is a key stopover area for thousands of birds within the East Atlantic Flyway (Delany *et al.* 2009; Catry *et al.* 2011), representing an important link between north western Europe and the wintering areas of the western coast of Africa. In the Tagus estuary, coastal saltpans support large numbers of these migratory birds. We experimentally drained a pond in the most important coastal saltpans of the Tagus estuary to determine the temporal pattern of shorebird and food density after drainage, as well as the potential influence of environmental factors such as tide in the use of harvested ponds by foraging shorebirds. We also assessed the impact of shorebird predation on benthic invertebrates after draining the pond, and their foraging behaviour on the drained pond. The results of this study will contribute to the environmental management of saltpans for shorebirds worldwide.

Methods

Study area

The study was carried out in a supply pond (9.1 ha) from Samouco saltpans complex (SW Portugal, 38°44'N, 8°59'W; Fig. 6), in the southern margin of the Tagus estuary. The pond is contiguous to the estuary and it has been used for artisanal fishing during the last decades. The Tagus estuary is the second most important wetland for shorebirds in Iberia (Delany *et al.* 2009), holding regularly c. 50,000 overwintering birds (Moreira 1995). Samouco saltpans are managed for waterbird conservation and are included in the special protection area of the Tagus estuary. This saltpans complex is the main high-tide roost for shorebirds using the estuary during the migratory periods, when 60–80% of all shorebirds passing through the Tagus estuary stay in this area (10,000 birds on average; Catry *et al.* 2011). This experiment was undertaken in September 2012, coinciding with the peak autumn migration for shorebirds.

Water management

We drained the supply experimental pond on 8th September 2012. We opened the sluice gate to allow the water flowing out by gravity during low tides, following the procedure used by the artisanal fishers. The experimental pond had remained flooded during the previous 7 months, which avoided the depredation of benthic invertebrates by shorebirds. Water depth (cm; measured with a ruler) and salinity (‰; measured with a salinometer HANNA HI 98402) were recorded regularly in 6 fixed stations, starting one week before dewatering. The water depth and salinity before draining were 49.4 ± 1.4 cm and 57.5 ± 0.7 ‰, respectively. After opening the sluice gate, the water level decreased rapidly, reaching 16.5 ± 1.5 cm after three days, and the first shorebirds started to forage in the drained pond (marking day 0 of the experiment). Six days after opening the sluice gate the water level decreased to 1.7 ± 0.9 cm and salinity was 70.6 ± 0.9 ‰, and finally, nine days after starting the drainage, the water level and salinity were 0.4 ± 0.3 cm and 78.0 ± 2.3 ‰, respectively. Thereafter the mud from the

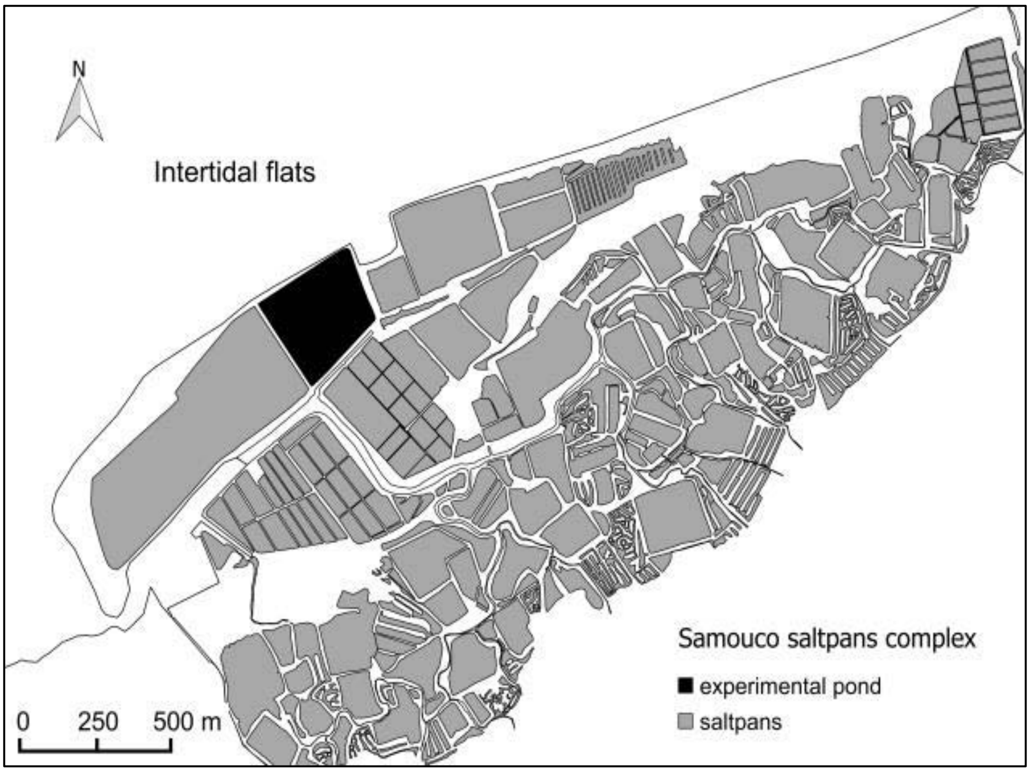

Figure 6. Study area in Samouco saltpans complex and the experimental supply pond (black).

pond bottom dried quickly. No water flowed into the pond during the experiment. After 15 days of sediment exposition, the number of shorebirds foraging in the pond was residual, the experiment was ended, and the pond flooded again.

Bird counts

Bird numbers were assessed daily from day 0 until day 15 at both the high and low tide periods. The counts were made every 30 minutes within two hours either side of high and low tide peak (i.e. four counts per tide period). A total of 128 counts were carried out. During each count we recorded the number of individuals of each shorebird species as well as their activity (feeding or non-feeding). For each tide period and day, the mean number of birds was first calculated by averaging the four counts and then this was converted to densities assuming that all area of the drained pond was available for foraging. Counts were performed by two observers during daylight inside a hide erected at the pond protection wall with the aid of binoculars (10×50) and zoom telescope (20-60×). Lastly, we calculated the proportion of foraging individuals in the drained pond in relation to the total population of shorebirds using the Tagus estuary during September (30,972 shorebirds; unpublished data).

Foraging behaviour, diet and gross intake rate

A subset of four shorebird species, dunlin *Calidris alpina*, ringed plover *Charadrius hiaticula*, redshank *Tringa totanus* and black-tailed godwit *Limosa limosa*, which comprised 95 % of the density of foraging birds (see Results), was used to analyse the foraging behaviour of shorebirds in the drained pond. These species use different foraging techniques (tactile foragers: black-tailed godwit and dunlin; visual foragers: redshank and ringed plover; e.g. Goss-Custard 1977, Moreira 1994, Nebel *et al.* 2005, Dwyer *et al.* 2013), and they also differ in the leg length (redshanks and godwits: 49 - 72 mm; dunlins and ringed plovers: 25 – 26 mm; Ntiamoa-Baidu *et al.* 2008). We collected data on bird foraging behaviour daily throughout the experiment at both high and low tide, using a hide. A total of 401 individuals (44 black-tailed godwits, 136 dunlins, 76 redshanks and 145 ringed plovers) actively foraging on the drained pond were randomly selected and videorecorded (range: 31.5 - 70.7 sec) with a digital camcorder (Sony HDR-CX115) equipped with an optimal zoom

(25×) and plugged to a telescope (20×). Sequences < 30 sec were excluded. To minimise the risk of pseudo-replication (Hurlbert 1984), each new bird filmed was at least 10–20 m from the previous one. Foraging sequences were examined in slow-motion using the software Kinovea 0.8.15. In addition we quantified the number of steps and number of pecks per unit time. Stitching behaviour was considered as a single peck when the bill was extracted from the sediment. Sediment penetrability affects the peck depth of tactile species (e.g. Mouritsen and Jensen 1992), thus we assessed sediment penetrability over the experiment according to the penetration of the bill into the sediment. Two different types of pecks were considered for tactile species: superficial pecks, when only the tip of the bill (dunlin: < 6 mm; black-tailed godwit: < 13 mm) was inserted into the sediment, and deep probes, when more than the bill tip was inserted into the sediment.

We also assessed diet and estimated intake rates from video sequences. The detailed analyses of these video recordings allowed the distinction of nine main prey items: the bivalves *Abra tenuis* and *Cerastoderma glaucum*, the gastropods *Hydrobia ulvae* and *Haminoea hydatis*, the crustacean *Palaemonetes varians*, insect larvae *Chironomus* spp., and the polychaete worms *Capitella capitata, Polydora* spp. and *Polychaeta* spp. We assumed that a peck was successful when we observed either a prey being ingested or swallowing movements. We also assumed that all small prey items classified in video recordings as unidentified prey (11.6 % of the total) were probably mudsnails *H. ulvae*. This is supported by the fact that mudsnails were the most abundant small prey in the sediment pond (see Results), and their capture is done exclusively with superficial pecks (Martins *et al.* 2013). The size of each consumed prey item was estimated in comparison to the birds´ culmen length, when items were held by the bill immediately before ingestion, split in eight size classes: prey larger than culmen, prey with equal length to culmen, prey length as a culmen fraction ($^3/_4$, $^2/_3$, $^1/_2$, $^1/_3$, $^1/_5$) and prey smaller than $^1/_5$ of culmen). Prey lengths larger than culmen or smaller than $^1/_5$ of culmen were estimated using the mean size of prey in the sediment (see below). Culmen lengths used for these estimates were 15, 33, 49, 67 and 88 mm for ringed plover, dunlin, redshank, black-winged stilt and black-tailed godwits, respectively (biometric data from birds captured in the study area during August - September, A. Rocha, unpublished data). The biomass of each prey item (expressed in ash free dry weight, AFDW) was estimated by converting their body size in biomass according to published equations relating body size and AFDM (Table 1). Due the lack of equations for *Polydora spp.* (the second most abundant polychaete worm) and *P. varians*, we used equations of related species (*Hediste diversicolor* and *Crangon crangon*, respectively). For the species *A. tenuis* and *H. hydatis* we used equations of species from the same *genus*, *Abra sp*. and *Haminoea orbygniana*, respectively.

Food availability and exclosure experiment

The abundance of benthic invertebrates was determined by taking 48 randomly sediment core samples. We assessed the abundance of benthic invertebrates 6 days before opening the sluice gate, i.e. the pond was still flooded, and every three days since until the end of the experiment. Invertebrate sampling after the sluice gate of the pond was opened was made outside the bird counting periods to minimise disturbance. Each corer (82 cm^2) was inserted into the sediment 5 cm (maximum depth used by foraging shorebirds in this benthic substrate; Pedro and Ramos 2009).

A shorebird exclosure experiment was performed to test their impact on shorebird predation on the benthic invertebrates. We selected six random sites, and at each site we sampled invertebrates in an exclosure cage and an

Table 1. Relationship used to predict prey biomass from prey size. DW: dry weight (mg); AFDW: ash free dry weight (mg); TL: total length (mm); APL: antero-posterior length (mm).

Species	Equations	Source
Hediste diversicolor	$AFDW=10^{(2.53LOG(TL)-5.94)}x0.771x1000$	Moreira 1996
Hydrobia ulvae	$AFDW=0.0154TL^{2.61}$	Santos et al. 2005
Abra sp.	$AFDM = 0.013APL^{2.23}$	Santos et al. 2005
Capitella capitata	$DW = 0.025556TL^{2.647}$	Méndez et al. 1997
Chironomids larvae	$DW = 0.0012TL^{2.787}$	Drake and Arias 1995
Haminoea orbygniana	$AFDW = 0.00006TL^{2.5246}x1000$	Malaquias and Sprung 2005
Crangon crangon	$AFDW = 0.2((TL+1.1295)/4.7906)^{3.0725}$	Viegas et al. 2007

control open area within 2m of each exclosure cage. Each exclosure (2 m × 2 m, 0.5 m high) was made using 2 mm plastic mesh and four wooden poles. We assessed the abundance of benthic invertebrates in the exclosure and adjacent open areas, as described above. In each sampling period we took a core from each exclosure and open area, i.e. a total of 12 sediment cores. The sediment samples were sieved (mesh 0.5 mm) and the invertebrates were fixed in 70% ethanol, and stained with Rose Bengal. In the laboratory, benthic invertebrate species were identified to the lowest taxonomic level possible using a stereomicroscope and a microscope.

We used a stereomicroscope fitted with a graduated eyepiece to measure body length (mm) of bivalves (shell length), gastropods (between the tip of the apex and the edge of the bottom lip) and chironomids larvae (from head to anus). In the case of polychaete worms, the field sampling procedures typically resulted in a large proportion of incomplete individuals for which body length could not be determined directly (Warwick & Price 1975). Therefore, the body length of *C. capitata* and *H. diversicolor* was assessed using the length of the thoracic region (Warren 1976) and the mandible length (Moreira 1994), respectively. We considered that all invertebrates present in the core samples were available to all shorebird species, with the exception of bivalves larger than 20 mm that were excluded (Moreira 1994). Prey biomass (expressed in AFDW·m^{-2}) was calculated as the difference between dry mass and ash mass.

Statistical analyses

For bird density, foraging behaviour, diet and gross intake rate analyses, data were grouped into five time periods: 0 (7 days before opening the sluice gate), 1 (0-3 days after opening the sluice gate), 2 (4-7 days after opening the sluice gate), 3 (8-11 days after opening the sluice gate) and 4 (12-15 days after opening the sluice gate). The effects of time elapsed after opening the sluice gate and environmental variables on bird density and foraging behaviour were analyzed using general linear models (ANOVA and ANCOVA), with exposure period (four levels: 1-4), tide (two levels: high and low-tide), and the interaction exposure period × tide as fixed factors. The analysis of bird density in the drained pond included tide amplitude as a covariate, since this variable may influence the number of shorebirds using supratidal areas at high tide (e.g. Navedo *et al.* 2010). Tukey's test was used in post-hoc comparisons. Shorebird density and foraging behaviour data were log-transformed to meet assumptions of normality and homoscedasticity.

The effects of the sediment exposure and bird predation on the density and biomass of benthic invertebrate were analyzed using two general linear mixed models (GLMM), with density and biomass as the dependent variables, using treatment (two levels: open areas and exclosure areas), exposure period (five levels: 0, 1, 2, 3 and 4) and the interaction treatment x exposure period as fixed factors, and sampling site as random factor.

The effects of the time elapsed after opening the sluice gate on the proportion of foraging birds and on the food supply (invertebrate biomass and density) were evaluated with Mann-Whitney and Kruskal-Wallis tests, because the data failed the assumptions of normality and homoscedasticity even after transformation. Data are presented as means ± S.E. All analyses were conducted using STATISTICA version 10.

Results

Shorebird density in the drained pond

After drainage, the pond was used by a large number of shorebirds and other waterbirds, particularly during the high-tide period (Appendix 1). Total shorebird density at high tide increased rapidly from 3.4 birds·ha^{-1} on day 0 to a maximum of 156.0 birds·ha^{-1} on day 6 (Fig. 7), while at

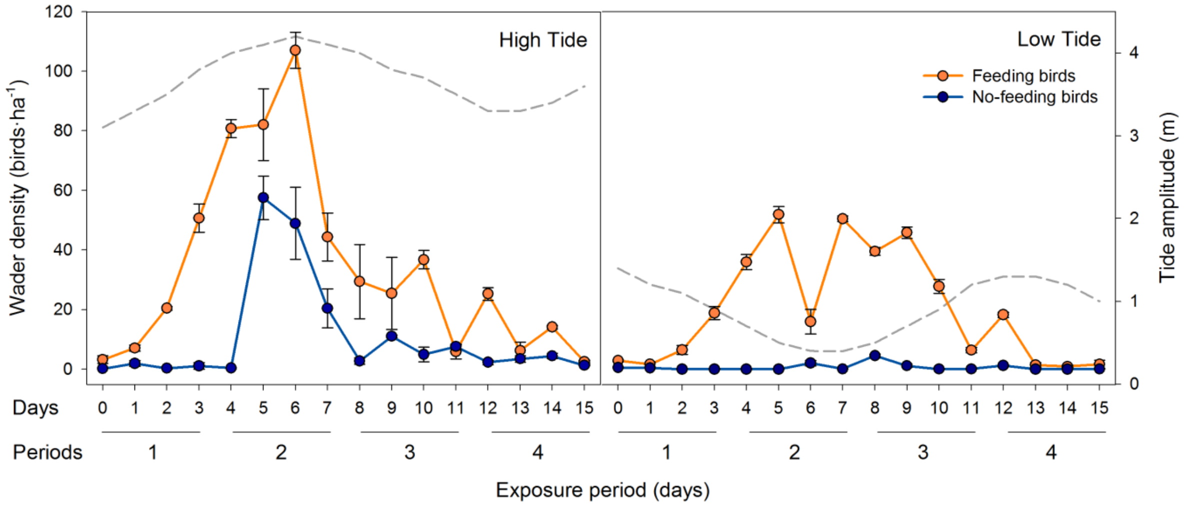

Figure 7. Number of feeding and no-feeding shorebirds (mean ± SE) per ha of the supply pond surface, recorded daily during high (left) and low-tide (right) periods, over the 16 days that the sediment was exposed. Days were grouped in four periods for statistical analysis. Tide amplitude is represented in a grey dashed line.

low tide the shorebird density increased from 3.4 birds·ha^{-1} on day 0 to a maximum of 52.0 birds·ha^{-1} on day 5. At one point the drained pond supported 6% of the total number of shorebirds in the Tagus estuary.

We recorded a total of 16 and 13 shorebird species at high-tide and low-tide, respectively. Dunlins (47%), ringed plovers (33%), little stints (7%), redshanks (4 %) and sanderlings (4 %) were the most abundant shorebirds at high tide. At low-tide the most abundant shorebird species were ringed plovers (37%), dunlins (26%), little stints (25%), sanderlings (6%) and redshanks (3%).

All shorebird species detected on the experimental pond were foraging birds except the oystercatcher *Haematopus ostralegus*. When the data for both tides were pooled together, the ANCOVA analysis showed that the density of feeding birds was not significant different (p > 0.05) between high (22.9 ± 11.8) and

Table 2. The most common feeding shorebirds species in the experimental pond in relation to the mean number of feeding birds at high tide (mean percentage ± SE). The mean density (birds·ha^{-1}) of feeding birds at high tide is indicated in parentheses. HT = high tide, LT = low tide periods. The effect of period on the proportion of feeding birds was tested with Kruskal-Wallis test (ns: non-significant; ***p < 0.001, **p < 0.01), followed by a multiple comparisons test (superscript with identical letters indicates significant difference at p < 0.05).

Species	Tide	Period 1	Period 2	Period 3	Period 4	H
Calidris alpina	HT	85.7 ± 3.8^{a}	63.5 ± 6.6	72.8 ± 7.1	55.8 ± 7.8^{a}	12.2**
	LT	56.2 ± 10^{a}	21.7 ± 3.6^{a,b}	78.5 ± 6.0^{b}	53.9 ± 14.2	22.2***
		(5.4)	(67.8)	(7.9)	(1.9)	
Charadrius hiaticula	HT	99.0 ± 1.0^{a}	90.1 ± 4.1^{b}	64.7 ± 13.1^{a,b}	76.8 ± 7.8^{a}	26.9***
	LT	24.0 ± 7.6^{a}	57.2 ± 13.6^{a}	69.6 ± 16.5^{a,b}	32.1 ± 12.3^{b}	20.1***
		(9.3)	(26.1)	(14.5)	(8.3)	
Calidris minuta	HT	94.4 ± 5.5^{a}	96.5 ± 2.5^{b}	73.1 ± 11.8	71.4 ± 11.9^{a,b}	17.2***
	LT	37.4 ± 12.8^{a}	79.0 ± 8.5	95.0 ± 3.5^{a}	58.7 ± 16.0	13.4**
		(1.7)	(5.9)	(3.7)	(2.7)	
Tringa totanus	HT	87.5 ± 7.7	97.8 ± 2.2	95.0 ± 5.0	-	2.4sn
	LT	57.35 ± 9.8	28.6 ± 3.2^{a}	79.2 ± 13.2^{a}	-	9.6**
		(2.7)	(4.5)	(0.3)	(0.4)	
Calidris alba	HT	100.0 ± 0.0	97.6 ± 2.4	74.7 ± 23.5	80.9 ± 11.1	9.8ns
	LT	-	86.0 ± 8.5	69.84 ± 18.3	77.67 ± 16.0	2.3ns
		(2.0)	(1.7)	(3.2)	(0.9)	
Limosa limosa	HT	98.1 ± 1.8	99.2 ± 0.8	-	-	0.0ns
	LT	71.6 ± 3.4	66.4 ± 20.2	-	-	0.1ns
		(0.5)	(1.8)	(0.1)	(0.5)	

low-tide (31.6 ± 12.0) periods. The densities of feeding birds in periods 2 (58.6 ± 3.0) and 3 (26.9 ± 3.1) were significantly higher than that in periods 1 (14.5 ± 3.1) and 4 (9.0 ± 3.1; $F_{3,113} = 35.22$, $p < 0.001$). There also was a significant interaction effect for period x tide ($F_{3,113} = 3.76$, $p < 0.05$). The proportion of feeding shorebirds was significantly higher during high-tide (83 ± 4.0 %) than low-tide (58 ± 5.4 %; $U = 42$, $p = 0.001$, $n_1=n_2=16$). However, for period 3, the proportion of feeding dunlins, ringed plovers and little stints was significantly higher during low tide than during high tide (Table 2). Foraging birds used the experimental pond differently according to body/leg-length size (Fig. 8). Small leg-length size shorebirds such as dunlin and ringed plover foraged at significantly higher densities during periods 2-3 than periods 1 and 4 ($F_{3,113} = 53.58$, $p < 0.001$; $F_{3,113} = 26.04$, $p < 0.001$, respectively). The greater mean bird feeding density for both high-tide and low-tide were observed during period 2, with 25.8 (± 3.5) dunlins ha^{-1} and 18.4 (± 1.3) ringed plovers ha^{-1}. We found a significant interaction in period x tide on dunlin feeding density ($F_{3,113} = 4.32$, $p < 0.01$) and on ringed plover feeding density ($F_{3,113} = 2.83$, $p < 0.05$). None of the interactions were significant for feeding

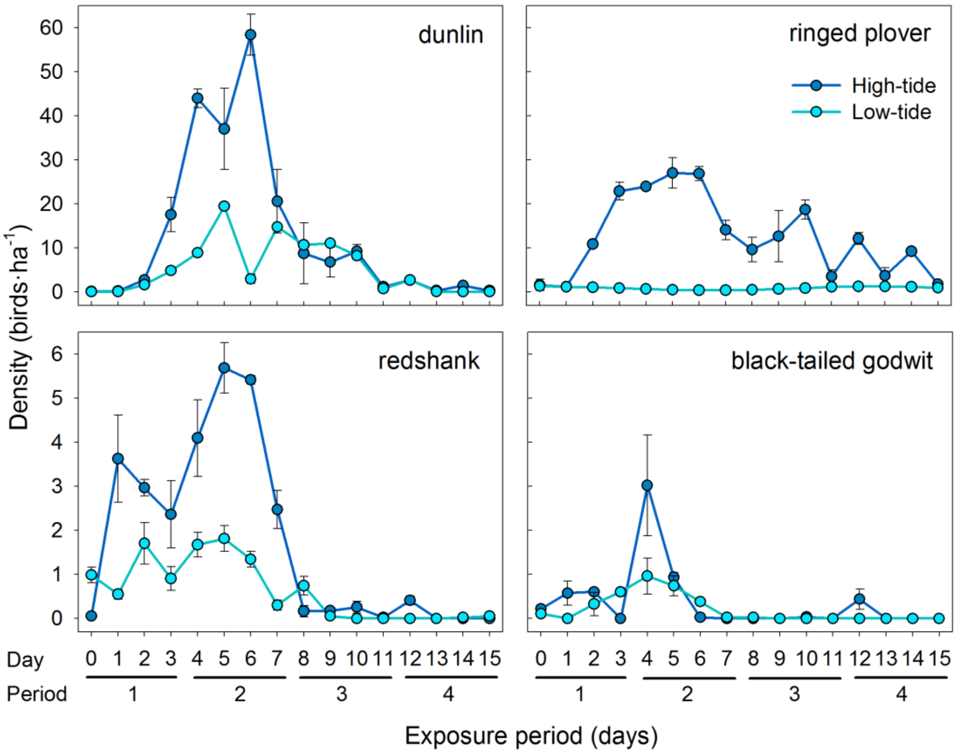

Figure 8. Density (mean ± SE) of four feeding migratory shorebirds recorded daily during high and low-tide periods, over the 16 days that the sediment from the supply pond was exposed. Days were grouped in four periods (1-4) for statistical purposes.

dunlins: density × tide amplitude and density × tide, nor for feeding ringed plovers: density × tide and tide × amplitude.

Medium leg-length size shorebirds such as redshank and black-tailed godwit fed preferably during the first two periods ($F_{3,113} = 77.49$, $p < 0.001$; $F_{3,113} = 15.01$, $p < 0.001$, respectively). The higher density for both high and low-tide were observed during period 2 with 3.8 (± 0.4) redshanks ha^{-1} and 0.8 (± 0.2) godwits ha^{-1}. The interaction terms density x tide amplitude and density x tide had no influence on the density of redshank. Tide amplitude had no effect on the density of feeding black-tailed godwits, however such density was significantly higher during the high-tide period (0.38 ± 0.12; $F_{1,113} = 4.04$, $p < 0.05$). There was a significant interaction in period × tide on the density of feeding redshank ($F_{3,113} = 4.95$, $p < 0.01$) but not on the density of feeding godwits.

Prey availability

Before opening the sluice gate the mean density of benthic invertebrates was 6,889.1 ± 916.7 individuals per m^{2} of sediment (range: 367-18,969 ind · m^{-2}; Appendix 2), and the most common benthic invertebrates were *C. capitata* worms (70%) and chironomid larva (14%). We found a significantly decrease in invertebrate density from periods 0 - 1 to period 4 ($F_{4,84} = 4.63$, $p < 0.01$), when density was reduced to 3,233 ind · m^{-2}. The mean biomass before dewater was 2.7 ± 0.3 g of AFDM·m^{-2} (range: 0.7 - 5.2 g of

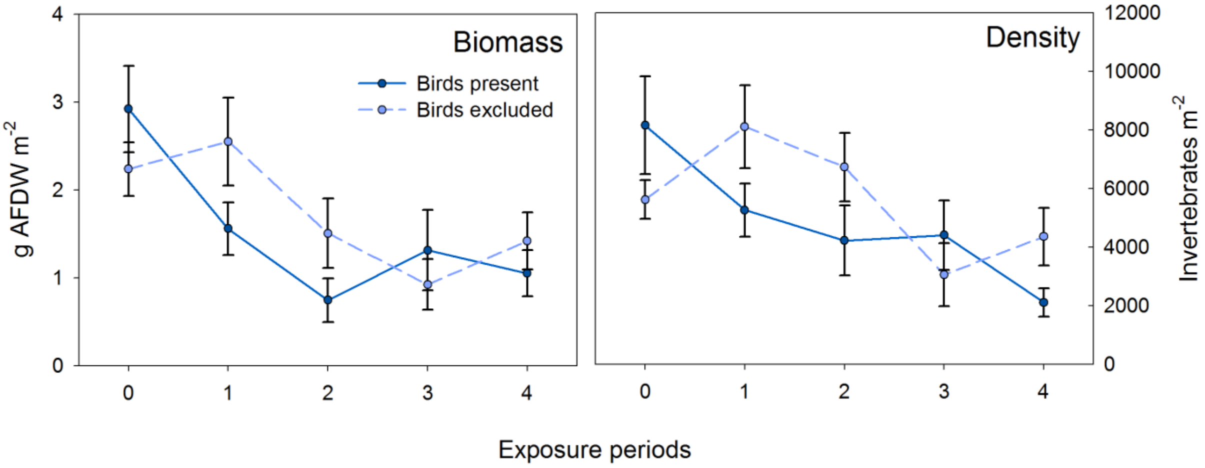

Figure 9. Biomass (g of AFDW·m^{-2}) and density (individuals·m^{-2}) of benthic invertebrates before (period 0) and after (periods 1-4) sediment exposure, for areas with and without shorebirds. Means are represented ± SE. Data were obtained from 24 core samples in period 0, and from 64 cores samples in periods 1-4. Bivalves longer than 20 mm were excluded.

AFDW·m-^{2}), and the benthic invertebrate community was characterized mainly by the polychaete worm *C. capitata* (56%; Appendix 3). After dewatering, we found a rapid and significant decrease in biomass until the exposure periods 3 - 4 ($F_{4,84}$ = 5.40 $p < 0.001$; Fig. 9), when biomass was reduced to 1.1 and 1.2 g·m^{-2}, respectively. The benthic community structure changed after draining, with *H. ulvae* (45%) and *C. capitata* (20%) becoming the most abundant species. We found a significant influence of period × sampling site on invertebrate biomass ($F_{3,113}$ = 4.95, $p < 0.01$).

We found no significant differences in invertebrate biomass, density and in the interaction between these two variables between bird present and bird excluded areas (Fig. 9).

Foraging behaviour

Dunlin and black-tailed godwit showed a significant increment on step rate ($F_{3,127}$ = 23.86, $p < 0.001$; $F_{1,40}$ = 5.14, $p < 0.05$, respectively; Fig. 10) over the exposure periods. Dunlin showed also a significant increment on peck rate ($F_{3,127}$ = 10.79, $p < 0.001$) but the black-tailed godwit did not ($F_{1,40}$ = 1.07, p = 0.307). The ringed plover

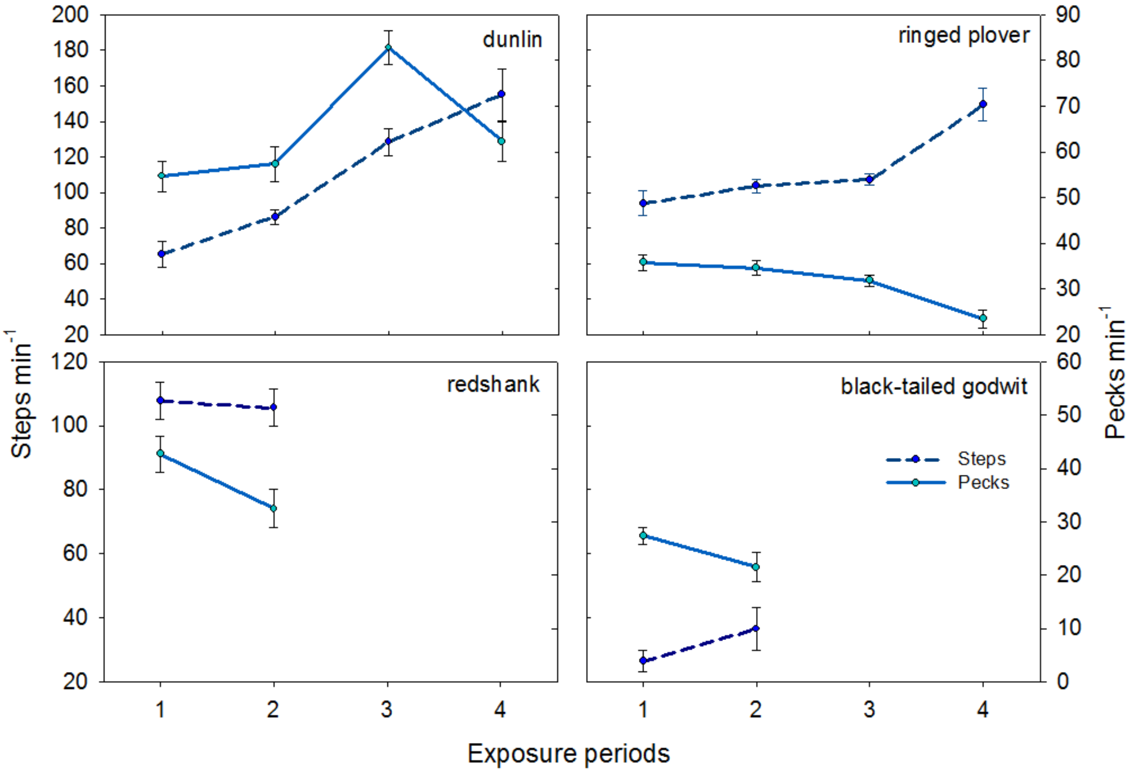

Figure 10. Feeding effort (mean ± SE) of four migratory shorebirds recorded daily (except redshank and black-tailed godwit, which were present mainly during periods 1-2) in the supply pond. The figures show the number of steps and pecks during prey search over the sediment during exposure periods.

showed a significantly increment on step rate ($F_{3,136}$ = 10.61, $p < 0.001$), and a significant decrease on peck rate ($F_{3,136}$ = 19.06, $p < 0.001$) along the exposure periods. Redshanks showed no significant differences on step ($F_{1,72}$ = 0.13, $p = 0.720$) or peck rates ($F_{1,72}$ = 0.0.42, $p = 0.517$) along the exposure periods. The searching effort of both tactile and sight feeders was not influenced by tide, and there was also no interaction period x tide (except for ringed plovers: $F_{3,136}$ = 10.69, $p < 0.001$).

Dunlins showed differences in foraging as the superficial pecking rate increased significantly in the last two periods ($F_{1,,132}$ = 22.61, $p < 0.001$), whereas the opposite trend was recorded for deep probing ($F_{1,132}$ = 28.67, $p < 0.001$; Fig. 11). The deep probing rate of black-tailed godwit decreased significantly from period 1 to period 2 ($F_{1,42}$ = 8.46, $p < 0.01$), but no significant difference was detected for the superficial pecking rate.

The intake rates of dunlin and ringed plover did not differ among the four periods of sediment exposure, their overall intake rate was 11.4 ± 2.1 and 33.0 ± 3.7 mg·min^{-1}, respectively. Dunlin preyed mainly on *C. capitata* and *H. hulvae*, while ringed plover fed almost exclusively on *C. capitata* (Fig. 12). No significant differences in intake rate were found for redshank and black-tailed godwit between periods 1 and 2. Black-tailed godwits fed mainly on polychaete worms and bivalves, with a mean intake rate of 24.6 ± 5.1 mg·min^{-1}. The redshank predated mainly on polychaete worms, *H. ulvae*, bivalves and *H. hydatis*, with a mean intake rate of 33.1 ± 6.3 mg·min^{-1}.

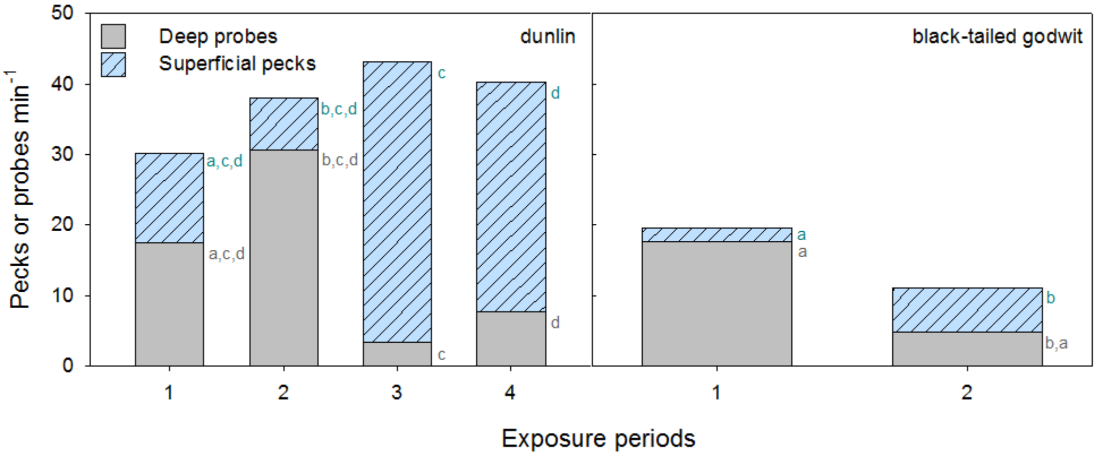

Figure 11. Mean rate of superficial pecks and deep probes of foraging dunlins (n = 136) and black-tailed godwits (n = 44) during the sediment exposure periods. Values represent mean ± SE. For multiple comparisons, equal letters (same colour) indicate significant differences (p < 0.05), while different letters indicate no differences between periods.

Discussion

The experimental drainage of a supply pond in coastal saltpans following a procedure of artisanal fishing provided a suitable foraging ground for a large number of migratory shorebirds that rely on the intertidal areas, mainly small-sized species (up to a 6% of the total population migrating through the Tagus estuary). These responded quickly to the availability of benthic macroinvertebrates in the drained pond, where they foraged successfully at high densities for approximately two weeks. The potential food supply available for shorebirds decreased significantly after sediment exposure, and the exclosure experiment showed that this decrease was due to factors other than shorebird predation.

Bird density

The number of migratory shorebirds foraging in the experimental pond increased rapidly as the water level decreased. Shorebirds are opportunistic foragers and they respond quickly to the availability of new food sources (e.g. Velasquez 1992, Masero *et al.* 2000,

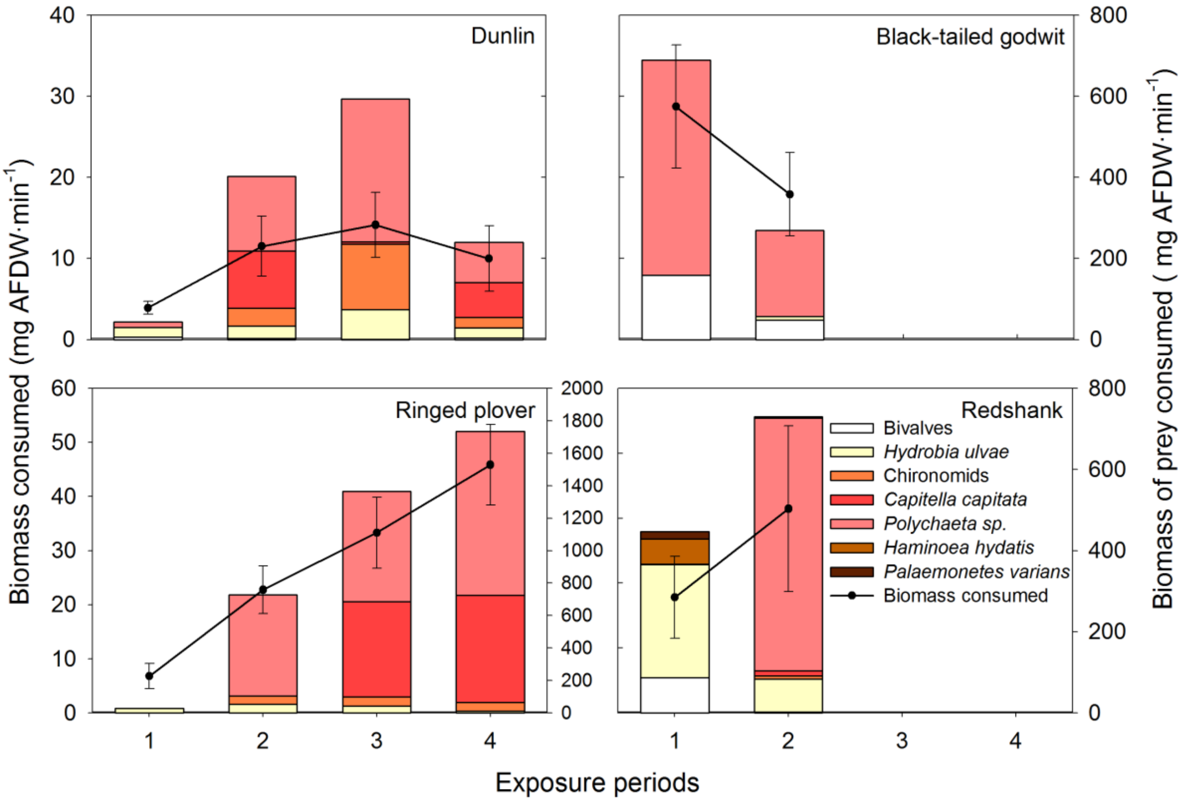

Figure 12. Diet and intake rate of visual (dunlin and black-tailed godwit) and tactile feeders (ringed plover and redshank) during the sediment exposure period of the supply pond. Intake rate (mg of AFDW·min^{-1}; line) was express as mean ± SE and biomass of each prey as total values of biomass consumed (mg of AFDW·min^{-1}; bars).

Masero and Pérez-Hurtado 2001, Taft *et al.* 2002). The density of foraging shorebirds on the pond was higher during high-tide when the adjacent intertidal foraging grounds were unavailable. This pattern supports that, in general, the drained pond functioned as a complementary feeding area. The high proportion of foraging birds during high-tide could indicate that they did not meet their daily energy requirements on the intertidal foraging ground due to low prey availability and/or an increased energy demand imposed by migration.

The experimental supply pond was used extensively as foraging area during a narrow window of time (6 - 12 days). This period was larger than that found by Navedo *et al.* (2015) on drained ponds from shrimp farms in Mexico, where shorebirds foraged during 2–3 days after draining and, overall, were absent from day 5 onwards. In our study area, the abandonment of the experimental pond as foraging ground was directly related to the reduction of the available food supply.

Desiccation was presumably a major factor affecting the benthic invertebrates community after dewater. The high daily temperature during September (22.3 ± 0.3°C on average) and windy conditions at the Tagus estuary could have a major role on sediment desiccation and on mud penetrability. The extended desiccation period could have led to the collapse of the benthic community due to declining water content of the substrate (Granadeiro *et al.* 2006; Kuwae *et al.* 2010), as well as the high temperature on the sediment surface. Mud penetrability affects the cost of bill insertion as well as the ability to detect and capture prey, since a lower penetrability limits bill movement for prey search and capture below the sediment surface (Myers, Williams & Pitelka 1980; Kuwae *et al.* 2010). It has been showed that sediment penetrability decreases with the time after dewater (exposure to air) due to the development of suction, i.e. negative pore water pressure relative to atmospheric air pressure (Sassa & Watabe 2007; Sassa *et al.* 2011). This could explain why godwits decreased their deep probes and why dunlins increased their superficial pecks through time.

The benthic invertebrate community was dominated by *Capitella capitata*, an opportunist polychaete (Méndez, Romero & Flos 1997). This dominance can be attributed to the high levels of organic matter in the bottom sediment. Overall, the increment of organic matter is related to low salinity, deep water, and a long flood period (e.g. Davis 2000, Tavares *et al.* 2009), which was the case of the our experimental pond. The density and biomass values of macroinvertebrates potentially available for shorebirds in our study were similar or higher that those recorded in supply ponds from saltpans in Iberia (Vieira & Amat 1997; Masero *et al.* 1999; Sánchez *et al.* 2006b), or in other geographical regions (Cordova & Ocaña 2007). But the biomass of the benthic macroinvertebrate community on the experimental pond was lower than that reported for intertidal mudflats of Tagus estuary (Silva *et al.* 2006; França *et al.* 2009). However, the experimental pond macroinvertebrate community was composed mainly by small-size individuals harvestable by shorebirds. On the Tagus intertidal mudflats, the bivalve *Scrobicularia plana* explains largely the high values of biomass during September (Silva *et al.* 2006; França *et al.* 2009). However, most of these bivalves are not harvestable by small-sized shorebirds due to large shell size (França *et al.* 2009), therefore the actual harvestable food supply by shorebirds on tidal mudflats during autumn migration may comprise a lower fraction of the biomass that is available. The high densities of polychaetes on the experimental pond can explain the large percentage (> 50% of the total individuals present in the saltpans at high tide) of dunlins, sanderlings, little stints, ringed plovers, redshanks and black-tailed godwits foraging in the pond during both low and high tide periods. This foraging preference was also found on saltpans in south Iberia, where more than half of the population of small-sized shorebird species prefer to forage in the saltpans during the migratory periods (Masero *et al.* 2000).

Foraging behaviour and intake rates

Although the food supply decreased through time, dunlins maintained the same intake rate by increasing searching effort through increasing both step and peck rates. During the first days after draining, foraging dunlins had lower step and peck rates than those reported on the Tagus intertidal mudflats during spring migration by Martins *et al.* (2013). This lower searching effort may be related to the type of prey items available in the pond, since dunlins' intake rate in the experimental pond was lower than that observed on the mudlats during the spring period by Martins *et al.* (2013). Nevertheless, it must be noted that foraging in the supratidal habitat is not tide limited, while the time window to forage on the intertidal mudflats is 7.5 h per low tide period (Granadeiro *et al.* 2006). By staying on the pond birds also reduce the energy cost of flying from feeding areas on mudflats to the high-tide roosts and vice versa (up to 5 km in the Tagus estuary; Dias *et al.* 2006). Ringed plovers fed mainly on polychaetes worms, maintaining a constant intake rate by increasing the step rate. The intake rate was higher than that reported for saltpans in south Iberia by Masero *et al.* (2007), and on intertidal mud and sandflats of north east England reported by Pienkowski (1982). Redshanks and black-tailed godwits foraged on the pond only during the first days and then left presumably due to sediment desiccation. Redshanks fed on a diverse range of prey items similar to those found in Tagus mudflats by Moreira (1996). On the pond they fed also on Atlantic ditch shrimp, captured mainly in the deep pools near the sluice gate. The pecking rate was similar to that observed in saltpans from south Iberia by Masero and Pérez-Hurtado (2001), however the intake rate reported in their study was lower than that observed on our experiment. Black-tailed godwits fed mostly on small size polychaete worms and bivalves. They maintained the same intake rate by increasing the step rate. The intake rate of black-tailed godwits was much lower than that reported on Tagus mudflats (75 mg·min^{-1}) by Moreira (1994) but similar to that recorded by Alves *et al.* (2012), ranging from 12 to 108 mg·AFDW min $^{-1}$.

The total daily food consumption of a dunlin foraging on the pond in autumn, assuming a mean body mass of 43 g, can be estimated at 4.8 g AFDM·d^{-1} according to Zwarts *et al.* (1990). Dunlin, with a mean intake rate of 11.42 mg·min^{-1}, need a foraging time of 7 h·d^{-1} and this should explain why birds did not increase their intake rate. According to the daily intake rate recorded for ringed plover, redshank and black-tailed godwit (assuming a mean correspondent body mass of 60, 121 and 287 g) they could reach their daily biomass needs foraging in the experimental pond for 3, 7, 13 h·d^{-1}, respectively. The reduced intake rate of black-tailed godwits should explain the low density of this species on the pond at later periods. Moreover, in late summer/autumn godwits could find higher densities and more profitable sizes of *Scorbicularia plana* and *H. diversicolor* on the Tagus mudflats (França *et al.* 2009). The highest fattening rates obtained in stopover areas by dunlins, ringed plovers and redshanks are, respectively, 3.9 %, 3.0

% and 1.0 % of their body mass (Zwarts *et al.* 1990b). Fattening rates of this magnitude, require an additional foraging time of 2.5, 0.9 and 0.6 $h \cdot d^{-1}$ by dunlins, ringed plovers and redshanks, respectively, which could be achieved in the pond.

During the study period we observed aggressive interspecific (with black-headed gulls *Larus ridibundus*) and intraspecific encounters on the pond. Social interactions during feeding activities resulting from high bird density (Ens & Goss-Custard 1984; Goss-Custard *et al.* 1995) may have forced part of the shorebird population to move to intertidal mudflats at low-tide, although dunlins, ringed plovers and redshanks could gain mass by just feeding on the experimental pond. In addition, we observed the regular presence of one peregrine falcon (*Falco peregrinus*) attacking shorebirds on the experimental pond, which could also explain the movements of shorebirds to the mudflats where predation risk is lower (Rosa *et al.* 2006), and may also compensate the flight cost.

Conservation implications

Migratory shorebird populations are globally experiencing declines (IWSG 2003) and the success of their migration relies on a network of high quality stopover sites between breeding and wintering areas, where they can rapidly rebuild their condition and pursue their journey. The present study showed that supply ponds after dewater could play a substantial role as foraging ground for migratory shorebirds during stopover periods. Shorebirds respond opportunistically in high densities to sediment exposure, and they could reach their daily biomass needs and gain mass foraging only or mostly on dewater ponds. The results of this study have major implications for the management of supply ponds: 1) we propose a mosaic sequential strategy for emptying supply ponds from saltpans after an extended flood period. A similar role was proposed for aquaculture fishponds in south Spain (Kloskowski *et al.* 2009) and shrimp farms in Mexico (Navedo *et al.* 2015); 2) it is clear that manipulating water level in a flood pond where a high density of benthic invertebrate occurs, dramatically increases the value of these areas as a foraging habitat for several species of waterbirds, mainly shorebirds; 3) managers should ensure water admissions during spring and summer invertebrate recruitment periods, which can enable to reaching high densities of benthic invertebrates in the sediment pond by autumn; 4) our study suggests that the use of the pond by feeding shorebirds could be enhanced by maintaining preferred lower water depths instead of a complete drawdown, since water content of sediments had an apparent direct negative influence on the availability of prey to birds. The presented management practices al saltpans during the migratory periods ensure quality feeding conditions for migratory shorebirds, and can contribute significantly to the maintenance of high density of foraging shorebirds on the intertidal mudflats.

Acknowledgments

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Appendix

Appendix 1. Bird surveys in the experimental supply pond after the water was drained. For each species we present the number of birds (mean ± SE) recorded daily during high-tide period over the 16 days after opening the sluice gate. Days were grouped in four periods: 1 (0-3 days), 2 (4-7 days), 3 (8-11 days) and 4 (12-15 days).

Shorebird species	Period 1	Period 2	Period 3	Period 4
Dunlin	26.47 ± 6.8	249.65 ± 24.3	36.58 ± 7.6	8.01 ± 1.0
Ringed plover	42.24 ± 8.5	121.99 ± 14.0	73.08 ± 9.7	34.57 ± 4.6
Little stint	4.36 ± 1.1	28.07 ± 3.9	22.42 ± 4.0	11.94 ± 2.0
Sanderling	3.93 ± 1.2	10.74 ± 1.9	13.00 ± 3.3	3.85 ± 0.8
Redshank	10.68 ± 1.9	19.46 ± 2.3	0.74 ± 0.2	0.32 ± 0.1
Curlew sandpiper	0.64 ± 0.4	12.06 ± 1.8	0.36 ± 0.3	0.00 ± 0.0
Black-tailed godwit	1.67 ± 0.4	3.99 ± 1.0	0.02 ± 0.0	0.24 ± 0.1
Turnstone	1.30 ± 0.3	0.61 ± 0.1	1.36 ± 0.3	1.15 ± 0.2
Kentish plover	0.10 ± 0.1	0.90 ± 0.3	0.46 ± 0.2	1.32 ± 0.4
Black-winged stilt	2.50 ± 0.4	0.08 ± 0.1	0.02 ± 0.0	0.00 ± 0.0
Greenshank	1.91 ± 0.4	0.36 ± 0.1	0.08 ± 0.0	0.00 ± 0.0
Common sandpiper	0.29 ± 0.1	0.13 ± 0.0	0.26 ± 0.1	0.11 ± 0.0
Grey plover	0.21 ± 0.1	0.11 ± 0.1	0.02 ± 0.0	0.01 ± 0.0
Oystercatcher	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.11 ± 0.0
Whimbrel	0.00 ± 0.0	0.00 ± 0.0	0.06 ± 0.1	0.00 ± 0.0
Avocet	0.06 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Snipe	0.00 ± 0.0	0.01 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
Total	96.37 ± 21.65	448.15 ± 49.94	148.46 ± 25.82	61.64 ± 9.34

Waterbirds species	Period 1	Period 2	Period 3	Period 4
Lesser Black-backed Gull	28.90 ± 4.6	250.36 ± 4.5	197.24 ± 5.4	17.89 ± 4.5
Black-headed Gull	41.47 ± 4.6	54.60 ± 4.5	24.74 ± 5.4	14.10 ± 4.5
Little egret	15.27 ± 4.6	12.43 ± 4.5	1.70 ± 5.4	0.28 ± 4.5
Sandwich tern	1.17 ± 4.6	3.07 ± 4.5	2.04 ± 5.4	0.26 ± 4.5
Grey heron	1.46 ± 4.6	0.38 ± 4.5	0.22 ± 5.4	0.04 ± 4.5
Black tern	0.00 ± 4.6	0.39 ± 4.5	0.10 ± 5.4	0.00 ± 4.5
Little tern	0.03 ± 4.6	0.26 ± 4.5	0.06 ± 5.4	0.13 ± 4.5
Flamingo	0.00 ± 4.6	0.00 ± 4.5	0.32 ± 5.4	0.00 ± 4.5
Yellow-legged Gull	0.00 ± 4.6	0.07 ± 4.5	0.04 ± 5.4	0.08 ± 4.5
Little greb	0.07 ± 4.6	0.00 ± 4.5	0.00 ± 5.4	0.00 ± 4.5
Cormorant	0.01 ± 4.6	0.00 ± 4.5	0.00 ± 5.4	0.00 ± 4.5
Total	88.39 ± 50.49	321.56 ± 49.78	226.46 ± 59.74	32.78 ± 49.78

Appendix 2. Benthic invertebrates density (ind · m^{-2}) before and after dewater on areas where birds foraged. Means are represented ± SE. Data were obtained from 12 core samples before dewater (period 0), and from 32 core samples after dewater (periods 1-4). Class size corresponds to > 90 % individuals collected during the experiment. Polychaetes range size was assess using the length of *H. diversicolor*. Bivalves longer than 20 mm were excluded. Biomass in the five periods were compared with a Kruskal-Wallis test (ns: non-significant; *P < 0.05; **P < 0.01), followed by a multiple comparisons test (superscript with identical letters indicates significant difference at P< 0.05).

	Exposure periods					
Taxa	**0**	**1**	**2**	**3**	**4**	**H**
Abra tenuis	195.81 ± 73.43	428.34 ± 183.58	367.15	458.94 ± 115.83	367.14 ± 244.76	6.5 ns
Cerastoderma glaucum	407.95 ± 147.09	275.36 ± 91.79	183.58 ± 61.19	-	489.54	1.3 ns
Hydrobia ulvae	367.15 ± 81.59	285.56 ± 107.93	611.92	917.88 ± 795.50	775.09 ± 341.31	2.9 ns
Chironomids larvae	1762.33 ± 534.30^{a}	1557.62 ± 426.54^{b}	514.01 ± 191.17	514.01 ± 118.65	163.18 ± 25.80^{a,b}	14.6**
Capitella capitata	6333.37 ± 1272.22^{a}	3953.01 ± 583.54	3328.85 ± 1356.97	2814.83 ± 1246.28	1346.22 ± 581.24^{a}	11.0*
Polydora sp.	815.89 ± 426.88	413.05 ± 65.16	954.59 ± 545.12	571.13 ± 389.16	538.49 ± 201.68	0.8 ns
Polychaetes	122.38 ± 0.00	122.38 ± 0.00	-	-	122.38 ± 0.00	
Total	10005	7158	5960	5377	3802	

Appendix 3. Benthic invertebrates biomass (AFDW g·m^{-2}) before and after dewater on areas where birds foraged. Means are represented ± SE. Data were obtained from 12 core samples before dewater (period 0) and from 32 core samples after dewater (periods 1-4). Class size corresponds to > 90 % individuals collected during the experiment. Polychaetes range size was assess using the length of *H. diversicolor*. Bivalves longer than 20 mm were excluded. Biomass in the five periods were compared with a Kruskal-Wallis test (ns: non-significant; ***P < 0.001), followed by a multiple comparisons test (superscripts with identical letters indicate significant difference at $p < 0.05$).

	Exposure periods						
Taxa	**0**	**1**	**2**	**3**	**4**	**Class size**	**H**
Abra tenuis	0.26 ± 0.06	0.21 ± 0.09	0.54	0.36 ± 0.16	0.71 ± 0.46	1–10mm	2.7 ns
Cerastoderma glaucum	0.07± 0.03	0.15 ± 0.3	0.03 ± 0.02	-	0.23	< 5mm	7.4 ns
Hydrobia ulvae	0.52 ± 0.13	0.32 ± 0.07	0.59	0.87 ± 0.67	0.95 ± 0.37	3-6mm	4.2 ns
Chironomids larvae	0.19 ± 0.06	0.21 ± 0.08	0.09 ± 0.03	0.07 ± 0.02	0.04 ± 0.01	5-15mm	2.2 ns
Capitella capitata	1.97 ± 0.38^{a}	0.92 ± 0.25	0.38 ± 0.15	0.54 ± 0.06	0.18 ± 0.08^{a}	10-30mm	20.4 ***
Polydora sp.	0.31 ± 0.12	0.15 ± 0.05	0.05 ± 0.03	0.11 ± 0.06	0.15 ± 0.03	-	7.6 ns
Polychaetes	0.35 ± 0.17	0.25 ± 0.18	0.07 ± 0.04	0.01 ± 0.00	0.06 ± 0.02	30-70 mm	9.5 ns
Total	3.69 g	2.22 g	1.76 g	1.95 g	2.33 g		

Chapter II

The effects of management suspension on roosting waterbirds


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Abstract

The selection of high-tide roosting sites in coastal saltpans by waterbirds is governed by several environmental factors, including water level and the existence of open dry areas in the evaporations ponds. However, little is known about the effects of the lack of management of these factors in the roosting consistency of different species. We study the effects of the suspension of management actions in a high-tide roost to estimate the impact on waterbird populations applying a before–after-control-impact design. Small and medium shorebirds were more vulnerable to the lack of management and thereafter showed a strong reduction in winter (38-50 %) and during migratory periods (spring: 77-79 %; autumn: 38-43%). The affected populations of small shorebirds and the spring population of medium shorebird did not reach the initial densities on the 6 years after management suspension. Our results demonstrate how the lack of management in a single year negatively influenced the density of roosting shorebirds in the following years to the management reestablishment.

Key words: saltpans, refuges, high-tide, shorebirds.

Introduction

Anthropogenic wetlands can provide alternative or complementary habitats for shorebirds (e.g. Lourenço *et al.* 2010; Navedo *et al.* 2015), and partially mitigate the loss of the natural habitats. When intertidal feeding grounds become unavailable as the tide rises, shorebirds and other waterbirds are forced to find refuge in supra-tidal habitats known as roosts. Shorebirds can use the same high-tide roosts over decades or even more than a century (Rehfisch *et al.* 1996; Rehfisch *et al.* 2003; Colwel *et al.* 2003). The availability of such roosts can be a key factor in maintaining high densities of non-breeding shorebirds on the intertidal mudflats (Velasquez & Hockey 1992; Masero *et al.* 2000) and the lack of nearby roosts may constrain the ability of shorebirds to exploit highly productive intertidal feeding areas (Dias *et al.* 2006a; Zharikov & Milton 2009). Man-made coastal saltpans used to obtain salt by solar evaporation of sea water, are key supratidal habitats for conserving shorebirds worldwide. They are used by shorebirds as both roosting and foraging grounds (e.g. Masero 2003; Sripanomyom *et al.* 2011; Warnock *et al.* 2012). Unlike natural wetlands, the water level and salinity in the evaporation ponds are stable and predictable as a result of management. Water depth is a major limiting factor in the selection of different ponds by shorebirds to roost in saltpans. This limitation is directly related with bird size and more specifically to leg length (Ntiamoa-Baidu *et al.* 2008). The maintenance of exposed margins and open areas in the dykes of evaporation ponds are also fundamental for large numbers of shorebirds roosting in saltpans.

In southwest Iberia the saltpans from Tagus estuary are key supra-tidal roosting sites for several waterbird species (Rosa *et al.* 2006; Catry *et al.* 2011). The Samouco saltpans complex is the most important high-tide roost in the Tagus estuary, harbouring several thousand shorebirds all year round, with 30–80% of all the shorebirds on this wetland (Catry *et al.* 2011). The Samouco saltpans are managed to provide suitable roosting and foraging sites for waterbirds, mainly shorebirds. Thus, the water depth (range 5-80 cm) is controlled in most ponds. During spring tides, the sluice gates from managed ponds are open manually allowing water from the estuary to enter the pond. The excess rainfall water is drained by opening the sluice gates during low tides.

Mechanic mowing is promoted seasonally to control the overgrowth of saltmarsh vegetation in dykes, improving their value as roosting sites for large numbers of shorebirds and breeding sites for a few waterbird species. These management actions were suspended during one year in most roosting sites of Samouco, so its quality for waterbirds was likely affected.

We used the management suspension to estimate the impact on waterbird populations in Samouco roost applying a before–after-control-impact (BACI) design. BACI design is well-suited to detect human disturbance or effects of activities on bird populations or habitats (Green 1979; Navedo & Masero 2008). It incorporates both time and control sites, thereby mitigating chances that unmeasured covariates are influencing observed effects (McDonald, Erickson & McDonald 2000). We predicted that small shorebirds such as sandpipers which rely in water less than 5 cm deep are more affected by the lack of management than large shorebirds such as godwits which roost in waters up to 15 cm depth or even other waterbirds.

Methods

Study area

The study was carried out in the 355 ha of Samouco saltpans complex (SW Portugal, 38º44'N, 8º59'W; Fig.13), located inside the Special Protection Area for Birds of Tagus estuary. The complex is divided in two separate management areas, one covering 190 ha and the other 26 ha, hereafter named Impact area and the Control area, respectively. Both areas comprise the water surface and dikes of active saltpans which are the main waterbird roosts, and non-active saltpans used mainly by larger shorebirds and other waterbird species. The suspension of management actions in the Impact area

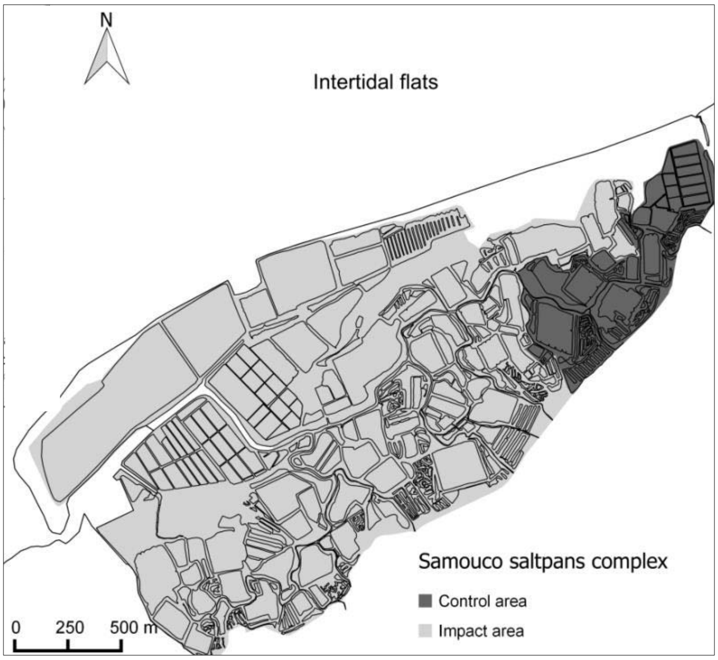


Figure 13. The Samouco saltpans complex indicating, on the left (in light grey) the Impact area, and, on the right (in dark grey) the Control area.

occurred between March 2008 and July 2009, allowing the overgrowth of saltmarsh vegetation, mostly in the dykes, and water depth presented large oscillations. For instance, during winter and spring the rainfall flooded the ponds, while in summer and autumn most ponds became dry or highly saline. Thereafter the management actions were re-established.

Bird surveys

The Samouco monitoring program provided information on monthly waterbird surveys, and included 4 years of data before suspension of the saltpans' management and 6 years of data after management reestablishment. The surveys were carried out between June 2005 and December 2014 (bird counts in the Control area began only in February of 2007 and no counts were performed in October 2007; in the Impact area counts were not performed in September and October of 2005). Both areas were counted by the same person sometimes with the help of a second observer from a vehicle, usually in a single day, sometimes in two consecutive days, and assessed over a track covering all saltpans. Counts were performed during a four hour period (± 2h from tide peak) in spring tides with amplitudes higher than 3.8 m, in order to ensure that all birds present in the estuarine flats had moved to the high tide roost (Rosa *et al.* 2006, pers. obs.).

Data Analysis

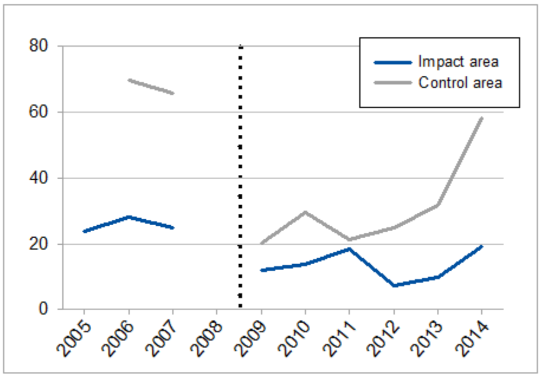
The BACI design study was conducted using data on Samouco waterbird surveys before (June 2005 – February 2008) and after the suspension of management (August 2009 – December 2014). The Control area was used to assess the temporal variation in the use of the roost by shorebirds. Monthly survey data were pooled seasonally: winter (November to February), spring migration (March to May), summer (June and July) and autumn migration (August to October). Bird species were grouped by their exposed leg length according to Ntiamoa-Baidu *et al.* (2008): small shorebirds (< 45 mm) from 10 species; medium shorebirds (71-86 mm) from 12 species; large shorebirds (111-174 mm) from 6 species; waterbirds (>200 mm, swimmers or divers) from 25 species including flamingos, herons, ducks and gulls. Waterbird density was calculated dividing bird abundance by the area of Impact and Control areas. Thereafter waterbird density was transformed (log x+1) to comply with the assumptions of normality and homoscedasticity of variances. A nested ANOVA was performed to detect differences in the density of each waterbird group with area (impact and control) nested in period (before and after management suspension) as a fixed factor. Data is presented as mean ± SE.

Results

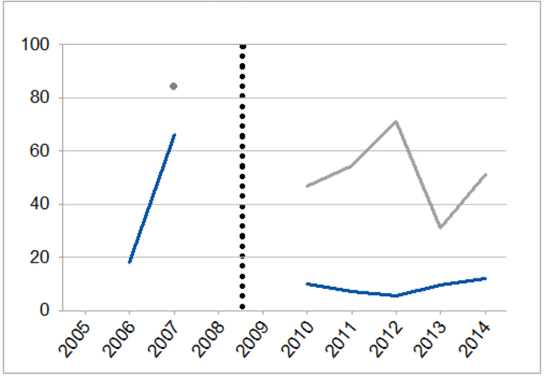
The Control area harboured the higher mean densities of small shorebirds, large shorebirds and other waterbirds populations in the Samouco roost during the study period, with 41.0 ± 3.60 birds·ha^{-1}, 6.7 ± 0.84 birds·ha^{-1} and 14.7 ± 1.68 birds·ha^{-1}, respectively (Fig. 14). The most common species of small shorebirds was the dunlin (*Calidris alpina,* 58-69%), of medium shorebirds was the redshank (*Tringa totanus*, 60-68%), and of large shorebirds were the black-tailed godwit (*Limosa limosa*, 51%) before and the black-winged stilt (*Himantopus himantopus*, 53%) after management suspension, and of other waterbirds was the lesser black-backed gull (*Larus fuscus,* 53-47%). The Impact area harboured 19.3 ± 1.85 small shorebirds·ha^{-1}, 7.4 ± 0.72 medium shorebirds·ha^{-1}, 5.2 ± 0.36 large shorebirds·ha^{-1} and 8.0 ± 0.37 other waterbirds·ha^{-1}. The most common species, before and after management suspension, of small shorebirds was the dunlin (76%), of medium shorebird was the grey plover (*Pluvialis squatarola*, 41-58%), of large shorebird was the black-tailed godwit (57-49%), and of other waterbirds was the lesser black-backed gull (*Larus fuscus*, 43-33%).

Small and medium shorebirds were more vulnerable to the lack of management and thereafter showed a strong reduction in wintering and migratory populations (Table 3). After management suspension small shorebirds showed a significant reduction in both areas (Impact and Control) of 50-57% during winter, 79-39 % in spring and 38-30 % in autumn periods. In the Impact area, the density of medium shorebirds decreased 38%, 77% and 43% during winter, spring and autumn periods, respectively. The opposite trend was observed in the control area due to the increase of bar-tailed godwit and redshank. Large shorebirds suffered a reduction of 30% on the wintering population in the Impact area, and an increment during migratory periods in both areas due to the increase of pied avocets in spring and black-tailed godwits in autumn. Wintering waterbirds decreased 9% in the Impact area, and 44% in the control area (decrease of lesser

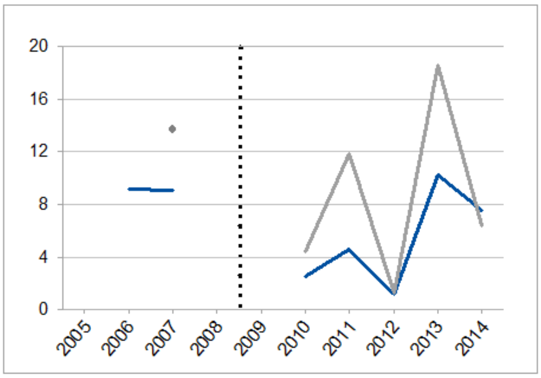
Small shorebirds - winter



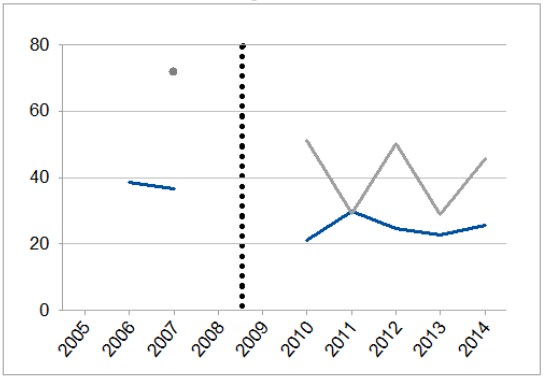
Small shorebirds - spring migration



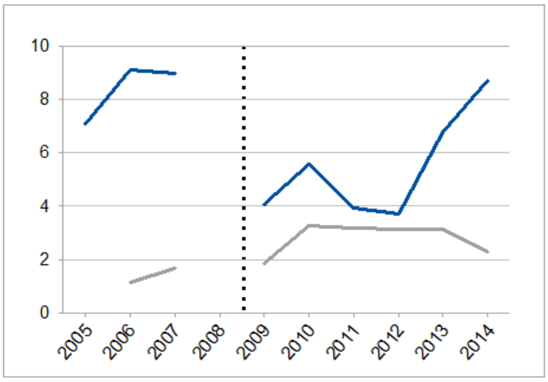
Small shorebirds - summer



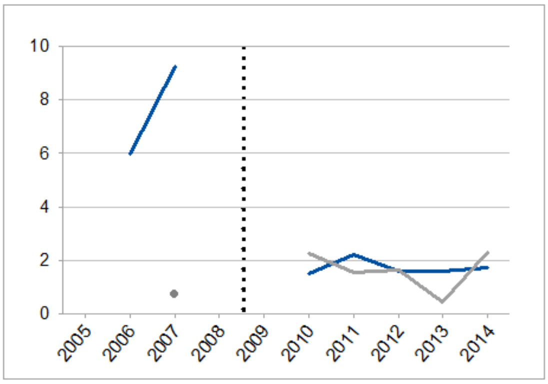
Small shorebirds - autumn migration



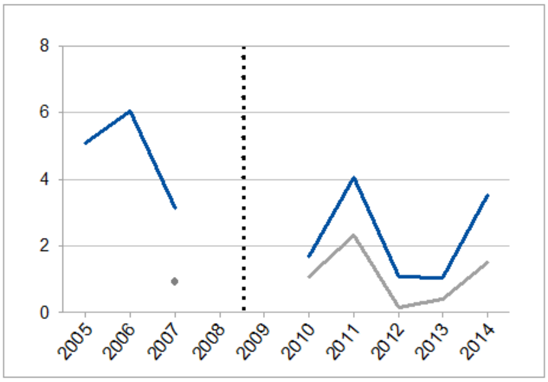
Medium shorebirds - winter



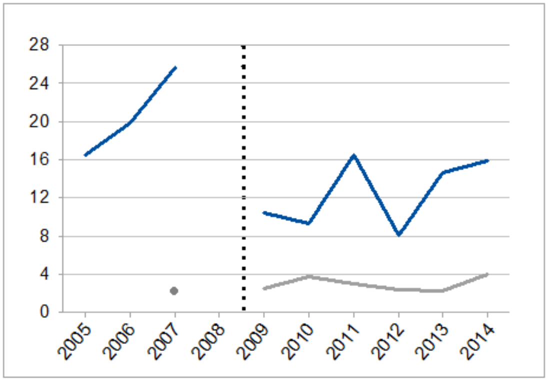
Medium shorebirds - spring migration



Medium shorebirds - summer



Medium shorebirds - autumn migration



Large shorebirds - winter

Large shorebirds - spring migration

Large shorebirds - summer

Large shorebirds - autumn migration

Other waterbirds - winter

Other waterbirds - spring migration

Other waterbirds - summer

Other waterbirds - autumn migration

Figure 14. Density of waterbird groups (small, medium and large shorebirds and other waterbirds·ha^{-1}) in four seasons (winter, summer, spring and autumn migration). The impact and control areas are represented with red and grey lines, respectively. The dashed line indicates the suspension of management activities.

black-backed gull and black-headed gull). None of the four groups showed significant changes in the density of summer populations.

The recovery of populations from the different waterbird groups differed once the management activities were re-established. The affected populations of small shorebirds and the spring population of medium shorebirds never reach its initial densities during the entire duration of the study. The winter and autumn migratory populations of medium shorebird recovered the initial densities after 6 and 3 years, respectively. The winter populations of large shorebirds and waterbird species recovered in the second and third years, respectively.

Discussion

Our results demonstrate how the lack of management in a single year (14 months) negatively influenced the density of roosting shorebirds in the saltpans for a much longer period than the suspension, despite management reestablishment. The reduced size of the Control area and its proximity to the Impact area could facilitate bird movements between them, and thus compromise the assumption of independence. Consistent changes over time in the waterbird populations at both areas should be the result of regional or extra regional trends on a major scale (Delany *et al.* 1999). However, changes in bird populations occurring at the impact but not at the control area, are likely to be related with changes at the impact area following the suspension of management actions. The effects in the density of migratory populations may not be as accurate as in the winter populations, since the density of staging populations detected in the counts are highly variable due to the potentially high turnover rates of migrants (Frederiksen *et al.* 2001; Gillings *et al.* 2009). However the strong decline (> 70%) in the spring populations of small and medium shorebirds supports the argument that the suspension of management actions was responsible for significant decreases in the use of Samouco saltpans as a high-tide roosting site. Wintering populations tend to be more stable (Metcalfe & Furness 1985), which enable a better assessment of the effects of habitat changes on the number of roosting birds.

The populations of dunlins and grey plovers in the Tagus estuary are in moderate declining over the last decade (25% reduction; Catry *et al.* 2011), which could justify the reduction during winter

Table 3. Nested ANOVA results to evaluate differences in bird density (data logx+1 transformed) for each waterbird group, for each season, with area (impact and control) nested in period (before and after management suspension) as a fixed factor.

Species and seasons	winter			spring migration			Summer			autumn migration		
	df	F	*p*	df	F	*P*	df	F	*P*	df	F	*p*
small shorebirds												
Control-Impact (before-after)	2	3.77	< 0.05	2	22.55	< 0.01	2	0.95	0.40	2	3.40	< 0.05
Error	55			35			24			42		
medium shorebirds												
Control-Impact (before-after)	2	24.62	< 0.01	2	4.58	< 0.05	2	2.01	0.16	2	40.62	< 0.01
Error	54			33			22			41		
large shorebirds												
Control-Impact (before-after)	2	3.99	< 0.05	2	13.67	< 0.01	2	0.05	0.95	2	12.80	< 0.01
Error	55			35			24			41		
other waterbirds												
Control-Impact (before-after)	2	8.91	< 0.01	2	0.78	0.47	2	1.92	0.17	2	1.66	0.20
Error	55			35			24			42		

and migratory periods of small (in both areas) and medium shorebirds, respectively. However this negative trend per se does not explain the strong reduction of these populations observed in the impact area. The populations of Icelandic black-tailed godwit and lesser black-backed gull that largely roosted in Samouco saltpans are increasing in Europe (Gill *et al.* 2007; Ross-Smith *et al.* 2014), but drive the decrease of large shorebirds and other waterbirds observed in the Impact area. These findings highlight the importance of the management actions in roosting sites, and demonstrated how the lack of management negatively influenced the density of roosting waterbirds in the years following management reestablishment.

Roost fidelity and roost preferences are thought to be variable among shorebirds and species exhibiting strong roost fidelity are likely to be most affected by their loss (Rehfisch *et al.* 2003; Conklin, Colwell & Fox-Fernandez 2008). The susceptibility of small shorebirds to the deterioration of roosts was already recorded in other important high-tide roost from the Tagus estuary. For instance, the conversion of saltpans with water level managed for birds, into a shrimp farm, led the decreased of the dunlin winter population from 5,000 to 2,000 birds (Lecoq 2002; Catry *et al.* 2011). Shorebirds are known to respond opportunistically to the increase of foraging space (e.g. Velasquez 1992; Taft *et al.* 2002) or to new high-tide roosts (Huang *et al.* 2012), however shorebird distinct populations do not reach the initial densities at the same time after the improvement of roosting conditions.

The flight costs may differently constrain shorebirds of using the same foraging grounds which are contiguous to the Samouco saltpans roost. Large shorebirds can fly farther in their routine movements between roosts and foraging areas (Santiago-Quesada *et al.* 2014). They may maintain the previous foraging grounds, roosting in alternative roosts and returning to Samouco roost right after the habitat conditions improve. Small shorebirds such as dunlin fly short distances (< 5 km) between roosts and foraging grounds (Dias *et al.* 2006a). Unpublished data from ringing recoveries and waterbird surveys, indicate the increase of the winter population of dunlins in two other roosts within Tagus estuary with birds from Samouco roost, after the suspension of management actions. These roosts are too distant from the Samouco roost (> 10km) and may prevent dunlins to identify restored conditions in Samouco roost after management has been re-established. Moreover, this potentially longer trips might increase the likelihood of predation while in flight (Goss-Custard *et al.* 2006a; Rosa *et al.* 2006), and therefore we expect the increase of small shorebirds populations in Samouco saltpans when the conditions of the habitat and safety in the actual roosts deteriorate. The abandonment of a particular roost can have severe negative consequences for some species, or for the entire shorebird community during defined periods of their life-cycle. Moreover, several studies have linked shorebird population declines with disturbance or loss of high-tide roosting sites (e.g. Burton, Evans & Robinson 1996; Martín *et al.* 2015; Green *et al.* 2015).

The highlight of this study is that saltpans rapidly lose their value for waterbirds as high-tide roost in the absence of adequate management. The suspension of management actions led a long-term negative effect on the density of small and medium shorebirds. Therefore, site-managers of coastal wetlands should establish efforts to ensure the continuous and adequate management in a network of high quality roosts for the conservation of the shorebird assemblage within the same wetland.

Acknowledgments

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Chapter III

Coastal saltpans are a good alternative breeding habitat for kentish plover *Charadrius alexandrinus* when umbrella species are present

Abstract

The loss and degradation of natural habitats in coastal areas worldwide has adversely affected many waterbird species, changing their breeding distribution and reducing their productivity. Anthropogenic habitats such as saltpans can provide alternative or complementary habitats for waterbirds and mitigate the increasing human impact on natural coastal habitats. Unvegetated linear paths between salt ponds are used by ground-nesting waterbird species to breed but their linear structure may facilitate the detection of nests by predators. This negative effect may, however, be counterbalanced by the advantages of breeding in mixed colonies. To evaluate the importance and the risks of breeding in saltpans we used the kentish plover *Charadrius alexandrinus* breeding in coastal saltpans of southern Portugal as a model species, to evaluate the role of nest-site characteristics, predation and nesting proximity to species with aggressive antipredator behaviour (black-winged stilt, *Himantopus himantopus*) on their nesting success. Kentish plovers selected nest-sites on the edges of the paths, with about 20% of water around the nest, and a mean visibility from above of 72 %; however nest-site characteristics were not correlated with nesting success. Predation was the main cause of nest loss in saltpans (42%); carrion crows *Corvus corone* were responsible for most diurnal nest predation (58%) and red fox (*Vulpes vulpes*; 73%) for nocturnal predation. A 8-year monitoring of kentish plover population showed a linear increase in their nesting success as their breeding season increasingly overlapped with that of the black-winged stilt. An experiment with artificial nests showed a significant increase in the number of exposure days (7 to 12) when kentish plover nests were within close proximity to black-winged stilt nests. Overall, our results show that saltpans are an important alternative breeding habitat for kentish plover, particularly when mixed species colonies are maintained.

Key words: artificial habitats, nesting success, colonially, predation timing.

Introduction

The increased human pressure on natural coastal areas throughout the world has contributed to marked changes in the structure of coastal ecosystems, leading to strong declines in biodiversity and services provided by these ecosystems (Fraser and Keddy 2005; Assefa *et al.* 2007). Global waterbird populations, for example, have been particularly affected (Butchart *et al.* 2010), with many species changing their breeding distribution and showing strong declines in productivity (Baillie *et al.* 2004; Beale and Monaghan 2004). At a global scale, anthropogenic habitats such as coastal pastures, rice fields, extensive aquaculture ponds or saltpans could play a significant role as buffer areas against the loss of natural coastal habitats for declining waterbird populations (e.g. Zwarts 2009; Sutherland *et al.* 2012; Navedo *et al.* 2014). Coastal saltpans are man-made habitats used for obtaining salt by solar evaporation of sea water in a series of shallow interconnected evaporation pans varying in size, water depths and salinity. Unlike natural wetlands, the water level and salinity in coastal saltpans are stable and predictable as a result of management. They are key coastal sites for conserving waterbirds in Europe, Asia, North America, Australia and Africa (e.g. Velasquez 1992; Alcorn and Alcorn 2000; Takekawa *et al.* 2001; Masero 2003; Sripanomyom *et al.* 2011; Dias *et al.* 2014). Saltpans are important roosting and feeding areas for non-breeding shorebirds and other waterbirds (e.g. Britton and Johnson 1987; Masero 2003; Dias *et al.* 2006; Takekawa *et al.* 2006; Sripanomyom *et al.* 2011), and important breeding grounds for waterbirds (Tinarelli & Baccetti 1989; Rufino & Neves 1991; Mcculloch & Borello 2000; Fonseca *et al.* 2005; Chokri & Selmi 2011). Waterbirds using coastal saltpans for breeding have to cope with a landscape that may differ significantly from natural coastal wetlands. For example, in most saltpans there is a path network used by workers to move around the saltpans. The unvegetated paths with reduced human presence during the breeding season facilitates the installation of colonies of ground-nesting waterbirds such as plover species *Charadrius sp*. close to the pans and channels of the salinas network. However, waterbirds using these paths might have to cope with a high predation pressure, because the linearity of paths could facilitate nest detection by predators, which are the main cause of nest failure for ground-nesting waterbirds in coastal natural habitats (Rönkä *et al.* 2006; Pietrelli & Biondi 2012; Pearson, Knapp & Sundstrom 2014). Moreover, the proximity of saltpans to urban areas is related with increased disturbance and predation by human-related opportunistic predators such as dogs and cats (Burger 1987). These potential limitations, however, could be counterbalanced by the advantages of breeding in mixed colonies. The reduced available nesting area on paths promotes the formation of relatively dense colonies of ground-nesting waterbirds (Sadoul *et al.* 1998). The increasing density of nesting birds could reduce the predation risk through early detection of predators (Brown and Brown 1987), reduce individual investment in vigilance (Terhunet & Brilliant 1996; Campobello *et al.* 2012) and the probability of a nest being predated through the dilution effect. The anti-predator benefits for a timid species nesting close to a species with strong anti-predator behaviour are often reported in terms of higher reproductive success than conspecifics nesting farther from the protector species (Campobello & Sealy 2011). These nesting associations can be vital to the reproductive success of protected species, with ecosystem-wide implications (Haeming 2001). For example, Piping Plovers *Charadrius melodus* nesting within colonies of mobbing least terns *Sterna antillarum* were more successful than plovers nesting outside colonies (Burger 1987; Powell 2001). In saltpans, American avocets *Recurvirostra americana* and black-winged stilts *Himantopus himantopus* usually nest colonially and defend their nests aggressively against terrestrial and avian predators (Sordahl 1990; Mayer & Ryan 1991). In southern Europe black-winged stilts breed mainly in active or abandoned saltpans (Rufino & Neves 1991; Tinarelli 1991), nesting colonially or semi-colonially (Cramp & Simmons 1983) and mob terrestrial and avian predators near their nesting sites (Godchfeld 1984). This aggressive antipredator behaviour may benefit more timid and solitary species by nesting in the area defended by these "protective umbrella" species. Coastal shorebirds such as plovers breed on sandy-beaches throughout the world (Cramp & Simmons 1983), and many of them are declining and in an unfavorable conservation status (Baillie *et al.* 2004). In Europe, kentish plover *Charadrius alexandrinus* breeding populations have declined or disappeared in many coastal regions (Schulz & Stock 1993; Domínguez & Vidal 2003), mainly due to the increase of tourism and the destruction of natural habitats (Bauer and Thielcke 1982, Lafferty *et al.* 2006, Delany *et al.* 2009). The traditional natural breeding habitats of kentish plovers include mostly coastal sandy beaches and inland lakes, lagoons, alkaline grasslands and seasonal watercourses (Cramp & Simmons 1983). Today, large numbers of kentish plovers breed in saltpans located in Southern Europe (Amat 1993, Pardal 2000, Equipa Atlas 2008) and in other geographical regions worldwide (Page & Stenzel 1981; Yang *et al.* 2011). Here, we conducted an eight years study of the breeding ecology of kentish plover in coastal saltpans of southern Portugal. We examined a series of factors that could possibly influence their nesting success in saltpans. Using kentish plover as a model species, we evaluated specifically if the aggressive antipredator behaviour of black-winged stilts breeding in sparse colonies may benefit a more timid and solitary species. Although previous studies have demonstrated that multi-species breeding is beneficial to plover species (Valle & Scarton 1999; Hanane 2014), most studies did not examine this aspect in relation to other factors influencing nesting success, and did not differentiate the effect of nest distance from nest density of protective umbrella species. We predicted that plovers breeding within a higher density of black-winged stilt nests have a higher nesting success.

Methods

Study area

Field work was carried at Samouco saltpans complex (38°44’N; 8°59’W) in the south bank of Tagus estuary, Portugal (Fig. 15). The study was conducted in the three major breeding areas for kentish plover: saltpans of Brito and Canto, with 9.4 and 12.8 ha respectively (active saltpans), and the saltpans of Providência, with 17.4 ha (these saltpans are no longer active for salt extraction but are managed for waterbirds, regularly controlling

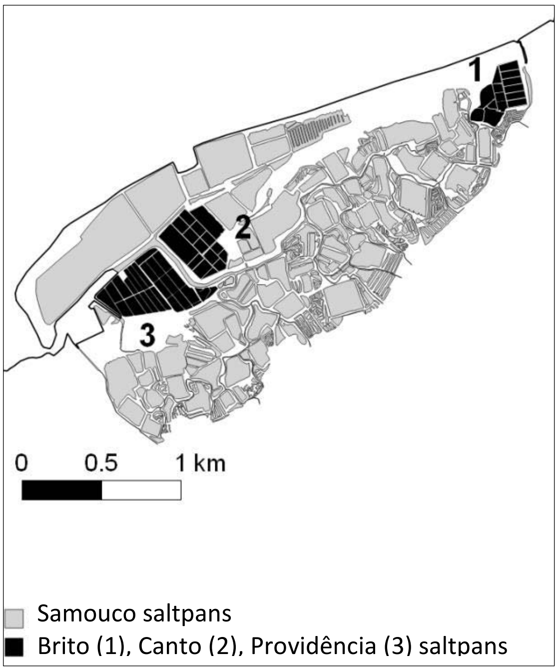

Figure 15. Samouco saltpans complex in detail. Locations of nest-site where characteristics were quantified (1 to 3) and predation experiments were performed (1 and 2) are shown in black.

vegetation and water levels). The remaining saltpans were inspected, but virtually no nests were found. Kentish plovers and black-winged stilts use the paths between the pans to nest. The paths in the main breeding grounds are an average 149.36 ± 12.8 m long, 270.08 ± 6.3 cm wide, and 33.93 ± 0.4 cm high from water. Vegetation in these paths was on average 10.70 ± 0.5 cm tall, covering 11.65 ± 0.6 % of the path surface. Structures such as rocks, wood and plastic debris covered 1.37 ± 0.2 % of the paths. The mean water depth in the pans adjacent to the paths was 5.16 ± 0.2 cm.

Data collection

This study comprised two periods of data collection: a long-term study, from 2006 to 2014, to study the nesting success of kentish plover in the saltpans, and a short-term study, from 2012 to 2014, to evaluate factors that could explain their nesting success in this habitat. Each year fieldwork was conducted during the breeding season (April–July).

Annual variations in nesting success

During the long-term study the nesting areas were visited by two persons twice a week, walking along the paths to find active nests. Nests were marked with numbered stones and their coordinates were recorded using GPS (Garmin, Oregon 550). Nest visits lasted no more than five minutes, and periods with extreme heat (noon and afternoon) were avoided. No predators were observed to take advantage of researcher-induced disturbance to the nests. The nesting population of kentish plover was estimated using the total number of active nests during the peak of the breeding period. This estimate was used because a higher number of pairs correlated with a higher number of nests ($r_s = 0.85$, $n = 8$, $P < 0.01$), so nest sample size was not an artefact of replacement clutches. A nest was considered successful when at least one egg hatched. Successfully hatching was assumed if (1) a recently hatched chick was found in the nest or nearby, (2) at least one egg showed evidence of imminent hatching (cracked or chipped eggshell), or (3) the date of egg disappearance matched the expected hatching date and no sign of predation was found. A nest was considered to have failed if (1) remains of the eggs were found, (2) the nest had been deserted (cold eggs), or (3) eggs disappeared prior to the expected hatching date. The fate of all other nests was considered unknown.

Black-winged stilt nests founded in the breeding areas were also marked with numbered stones and their coordinates recorded using a GPS.

Nest-site characteristics and nesting success

During the 2012 breeding season, we compared nest-site characteristics (see Table 4) of active nests with those of random points along the path. A random point was selected every 5 -10 meters using a table of random numbers. The selected number corresponded to the distance (cm) from the center to the edge of the path, alternating each time the side in which the measurement was made. If a random point was within 1 m of a kentish plover nest, a new point was randomly selected to avoid spatial overlap with nest sites. Nest characteristics were measured using a square grid of $1m^2$ divided into 100 equal parts, centered over each nest and random point. The kentish plover clutches are predated mostly by aerial predators such as gulls, crows or gull-billed terns (Page *et al.* 1983; Amat, Fraga & Arroyo 1999; Pietrelli & Biondi 2012). We visually estimated the visibility of each nest by looking at a fixed circular area of 20 cm^2 centered in the nest, and observed from 100 cm high. In order to get robust samples sizes, we also examined the patterns of nest predation by placing artificial plover nests, in four different visibility classes: 0-25%, 26-50%, 51-75% and 76-100%. Each artificial plover nest consisted of a shallow scrape containing two domestic quail *Coturnix coturnix* eggs, which are comparable in size, shape, and

Table 4. Comparison (mean ± SE) between characteristics of active nests of kentish plovers (n = 88) and random points (n = 275). *: variables with p < 0.05 in a GLM univariate analysis. See text for details.

	Variables	Description	Nests	Random points
1	Vegetation cover (%)	Percentage of vegetation with more than 5 cm height	9.78 ± 1.4	9.00 ± 0.9
2	Water (%) *	Percentage of water	19.62 ± 1.9	7.49 ± 0.8
3	Vegetation height (cm) *	The height of the tallest vegetation	11.67 ± 1.5	6.87 ± 0.6
4	Structures (%) *	Percentage of structures (mainly rocks and pieces of wood) with more than 5 cm height	3.52 ± 0.66	1.38 ± 0.2
5	Water distance (cm)	Shortest distance from the nest to the water	77.90 ± 18.4	91.37 ± 3.6
6	Nest visibility (%) *	Visibility of the nest as measured by looking directly from a height of 1 m and 20 cm^2 around.	72.02 ± 3.2	92.93 ± 0.9

colour to kentish plover eggs (e.g. Marini and Melo (1998). We compared the number of days that kentish plover and artificial nests were active (nest exposure days; see statistical analysis below) according to the visibility classes. During June 2012, we placed nine artificial nests in each visibility class along different paths. Nests were marked with numbered stones and their coordinates were recorded using a GPS. The nests were visited twice a week. If all eggs from one path were predated, we placed the artificial nests in a new path.

Proximity to black-winged stilt nests and nesting success of plovers

We evaluated the role of nesting associations between kentish plover and black-winged stilt in the nesting success of plovers during the 2014 breeding season. We performed an experiment using artificial nests (see above). Two stilt sparse colonies (density: 0.0033 - 0.0035 nests m^{-2}) located on paths between pans were selected. From every active stilt nest (center nest), we placed on the path two artificial plover nests per distance class (<20, 20-40, 40-80, 80-100 and >100m). Within each distance class, the precise location of artificial nests were defined by selecting two random points from a table of random numbers, the first corresponding to the distance (m) from the stilt nest and the second to the distance (cm) from the edge of the path. Nests were marked with numbered stones and their coordinates were recorded using a GPS. The nests were visited twice a week and were replaced in a new location when both eggs were predated or after 27.2 days, the mean hatching period for kentish plover (Fraga and Amat 1996).

Predator identity and activity in the breeding colonies

During the 2014 breeding season, we examined the composition of the predator community, the relative importance of each predator, daily predatory activity, and the monthly patterns of nest predation in saltpans. We performed diurnal observations, and set infrared-triggered cameras in artificial and natural kentish plover nests. We spent 825 minutes in May and 620 minutes in June, in two hour periods (early morning and late afternoon), twice a week, observing the breeding sites to record the number of each potential predator species and the number of breeding waterbirds involved in anti-predatory displaying. The anti-predatory behaviour by breeding waterbird species was recorded in four categories: no action, alarm calls, chasing and attacking potential predators. The same variables were recorded in occasional observations during the visits to the breeding sites. Nocturnal and also diurnal nest predators were monitored using movement triggered cameras (Moultrie A-5). Each camera was positioned on the ground, at four meters distance from artificial plover nests (as described above). When a predator approached the nest, the motion beam was cut and it was photographed. Eight cameras were set to work constantly all day i.e, for 24 hours with 1 minute of trigger delay during three months (14,372 hours), recording the date and time of each taken photo. Each camera was moved to another nest after egg predation or after 15 days in the same location.

Statistical analyses

Kentish plover nesting success was calculated using the Mayfield method (Mayfield 1961, 1975). The exposure days (observation days) were measured in relation to the outcome of the nests. Successful nests and failed nests had an exposure equal to the number of days since the discovery of the nest, until the day in the middle of the period between the day that the clutch disappeared and the last day the nest was known to be active. Nests with an unknown outcome had an exposure equal to the number of days since their discovery until their last active known day (Manolis, Andersen & Cuthbert 2000). The probability of nest success (PEN) was obtained by raising the daily survival estimate (p) to the mean incubating days of kentish plover (25.5 days): $PEN = p^n$. The daily survival estimate (p) was calculated by: $p = 1 - (L/E)$, where L was the total number of nests losses during the time the nests were observed and E was the total number of exposure days for all nests. We used one-way ANOVA (after checking normality and the homogeneity of variance) to examine differences in the number of exposure days between: a) years, and b) the four different nest visibility classes (for natural and artificial nests separately). Post-hoc Tukey tests for multiple comparisons were used when appropriate. We analyzed kentish plover nest-site selection with a generalized linear model (GLM, binomial distribution model with logit link function) using nest type as the dependent variable (1 = nest, 0 = random point) and the independent variables from Table 4. Firstly, we performed a univariate analysis to verify the relationship between nest type and each nest-site characteristic, and retained all variables with a $P < 0.25$. Secondly, we built a multivariate model using all variables with a $P < 0.25$ in the univariate analysis (Hosmer, Lemeshow & Sturdivant 2013). Then we verified step by step the importance of each variable and retained in the final model all variables with a $P < 0.05$.

A Spearman rank correlation was used to relate estimated number of pairs and total number of nests with nesting success (PEN). Nest-site characteristics were transformed (log + 1), and subjected to a Principal Component Analysis (PCA) due to collinearity between variables and to reduce the number of independent variables into three principal components, that were correlated (Pearson correlation) with nesting success (PEN). We divided the number of days with active kentish plover nests by the number of days with active nests of black-winged Stilt for each year to assess the breeding season overlap between the two species. This variable was used in a linear regression to assess the potential influence of the black-winged stilt anti-predator behaviour on kentish plover nesting success (PEN). We assessed whether the number of exposure days differed between kentish plover and artificial nests using a GLM, with month (May, June, July) as a random factor, in order to control for differences in success between the three months of the breeding season.

The potential benefit of kentish plover breeding in the area defended by black-winged stilts was analyzed performing a GLM mixed model with type III Sum of Squares and using transformed (log + 1) variables: number of exposition days (dependent variable), breeding month (May, June), the number of black-winged stilts active nests inside a 40 m radius around each stilt nest, and the distance to the nearest black-winged stilts nest (independent variables). The number of artificial nests inside a 40 m radius was used as random factor, in order to control for the possible effect that artificial nests could have in attracting predators. A 40 m radius circle was selected because this is the distance that typically Recurvirostrids perform distraction displays to predators (Sordahl 1989). The number of black-winged stilt and artificial nests (with at least one egg) were counted inside the 40 m radius of each stilt's nest, as the mean number of nests per day during the period that the center nest was active. The breeding period started when the first kentish plover nest was found (day 1 = 14 of April). For each artificial nest we measured the distance (m) to all active black-winged stilt nests in the saltpans. A Chi square test was performed to assess differences in predation rate of trigged kentish plover and artificial plover nest by the most important nest predators. All results are presented as mean ± SE.

Results

Annual variations in nesting success

A mean of 56.0 ± 5.5 breeding pairs of kentish plover were found in Samouco saltpans (range: 29 pairs in 2009 to 76 pairs in 2011). The first clutches were found between 26 March and 14 April, and the peak of breeding activity occurred from mid-May to mid-June, with an average of the 43.0 ± 2.8 % of the total number of nests. The last nests were found between 1 and 27 of July. Most of the clutches were found along the paths between pans (70.0 ± 9.8 %). The mean annual nest success of kentish plover (PEN) was 0.25 ± 0.1, and was very variable over the years, with values ranging from 0.04 to 0.50. The mean annual exposure was 7.9 ± 0.9 days, ranging from 4.6 ± 1.0 to 12.8 ± 1.1 days (Fig. 16). The number of exposure days differed significantly among years ($F_{7, 1145}$ = 10,401, P < 0,001), with the highest value in 2010, and the lowest values in 2009 and 2012. There was no correlation between either number of pairs or number of nests with nesting success (r_s = 0.19, P = 0.65 and r_s = 0.06, P = 0.89, both n = 8).

Relationship between nest-site characteristics and breeding success

Kentish plovers selected nest-sites in the

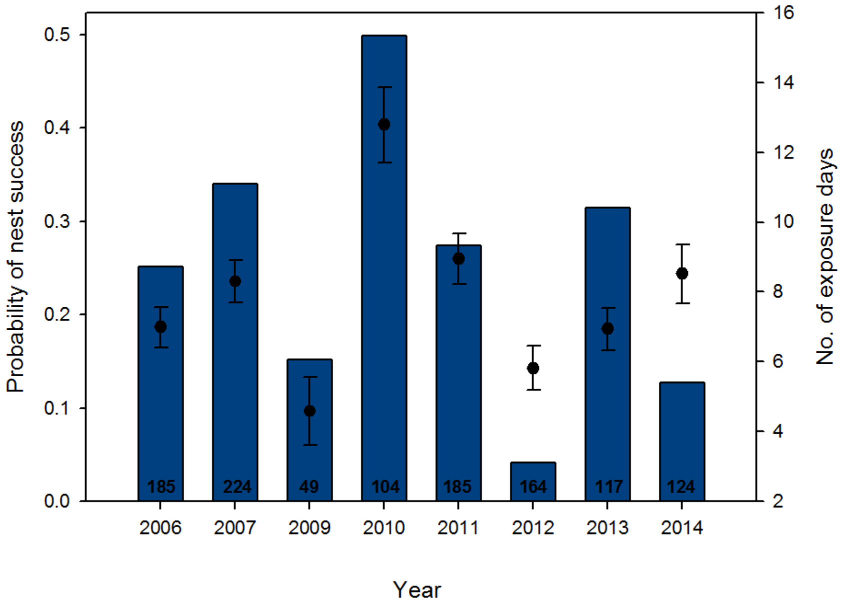

Figure 16. Annual variation in kentish plover nesting success in the study area using the Mayfield method. The probability of nest success (bars) is shown on the left y-axis and the mean number of days (± SE) that nests were exposed is presented on the right y-axis. Sample size (number of nests) is presented in the bars.

edges of the paths, closer to the water, taller vegetation, closer to structures, and with less visibility from above, when compared with random points (Table 4). A multivariate GLM with a binomial distribution and logit link function showed that only two variables were important in separating random points (reference category) from nest-sites: percentage of water around the nest (coefficient = -0.034 ± 0.08, Wald $X^2 = 17.20$, $p < 0.001$) and nest visibility (coefficient = 0.027 ± 0.05, Wald $X^2 = 28.96$, $p < 0.001$). In the paths, more than 50% of kentish plover nests had little or no vegetation cover (76-100% visibility from above). Despite the fact that less visible nests had a higher number of exposure days, nest visibility did not influence the number of days that both natural and artificial nests were exposed ($F_{3,\,125} = 0.58$, $P = 0.63$ and $F_{2,\,96} = 1.14$, $P = 0.33$; Fig. 17).

The six nest-site variables were reduced by the PCA to three main independent components that explained 88.05 % of the total variance. The first component (PC1) was positively correlated to distance to water and negatively correlated with percentage of water. The second component (PC2) was positively correlated with vegetation cover, vegetation height and nest visibility. The third component (PC3) was positively correlated with the presence of structures. However, none of these nest-site characteristics were significantly correlated with nesting success (PC1: r = -0.25, $p = 0.39$; PC2: r = -0.06, $p = 0.83$; PC3: r = 0.38, $p = 0.18$).

Presence of black-winged stilt nests and nesting success

Overall, kentish plover nested in mixed

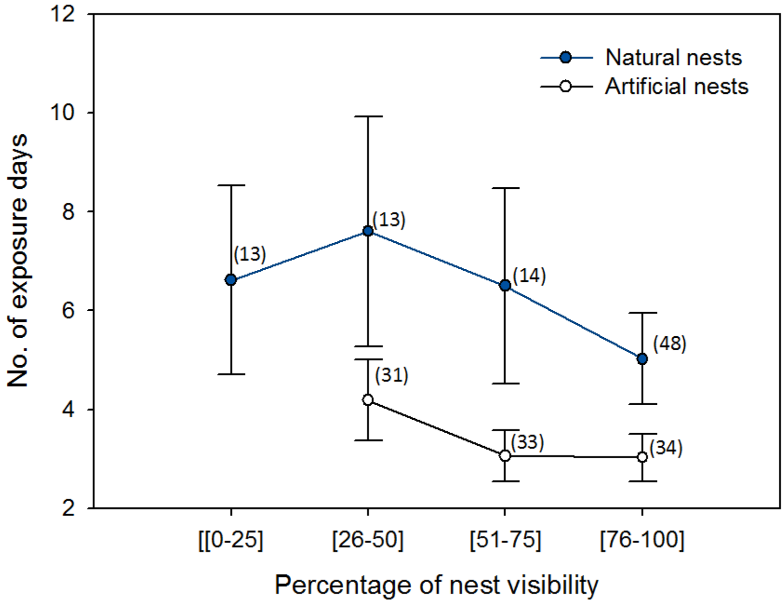

Figure 17. Number of exposure days (mean ± SE) of kentish plover nests (n = 129) and artificial plover nests (n = 99) according to visibility categories. No artificial nests were placed in the visibility category of 0-25%.

colonies with black-winged stilts and little terns *Sternulla albifrons*. The presence of breeding black-winged stilts had a significant and positive effect on nesting success of kentish plover in saltpans (Fig. 18). The nest success of the kentish plover increased when their breeding season coincided with that of black-winged stilts during a 8 year period [PEN = -0.1994 (SE = 0.1151) + 0.6749 (SE = 0.1676)*proportion of coincidence, adjusted r^2 = 0.73, $F_{1,6}$ =16.22, P < 0.007]. During the breeding season of 2014, when potential predators, were present, black-winged stilt was responsible for 76% of alarm calls and 93% of predator chasing, and little terns were responsible for 61 % of predator attacks. Similar results were obtained for the occasional observations (see Appendix 4).

During the entire breeding season we placed a total of 454 artificial nests close to the black-winged stilt colonies, ranging from 1.3 to 270.1 m from black-winged stilt nests. Within the 40 m radius circle from black-winged stilts nests, we recorded a mean of 6.11 ± 0.1 artificial plover and 0.80 ± 0.1 black-winged stilt nests. The number of exposure days of artificial nests was significantly and positively affected by the density of black-winged stilt nests ($F_{1,349}$ = 12.546, p < 0.001; Fig. 19) and the breeding month ($F_{1,349}$ = 9.70, P = 0.002): the number of exposure days was higher with increasing number of black-winged stilt nests for the last month (June) of the breeding period. There was no effect of the distance to nearest black-winged stilt nest ($F_{1,349}$ = 0.05, p = 0.831) in the number of exposure days of artificial plover nests.

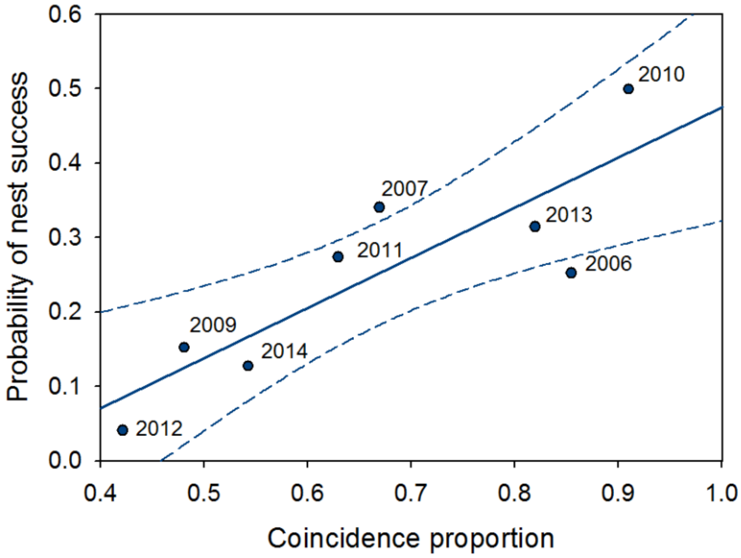

Figure 18. Linear regression with 95% confidence intervals between kentish plover probability of nest success with the proportion of coincidence between the breeding season of kentish plover and black-winged stilt (obtained dividing the number of days with active kentish plover nests by the number of days with active nests of black-winged stilt).

Nest predators

During the 2014 breeding season we observed 87 events of potential nest predators: 44% corresponded to carrion crows *Corvus corone*, 16 % to marsh harriers *Circus aeruginosus*, and 15% to common kestrels *Falco tinnunculus* (Appendix 4). Only one mammal species, domestic cat *Felis silvestris catus*, was detected during our diurnal observations. We repeatedly observed two carrion crows working together, the first bird flying over the colony and when breeding birds left the nests to chase it, the second crow landed and searched for eggs along the paths.

We monitored 51 nests of kentish plover and 108 artificial nests with cameras, and 42% of these nests were predated. We found no differences in the number of exposure days between kentish plover (2.35 ± 0.5 days) and artificial (3.34 ± 0.8 days, $F_{1,62} = 0.32$, $p = 0.575$; using month as a random factor) nests. Birds were responsible for most nest predation (58%), followed by mammals (33%; Appendix 5). Bird predation was mainly by carrion crows (58%), magpies *Pica pica*, (34%) and turnstones *Arenaria interpres* (8%). On three occasions, turnstones were recorded chasing kentish plover from their nests and then predating the eggs. The main mammal nest predators were red foxes *Vulpes vulpes* (73%), brown rats *Rattus norvegicus* (23%) and domestic cats *Felis silvestris catus* (4%). The predation rate of kentish plover nests did not differ between avian (29%) and mammalian (53%) predators ($X^2 = 1.1$, df = 1, $p = 0.28$, with Yates correction), but a significant difference was detected for the artificial nests (birds = 70%, mammals = 28%; X^2 =

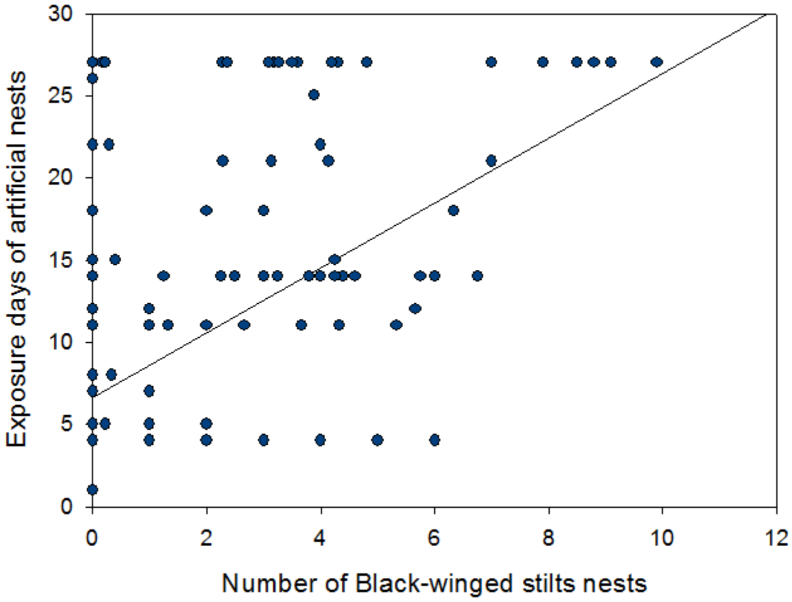

Figure 19. Relationship between number of days that the eggs were present in artificial kentish plover nests and mean number of black-winged stilt nests per day during the period that the artificial nest was active.

7.7, df = 1, *p* = 0.005, with Yates correction). Carrion crows were responsible for most nest predation during May (38%) and July (54%), and red foxes during June (33%). Predators were recorded in the colonies during different times of the day. During daytime, carrion crows predated nest in the first hours of the day, while magpies predated in late afternoon (18 – 20 h; Fig. 20). Mammal predation was detected only during night, with red foxes predating eggs between 22:00 and 04:00 h.

Discussion

Our study showed that coastal saltpans are an important alternative breeding habitat for kentish plover, as their nesting success was similar or even higher than that of natural habitats (see Norte and Ramos 2004). In both natural (Székely 1990; Schulz & Stock 1993; Domínguez & Vidal 2003; Pietrelli & Biondi 2012) and alternative habitats (Székely 1992, this study) predation is the main cause of nest loss. We found that breeding close to semi-colonial species with marked anti-predator mobbing behaviour significantly

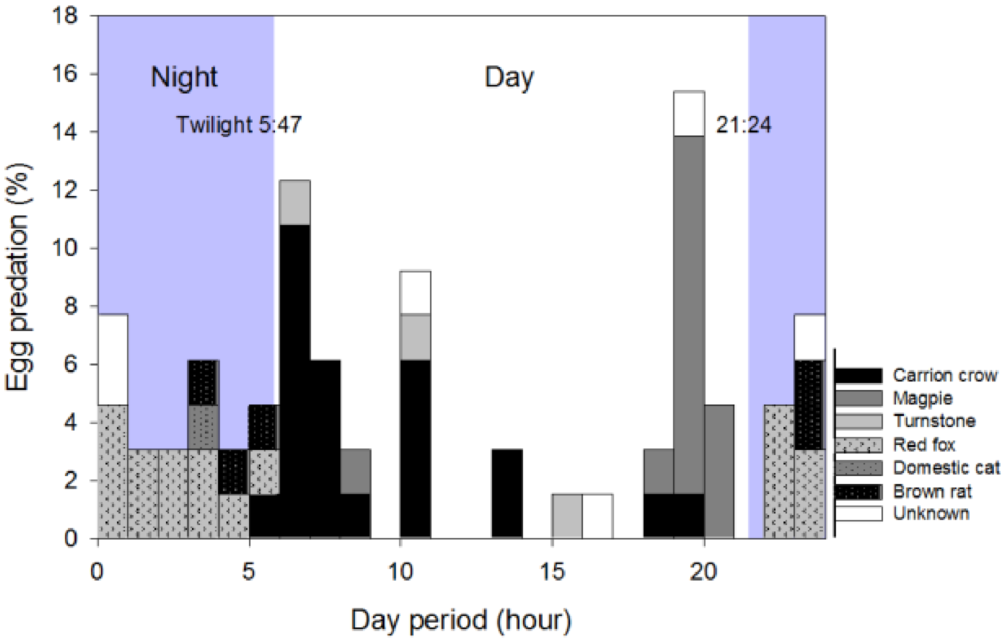

Figure 20. Egg predation recorded by triggered cameras in the breeding areas during a 24 h period between May and July of 2014. The day period was considered between the mean morning and afternoon twilights. Triggered cameras detected two mammals species: red fox *Vulpes vulpes* and brown rat *Rattus norvegicus*, three bird species: carrion crow *Corvus corone*, magpie *Pica pica* and turnstone *Arenaria interpres* and six none identified predators (unknown).

increased nesting success in a more timid and non-colonial shorebird species such as kentish plover.

The mean nesting success of kentish plover in our study site (0.25) was higher than that in natural sandy beaches of the Portuguese west coast (0.12; Norte and Ramos 2004), and in natural inland saline lakes of southern Spain (0.19; Fraga and Amat 1996), and similar to coastal lagoons of the United Arab Emirates (0.22; Kosztolanyi *et al.* 2009). We found that neither the number of nests nor the number of breeding pairs at the peak of the breeding period was related with nesting success of kentish plovers. Norte and Ramos (2004) compiled data from man-made and natural habitats using traditional methods over one or two breeding seasons, and they found no differences in nesting success between both habitats. Also, Toral and Figuerola (2012) found that nesting success of kentish plover in rice fields (0.45) was similar or even higher than that in marshes in southwest Spain (0.29). Studies of other waterbird species that nest in artificial and natural coastal habitats found similar results. For example, Yasué *et al.* (2007) found no significant differences in nesting success of malaysian plover *Charadrius peronii* breeding in sandy beaches and semi-natural saltflats in Thailand; in Hungary the hatching success of pied avocets *Recurvirostra avosetta* was higher in semi-natural sites (dry fishpond, reconstructed wetlands) than in natural alkaline lakes; Catry *et al.* (2004) found no significant difference in the nesting success of little tern population between sandy beaches (30.9 %) and saltpans (30.1 %) in coastal Portugal. Our long-term study showed a strong inter-annual fluctuation in the kentish plover nesting success in Samouco saltpans, which is similar to the variability among sites compiled by Norte and Ramos (2004), and also with a number of studies of snowy plover (*Charadrius nivosus*; (Wilson-Jacobs and Meslow 1984, Powell 2001, Colwell *et al.* 2010). Therefore, annual and site specificity differences are likely to overcome the habitat per se in explaining nesting success of plover species.

Our nest-site selection study in the saltpans showed that kentish plovers prefer to nest on paths' edges. The elevation of paths and the relatively constant water level in the adjacent pans allowed the selection of nest-sites near the water. The distance to water was much longer in natural habitats, e.g. sandy beaches (Rocha, Trigo & Luís 2004) where high tides could cause important nest failure (Norte & Ramos 2004). Distance to water is an important factor under very hot conditions, because incubating birds are probably unable to maintain homeostasis for long periods. In nest-sites close to water it was possible to quickly soak the belly (i.e. wetting their ventral plumage; Maclean 1975; Kalsi and Khera 1986), which would allow a more continuous nest attendance (Amat & Masero 2004). Furthermore, nesting in the path's edge would allow a better view of predators approaching benefiting from its linearity and elevation relatively to water. As in the natural habitats kentish plover selected nest-site with sparse vegetation cover (Cramp & Simmons 1983; Székely 1990) or built the nests in close proximity to structures (Page *et al.* 1983; Powell 2001). Structures can work as a shelter from predominate winds (Norte & Ramos 2004), vegetation cover near the nest can shelter the chicks after hatching (Lorenzo & González 1993), and both can also help to dissimulate the nest-site, and difficult the detection of possible terrestrial predators by incubating birds (Walters 1984). The presence of structures and sparse vegetation should also reduce the thermal stress of the incubating adults, especially when the ambient temperatures at ground level might be > 50 ºC. Kentish plover nesting in sites with no or little cover may be unable to maintain homeostasis for long periods and the nests may be deserted (Amat & Masero 2004), however, it is clear that kentish plover do not select dense and tall vegetation to nest (Norte and Ramos 2004; this study), presumably because those characteristics prevent the birds from detecting predators (Amat & Masero 2004; Medeiros *et al.* 2012). Similarly to other studies on both kentish plovers (Norte & Ramos 2004) and other coastal species (Powell 2001; Medeiros *et al.* 2012; Pearson *et al.* 2014) we did not find a relationship between selected nest site characteristics and nesting success. This suggests that selected physical habitat characteristics may be more important for thermal regulation of eggs and incubating birds than to dissimulate the nest from predators or that predation is highly stochastic.

Based on the strong variability in nesting success among years and sites (Norte & Ramos 2004), and on our observations of potential nest predators that used the study area we expected that predation would be the main factor explaining nesting success of the kentish plover in this study. The use of Infrared-triggered cameras on both natural and artificial nests clearly showed that around 50% of the nests were predated. The diversity of nest predators in our study was lower than that reported in most studies with breeding plovers in natural habitats (Mayer & Ryan 1991; Fraga & Amat 1996; Brunton 1999). The variability of predators among sites suggests that the breeding location is more important to explain the net effect of predation than habitat type. Our study was the first using infrared-triggered cameras to provided novel information regarding predator identification and timing of predation for kentish plover nest: most nests were predated from 19:00 to 11:00 h, and very few were predated during the hotter part of the day. As expected, avian predators were most active early in the morning and late in the afternoon, and mammal predators were active during the entire nighttime. We found also that the number of diurnal species involved in nest predation was much lower than the number of potential predators observed. Carrion crows were the main observed potential predator and were effectively responsible for most nest predation in our saltpans study site, predating eggs in the morning period. This species was also observed as the main predator of kentish plover nests in coastal natural habitats (Pietrelli and Biondi 2012; Domínguez and Vidal 2003). The predation by magpies in late afternoon may be related with the avoidance of midday high temperatures (Birkhead 1991) and competition with carrion crows in the morning. This species was identified as the main nest predator of kentish plover nests at the Llobregat delta in Spain (Ballesteros & Torre 1993). Corvids or their signs were reported as the main snowy plover nest loss in natural habitats of Western North America (Powell 2001; Colwell *et al.* 2005). However, when gull *Larus sp.* or gull-billed tern *Gelochelidon nilotica* colonies are present close to plovers breeding sites, they seem to be responsible for most nest losses (Page *et al.* 1983; Fraga & Amat 1996). Turnstones were observed preying eggs from kentish plover nests (Fraga & Amat 1996) or from other breeding species in saltpans (Catry *et al.* 2004). In our study we recorded egg predation by migratory turnstones when breeding sites were used as a high-tide roosting site by large numbers of shorebirds. In evaporation salt ponds in California, nests of western snowy plover *C. n. nivosus* monitored with remote surveillance systems showed a 43% nest predation. Egg loss was recorded mainly by bird predation, most by California gulls (*Larus californicus*), ravens (*Corvus corax*), birds of prey and turnstones (Demers & Robinson-Nilsen 2012). Contrary to this study, we did not record birds of prey predating on kentish plover eggs, although marsh harrier and common kestrel were the second and third most observed potential predators in the colonies. This may be due to anti-predatory behaviour of black-winged stilts (see below), more than the detection failure of fast predation events by trigger cameras as suggested by Demers and Robinson-Nilsen (2012). Of the three recorded mammal species, Red foxes were responsible for most nest losses, and were active only during the night. Foxes are mainly active at night in areas with regular human presence (Lucherini, Lovari & Crema 1995) such as the man-made saltpans. Foxes were also recorded (by signs) as the main mammal predator of kentish plover nests in natural habitats (Norte & Ramos 2004; Lengyel 2006; Pietrelli & Biondi 2012), and were even observed predating incubating birds (Amat & Masero 2004). Due to the high abundance of Red foxes, they were the most common mammalian nest predator of other ground breeding bird species (lapwing *Vanellus vanellus* and great bustard *Otis tarda*) recorded by trigger cameras (Bolton *et al.* 2007; Parker 2014). In saltpans, Catry *et al.* (2004) found signs of predation by dogs, domestic cats and brown rats of little tern nests. We detected the same mammal nest predators in our study site, except dogs.

We showed that when the breeding season of kentish plover was broadly coincident with that of the black-winged stilt, the nesting success of the first species was significantly higher. The results of the artificial nests experiment showed that kentish plover should benefit from the presence of nesting black-winged stilts. Kentish plover is a timid species and in the presence of potential predators perform distraction-lure displays (Cramp & Simmons 1983). Predator-mobbing (the tendency to chasing and attack potential predators) occurs only in larger shorebird species such black-winged stilts (Sordahl 1981). However, black-winged stilt anti-predator behaviour seemed less effective when aerial predators could land in the colony or worked in group. This predatory behaviour and the presence of mammalian predators could explain why nest visibility from above had no influence in the predation rate. Our experiment suggests that even a reduced density of shorebird breeders with strong anti-predator behaviour in saltpans, should be more important for the nest success of smaller shorebird species, than only the distance to their nearest nest. A similar result was found by Mayer and Ryan (1991) in alkali wetlands of North Dakota, where they found no evidence of increased survival rates of artificial piping plover *Charadrius melodus* nests located in proximity to American avocets *Recurvirostra Americana* nests. These authors suggests that colonies must reach a critical size and density before avocets can successfully repel nest predators, also American avocets should be less effective at driving off mammalian predators, which are responsible for most piping Plover nest losses. A different result was found by Powell 82001) in California, where the nearest distance to least tern *Sterna antillarum* nest influence the predation risk of snowy plovers but not tern colony size. Lengyel (2006) found that the hatching success of pied avocets in Hungary was higher in colonies with at least 15 pairs. The hatching success of piping plovers nesting in New Jersey was explained by the density of Least Tern nests within colonies where both avian and mammal predators were present (Burger 1987). Likewise, Brunton (1999) found that predation by small mammals, gulls, and crows was dependent on Least tern colony size and these predators were deterred from colonies with more than 100 nests. We concluded that kentish plover nest success benefited from the density of black-winged stilts nests and the capability of stilts to mobbing potential predators varies. Black-winged stilts proved to be a strong anti-predator against birds of prey, but were less efficient against carrion crows and magpies (on the ground) and even less so with mammals.

Conservation implications

Given that the natural coastal habitats will continue to be lost owing to the increase of human disturbance, development, sea level rise and coastal erosion, man-made habitats such as saltpans will be increasingly important as an alternative nesting habitat for coastal bird species. In this study we demonstrated that saltpans provide at least similar breeding conditions for kentish plover in terms of nesting success and predation pressure to those of natural habitats. Moreover, saltpans could play an important role in the conservation of kentish plovers, if they are able to nest in mixed colonies with black-winged stilts. Therefore, large breeding populations of both small and large wader species will have a positive feedback on each other's breeding success. This must be born in mind when planning conservation strategies for wader species breeding in alternative habitats. Despite the importance of saltpans for waterbirds, they have been progressively abandoned (Múrias *et al.* 2002), leading to vegetation overgrowth and flooding, which does not benefit kentish plovers. Therefore, preventing abandonment and limiting predation are the most urgent actions in order to maintain nesting habitat for kentish plovers and other coastal breeding waterbirds.

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Appendix

Appendix 4. Number of records of potential nest predators obtained during observation periods (total number of minutes per month) and in occasional observations (n) on the main breeding areas. During two months 12 bird and one mammal species were detected. The anti-predator behaviour of breeding black-winged stilt (BWS) and little tern (LT) was recorded in 4 categories (see text for details).

Observations	Periods				Occasional			
Months	May		June		May		June	
Duration time and events (n)	825 min		620 min		22		7	
Diurnal predators	47		40		22		7	
Carrion crow	15		23		8		0	
Marsh harrier	13		1		8		2	
Common kestrel	3		10		1		0	
Little egret	3		2		0		1	
Black kite	3		0		0		0	
Peregrine falcon	1		1		2		0	
Lesser black-backed gull	2		0		2		3	
Black-headed gull	1		1		0		1	
Whimbrel	2		0		0		0	
Magpie	1		1		0		0	
Spotless starling	2		0		0		0	
Turnstones	1		0		1		0	
Domestic cat	0		1		0		0	
Anti-predator behaviour	**BWS**	**LT**	**BWS**	**LT**	**BWS**	**LT**	**BWS**	**LT**
1 - No action	12		4		2		1	
2 - Alarm calls	10	5	6	0	7	0	0	0
3 – Chasing	61	1	39	7	54	2	16	3
4 – Attack	9	10	0	4	3	6	0	1

Appendix 5. Nest predation recorded by cameras in natural nests of kentish plover (KP) and artificial plover nets (AN) during three months. Monitored time corresponded to the period of time (Mean ± SE) between visits from cameras setting to collecting (after predation or 15 days without predation) and time to predation event and time (mean ± SE) from setting to the predation of both eggs.

	May		**June**		**July**	
	KP	**AN**	**KP**	**AN**	**KP**	**AN**
Nests (n)	20	44	17	39	12	25
Monitored time (min)	6375.68± 634.15	5621,86± 419.98	4535.59± 1458.40	8069.13± 800.05	4778.92± 456.73	11432.20± 1004.04
Time to predation event (min)	4799.44± 1283.17	1946.74± 552.59	2246.20± 1203.62	4373.75± 967.14	456.74± 576.77	8130.33± 1454.98
Predation (%)	41	55	24	41	33	36
Birds (n=38)	**22**		**9**		**7**	
Turnstone	2	1	0	0	0	0
Carrion crow	1	10	1	3	2	5
Magpie	1	7	0	5	0	0
Mammals (n=22)	**7**		**9**		**6**	
Brown rat	0	0	1	2	1	1
Domestic cat	0	0	0	0	1	0
Red fox	5	2	2	4	0	3
No identified predator	0	4	0	2	0	0

Chapter IV

Morphological, physiological and behavioural responses to salinity during growth: an experiment with black-winged stilt chicks


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Abstract

Combined physiological and behavioural responses to salt loads during growth have rarely been studied in air-breathing vertebrates able to inhabit in hypersaline habitats, but they may be of particular importance in understanding the differences among species in patterns of habitat use or ontogenetic diet switches. The black-winged stilt *Himantopus himantopus* is a waterbird species that breeds in a range of habitats from freshwater to hypersaline wetlands. Here, we compared basal metabolic rate (BMR), heat shock proteins (Hsp70), plasma ions, hematocrit, body mass, growth rate and behavioural responses of captive-reared black-winged stilt chicks maintained under three salinity regimes: 0 ‰, 20 ‰, and 60 ‰. Additionally, we compared BMR and saltgland mass in wild fledglings from freshwater and hypersaline water. Contrary to expectations, none of the physiological and morphological variables measured differed significantly among treatments. Likewise, the BMR of wild and captive-reared fledglings was similar. Surprisingly, the saltgland mass of wild fledglings from freshwater and those from hipersaline habitats was also similar. However, head-shaking, a behavioural response associated to minimise salt intake and to expel the secretions of salt glands, differed according to salinity level, with head-shaking rate increasing with salinity. The results of this study support the key role of behavioural osmoregulation in avoiding salt stress during growth.

Key words: basal metabolic rate, behaviour, heat shock proteins, salt stress, shorebirds

Introduction

Salinity is a crucial environmental factor affecting physiological performances in all organisms, and represents one of the main natural sources of stress shaping the biodiversity of ecosystems (Odum 1985). During their seasonal movements, billions of vertebrates as diverse as fishes, birds, reptiles, marine mammals and amphibians are subjected to large changes in the salinity of their environments. A suit of physiological, anatomical, morphological and behavioural adaptations ensure that they are able to maintain osmotic homeostasis even under highly saline conditions (Schmidt-Nielsen 1997; Bentley 2002; McNab 2002; Gutiérrez 2014).

Salinity fluctuations have a significant negative effect on the growth of fishes, amphibians, and marine invertebrates (e.g. Martins *et al.* 2014; Bai *et al.* 2015; Nakkrasae *et al.* 2015). In birds, laboratory and field studies have demonstrated that birds developed under highly saline conditions often exhibit depressed growth rate and, body mass, reduced immune response, and dehydration (reviewed in Gutiérrez 2014). However, the life history of a large number of waterbirds shows that they typically rely on saline and hypersaline wetlands as breeding habitats. Many of these waterbirds are precocial species with self-feeding chicks (for example Charadriiformes), hence they must have a set of co-evolved traits which enable them to deal with saline and hypersaline water. Unfortunately, studies about the effects of salt intake in precocial chicks that rely on hypersaline wetlands for feeding are very scarce (but see Hannam *et al.* 2003). As in the case of adult birds, the responses of precocial chicks to salinity should be interpreted in the context of combined physiological and behavioural responses (Mahoney 1985; Mahoney & Jehl 1985; Gutiérrez 2014), as the effectiveness of behavioural responses might eliminate the need to invoke comparatively more expensive physiological responses (Dunson 1981; McNab 2002). Knowledge of these responses will be critical in understanding the impact of anthropogenically-caused salinization on wildlife, which is a conservation issue of global concern (Rubega & Robinson 1996; Hannam *et al.* 2003; Gutiérrez 2014).

The basal metabolic rate (BMR) represents an animal's maintenance cost, and is useful as a physiological standard for animal performance (e.g. Rønning *et al.* 2007). In birds, this flexible physiological trait is known to show physiological adaptations to ecological conditions (e.g. Lindström and Klaassen 2003; Gutiérrez *et al.* 2012). A recent study showed that adult individuals of a shorebird species, the dunlin *Calidris alpina,* increased their BMR with increasing salinity (Gutiérrez *et al.* 2011). This finding provided strong support that maintaining a functional osmoregulatory machinery to excrete excess salt imposed significant energy costs, probably owing to the increased size and metabolic intensity of supraorbital saltglands (Gutiérrez *et al.* 2011, 2015), the key organ used by waterbirds to ensure survival in saline environments (Gutiérrez 2014). It is believed that such costs are unequal in adults and chicks, but studies addressing this question are scarce (reviewed in Gutiérrez 2014). In the case of shorebirds inhabiting hypersaline habitats, precocial chicks of species such as the American avocet *Recurvirostra americana* have relative large saltglands (Rubega & Oring 2004). The size of bird saltglands is strongly influenced by salinity levels (Staaland 1967; Gutiérrez *et al.* 2012, 2015), so self-feeding chicks of shorebird species breeding across a gradient of environmental salinity could adjust their basal metabolic rate (BMR) according to salinity levels. Studies involving the basal metabolic response of precocial chicks in relation to water salinity will provide new insights into the potential energy costs of inhabiting hypersaline habitats, and the relative flexibility of this physiological trait to deal with salinity.

The ability of some waterbirds to inhabit hypersaline and alkaline wetlands has been suggested to rely on behavioural and, perhaps, anatomical adaptations for reducing the intake of saline water, as well as on the hypo-osmolality of their food supply (Mahoney and Jehl, 1985; see reviews by Sabat 2000; Gutiérrez 2014). For example, head-shaking is a behavioural response associated to minimize salt intake and to expel the secretions of salt glands (Peaker & Linzell 1975; Mahoney & Jehl 1985). The behavioural and anatomical adaptations are not as well studied as physiological mechanisms, but they may be crucial to maintain the osmotic balance in these species (reviewed in Gutiérrez 2014). The combined study of physiological and behavioural responses of precocial chicks dealing with different salinities may provide explanations for differences among species in patterns of habitat use, ontogenetic diet switches or in estimating the optimal diet.

Here, we examined BMR, body mass, growth rate, plasma concentrations of sodium and chloride, hematocrit, heat shock proteins (Hsp70), and shaking-behaviour of precocial shorebird chicks reared in freshwater, saline, and hypersaline water. This set of physiological variables were measured because they may reflect saline stress in birds (e.g. Gutiérrez *et al.* 2015). We used the black-winged Stilt *Himantopus himantopus* (here onwards, stilt) as model species, a precocial shorebird that inhabits a variety of habitats during breeding, ranging from inland freshwater, to brackish, saline and hypersaline wetlands (Cramp & Simmons 1983; Pierce 1996). We also compared BMR and saltgland mass in wild fledglings from freshwater and hypersaline water. We predicted that captive-reared stilt chicks would increase significantly their BMR and head shaking-behaviour with increasing salinity. If stilt chicks ingest large amounts of water and its associated ionic load, we expected that chicks coping with saline and hypersaline water during growth show changes in plasma ions, hematocrit, body mass, growth rate, and Hsp70.

Methods

Captive-reared chicks

Wild chicks were captured during spring 2013 in the complex of Samouco saltpans, in the Tagus estuary, Portugal (38°44’N, 8°59’W). The salinity of saltpans ranged from 60 to >300 ‰ during the breeding season (April-July). At the saltpans, stilts use the dykes of the hypersaline pans to place their nests, so their precocial chicks have to cope with a saline environment just after hatching. We randomly chose a chick in broods with more than two chicks. Captured chicks were aged according to bill-length (age [days] = -15.67+1.28*bill length [mm]; unpublished data), identified individually with flag codes and transported immediately to outdoor cages (2 × 2 × 2 m; Fig. 21) located in the field station of Samouco saltpans, where they were reared in captivity until they fledged. Chicks were randomly assigned to one of the three salinity regimes: 0 ‰, 20 ‰, and 60 ‰. All cages had available heat lamps, controlled vegetation for shelter, and the same food type. Water was provided in a tray (26 cm diameter, 4 cm high), and its salinity changed according to treatment. The food consisted of alive fly larvae (*Protophormia* sp.) and brine shrimps (*Artemia* sp.); both prey were placed into the water tray. Therefore, prey items were always submerged below water surface, so chicks inevitably had to ingest water while feeding (similar to wild conditions). Shorebirds inhabiting hypersaline habitats rely on hypo-osmotic prey such as brine shrimp and diptera larvae and adults, whose body water content is about 78-87% of body mass (Mahoney & Jehl 1985). The water content of fly larvae provided in our experiment ranged 70-75%. The food and water were renovated three times a day, and salinity levels of residual and fresh water were measured using a conductivity meter (HI 98402).

Morphological and physiological measurements

All chicks were weighed (0.1 g) and measured (bill length and tarsus-plus-toe; 0.01 mm) daily by the same person (AR) around 14:15 h. For each treatment group, we defined chick growth rate (mm·day-1) as the coefficient of a regression of mean body length (tarsus-plus-toe length) on chick age (Reed *et al.* 1999).

After three weeks, experimental fledglings were transported in late afternoon to the laboratory of the University of Extremadura to measure their BMR, in terms of oxygen consumption, using standard flow-through respirometry (see detailed procedure in Gutiérrez *et al.* 2011, 2012). We performed BMR measurements at night (resting period of their circadian cycle) and in post-absortive state (fledglings were placed in outdoor cages without food about 3 h but with water *ad libitum* − salinity according to treatment). Fledglings were individually

Figure 21. Wild black-winged stilt chicks in (a) Samouco outdoor cages; stilt chick in (b) 15-l metabolic chamber prior to the measurements of basal metabolic rate at Extremadura laboratory; stilt chicks in (c) the hacking outdoor cage in the pan before their release.

placed in metabolic chambers (15 L) in darkness and housed in a temperature-controlled cabinet at a constant temperature (27°C; within the thermoneutral zone; Fig. 21). The calibration and technical characteristics of the respirometer apparatus followed Gutierrez *et al.* (2011). Birds were weighted prior to and after BMR measurements, and their mean body mass was used in the analyses (see below).

In addition to captive-reared individuals, we also measured BMR in wild fledglings captured in Samouco saltpans and freshwater reservoirs (Caia, Portugal). These birds were captured in the field in late afternoon and then immediately transported to the University of Extremadura for metabolic measurements. The wild fledglings from saltpans were captured in hypersaline pans (67 ‰), while wild fledglings from reservoirs only had available freshwater. The procedure for metabolic measurements was identical to that described for captive-reared fledglings.

After completing the BMR measurements, we collected a blood sample (about 70 µl) from the brachial vain using microcapillary tubes. These blood samples were used to measure hematocrit, plasma concentrations of sodium and chloride, and heat shock proteins (Hsp70). Blood samples were centrifuged for 10 minutes at 11,800 rpm at 4°C to separate plasma from red blood cells, and stored at -20ºC until analysis.

Hematocrit was measured as the percentage of blood cell volume to total volume within the microcapillary tube. We used the SPOTCHEM E-Plate with the SPOTCHEM-SE and SPOTCHEM El Analyser, to determine Na^+ and Cl^- concentrations. The SPOTCHEM E-Plate measures simultaneously both electrolytes.

Concentrations of Hsp70 were determined from the cell lysates by means of an enzyme-linked immunosorbent assay. For details about the heat-shock proteins protocol see Gutiérrez *et al.* (2015).

After metabolic measurements and blood collection, wild chicks were released at the capture site. Captive-reared chicks were transported again to the outdoor cages at the Samouco's saltpans. After one day in these cages, chicks were released by 'hacking'. They were placed in a large outdoor cage of mesh and iron bars built in a pan where usually there are flocks of wild stilts. After a few days under these conditions, chicks were released in this pan.

Behavioural response

We videotaped the chicks while feeding on fly larvae offered into the water tray. As previously described, fly larvae were always submerged below water surface, simulating when chicks feed on diptera larvae in wild conditions. Therefore, chicks had to cope with the adherent water on each prey item before swallowing it. Each chick was videotaped during the first two minutes of the feeding period, just after renovating the water and food. Thus, for each prey item ingested during the period of recording, we calculated the mean number of head shakes when (a) the prey is handled in the bill, before ingestion, and (b) after ingestion. Each chick was recorded eight times through the growth period.

Saltglands

We examined the saltglands of freshly fledglings found dead in hypersaline saltpans (n = 14) and freshwater (n = 6) habitats of the study area to assess their dry mass. We performed an incision on birds head scalp with a scalpel to expose salt glands (see Fig. 24), removed it, dried to constant weight in an oven (60ºC), and weighted it in a precision balance to the nearest 0.0001 g.

Statistical analyses

The effect of salinity on fledgling body mass, chick growth rate, plasma concentration of sodium and chloride, hematocrit, Hsp70, and BMR was assessed using general linear models (GLM), with treatment (three levels) as fixed factor. The effect of salinity in mass-corrected BMR was also assessed using GLMs, with treatment as fixed factor (three levels) and body mass as covariate. A General Linear Mixed Model (GLMM) was used to test the effect of salinity in the rate of head-shaking, with treatment as fixed factor (three levels) and individual identity as random factor to avoid the lack of independence in the observations (note that each chick was videotaped several times; see above). Morphological and physiological differences between wild and captive-reared fledglings according to salinity were tested using *t*-test for independent samples, except in mass-corrected BMR for which we also used a GLM with body mass as covariate.

BMR, body mass, tarsus length, plasma concentration of ions and lateral head-shakes were log transformed to achieve normality and homoscedasticity of variances. Data are shown as mean ± SE. All statistical analyses were performed with STATISTICA v10.

Results

Prior to the experiment, body mass and body size (tarsus) of chicks were similar among treatments ($F_{2,23}$ = 2.54, P = 0.10, $F_{2,23}$ = 1.87, P = 0.18, respectively). The age at capture differed to some extent among captive-reared chicks, but there was no significant differences in the mean number of days in each treatment ($F_{2,22}$ =1.48, P = 0.25). We did not find significant effects of treatment in final body mass ($F_{2,22}$ = 2.30, P = 0.12), BMR ($F_{2,22}$ = 1.21, P = 0.32) and mass-corrected BMR ($F_{2,22}$ = 0.57, P = 0.57) of fledglings (Fig. 22). We also didn’t found significant differences among treatments in growth rate ($F_{2,18}$ = 1.47, P = 0.26), plasma ions (Na^+: $F_{2,15}$ = 0.24, P = 0.79; Cl^-: $F_{2,15}$= 0.01, P = 0.99), hematocrit ($F_{2,15}$ = 0.99, P = 0.39), and Hsp70 ($F_{2,15}$ = 1.69, P = 0.22) of fledglings (Fig. 23).

None of the physiological variables measured differed between wild fledglings from hypersaline habitats and those captive-reared at 60 ‰ (BMR: t_{15} = -0.73, P = 0.48; mass-corrected BMR: $F_{1,15}$ = 0.22, P = 0.65; Na^+: t_{12} = 1.25, P = 0.24; Cl^-: t_{12} = 0.53, P = 0.61; Hematocrit: t_{12} = 0.52, P = 0.52, P = 0.38; mass-corrected BMR: $F_{1,15}$ = 1.22, P = 0.29; see mean values in Fig. 22).

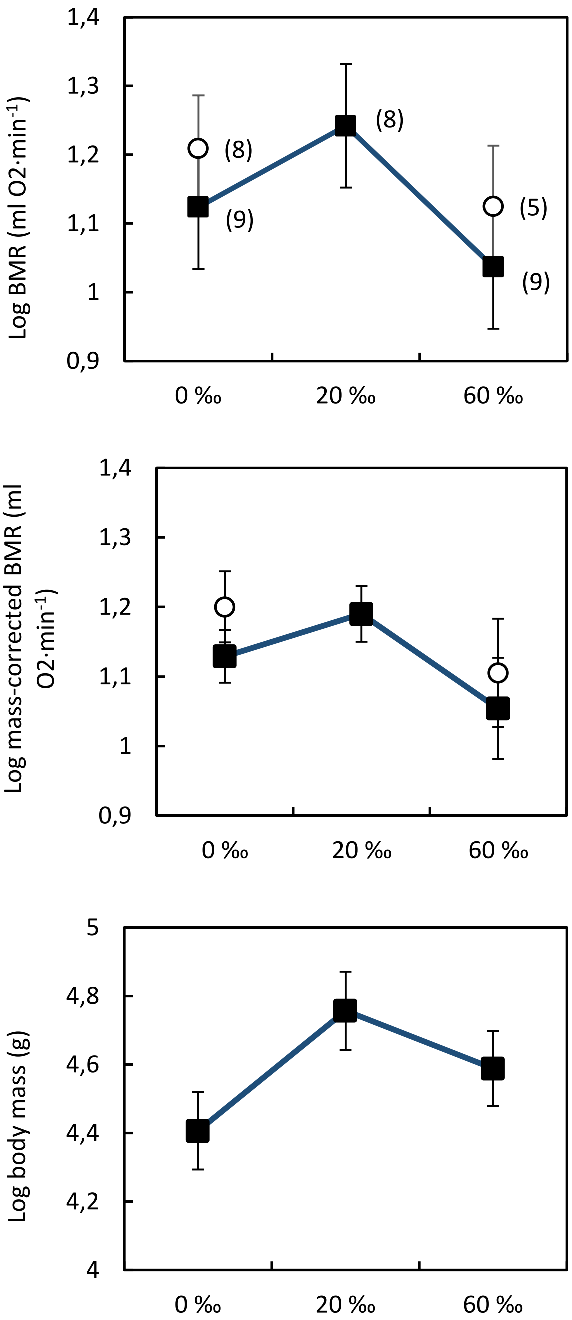

Figure 22. Body mass (bottom), whole-animal (middle) and mass-corrected BMR (top) of captive-reared chicks at 0, 20, and 60 ‰ (black squares). The whole BMR and mass-corrected BMR of wild fledglings from hypersaline pans and freshwater reservoirs also are showed as reference values (empty circle). Data are means (± SE) and sample size is showed in brackets. There were no statistically significant differences among treatments (see text for details).

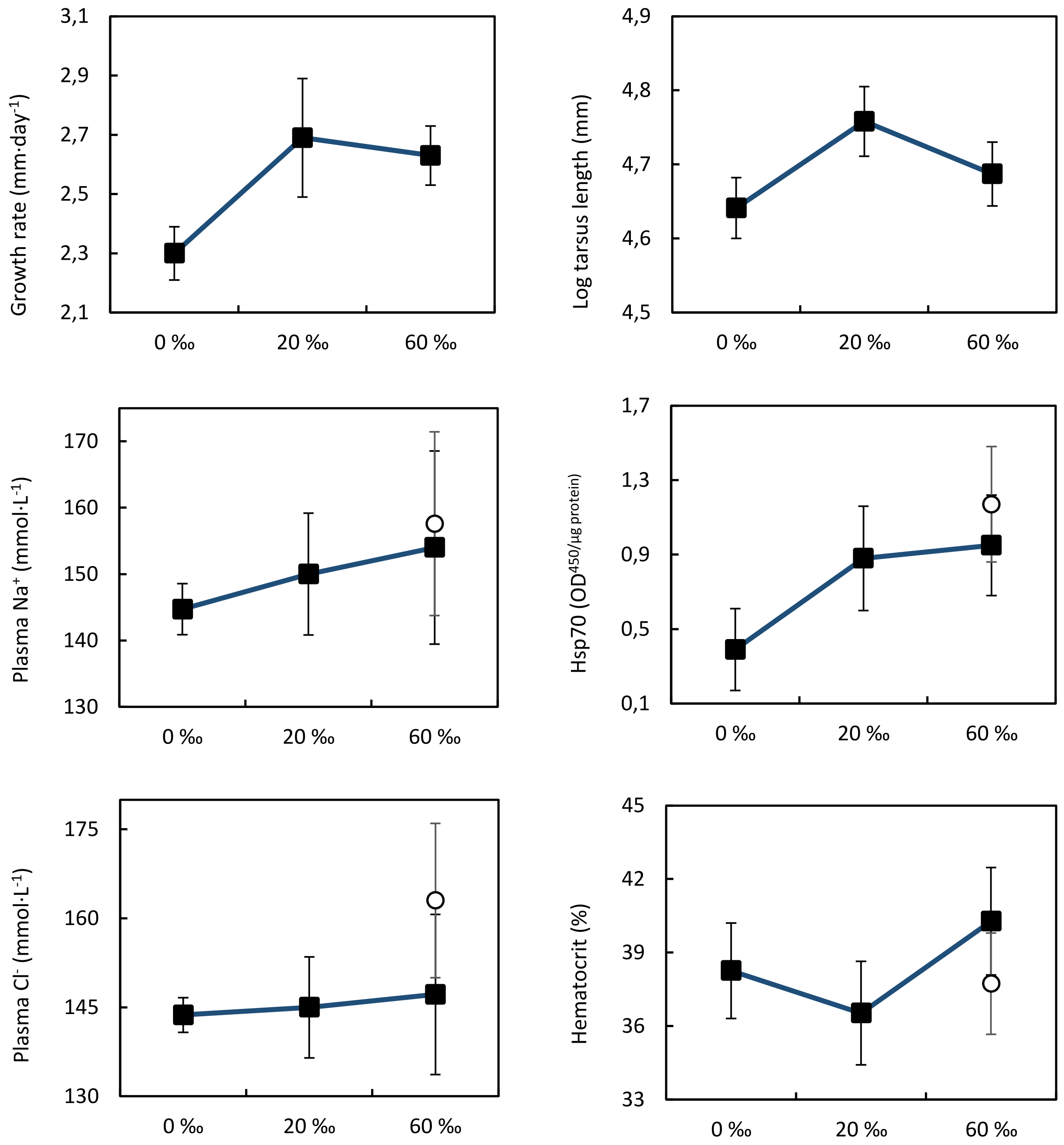

Figure 23. Morphological and physiological measurements of black-winged stilt fledglings captive-reared at 0, 20, and 60 ‰ (black squares). These measurements are also shown as reference values (empty circle) for wild fledglings from hypersaline pans and freshwater reservoirs. Data are means (± SE), and sample size is showed in Figure 22. There were no statistically significant differences among treatments (see text for details).

Likewise, BMR, and mass-corrected BMR of wild freshwater fledglings and those captive-reared at 0 ‰ were similar (BMR: t_{12} = -0.52, P = 0.38; mass-corrected BMR: $F_{1,15}$ = 1.22, P = 0.29; see mean values in Fig. 22 and 23). Unfortunately, some blood samples of wild freshwater fledglings were unfrozen accidentally before analyses and were discarded from further analyses. Due to the low size sample, we could not compare plasma ions, hematocrit, and Hsp70 in both groups (freshwater fledglings and captive-reared at 0 ‰).

The saltgland dry mass was similar in wild chicks from hypersaline pans (43.0 ± 12.3 mg) and freshwater reservoirs (55.0 ± 10.8 mg; $F_{1,6} = 0.48$, $P = 0.51$; Fig. 24).

Head-shaking behaviour differed significantly among treatments, with chicks from hypersaline water showing the highest rate of head-shake movements (before prey ingestion: $F_{2,19} = 30.34$, $P < 0.001$; after prey ingestion: $F_{2,19} = 13.04$, $P < 0.001$; Fig. 25). Post-hoc Tukey test showed significant differences among all treatments ($P < 0.001$), except between treatments 0 and 20 ‰ after prey ingestion ($P = 0.11$).

Discussion

Feeding on hypo-osmotic prey items allowed stilt chicks to inhabit hypersaline environments during their growth phase. The lack of significant differences in growth rate and body mass and the suite of physiological measurements among fledglings reared in freshwater, saline and hypersaline water, is consistent with the notion that stilt chicks are able to successfully maintain their ionic and

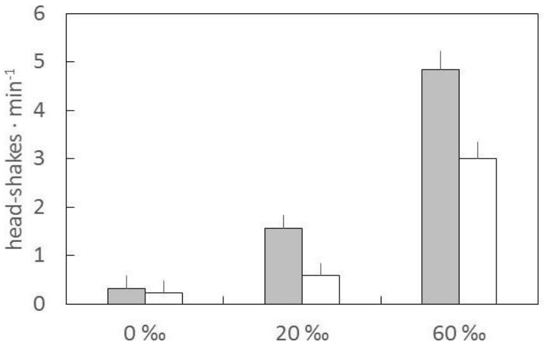

Figure 24. Head-shaking behavior (mean ± SE) of captive-reared stilt chicks before (grey bars) and after (white bars) ingesting diptera larvae at 0, 20, and 60 ‰. There was statistically significant differences among treatments (see text for details).

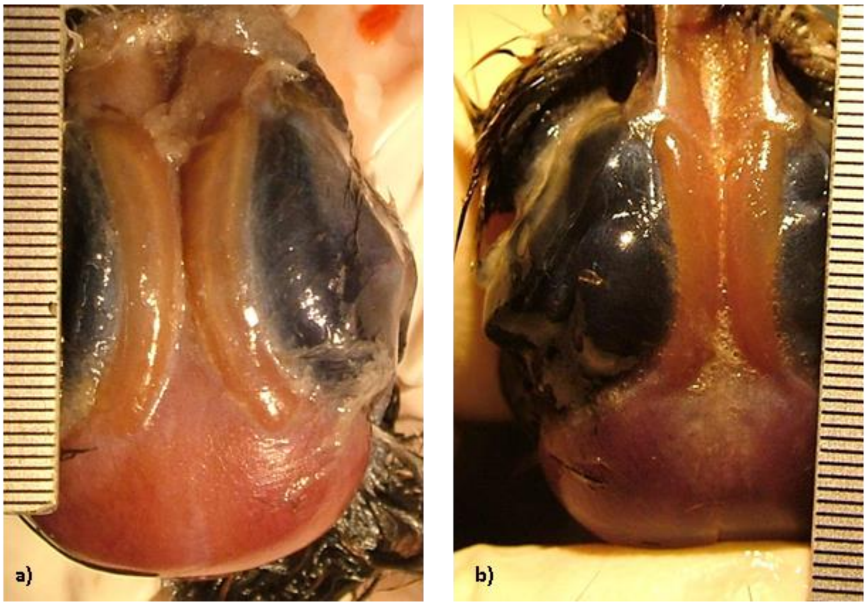

Figure 25. Photographies showing saltglands of wild fledglings of black-winged Stilt found dead in (a) freshwater and (b) hypersaline.

osmotic homeostasis in challenging hypersaline habitats. Our findings support that behavioural responses such as head-shaking play a significant role in the avoidance of salt-loading by precocial shorebird chicks in species such as black-winged stilt.

Maintaining and using large, active saltglands is assumed to impose significant energetic and physiological costs, and adult waterbirds adjust the mass of saltglans to changing osmoregulatory demands (reviewed by Gutiérrez 2014, Gutiérrez *et al.* 2015). As noted earlier, a recent study by Gutiérrez *et al.* (2011) found that mass-corrected BMR of adult dunlins increased significantly by 17 % between freshwater and seawater, suggesting that developing and maintaining active saltglands is energetically expensive. In contrast to our prediction, BMR and mass-corrected BMR of captive-reared fledglings were similar among treatments, which suggests that the energetic costs of maintaining an active osmoregulatory machinery were low or masked by the costs of growth. The BMR values from wild fledglings corroborated that the captive-reared fledglings' BMR were within natural ranges. We expected that the saltglands of fledglings from hypersaline habitats should have the highest mass, but hypersaline and freshwater fledglings had similar saltgland mass. This could explain, at least in part, the lack of an effect of salinity on BMR. The degree of hypertrophy of salt glands in adult shorebirds is strongly influenced by salinity levels, but also by factors such as ambient temperature, prey type, and energy intake rates (Gutiérrez *et al.* 2012). Almost all data on saltgland mass of shorebirds are based on studies performed with adult individuals. Rubega and Oring (2004) found that American avocets, a related shorebird species of the same family that also uses habitats across a very wide range of salinities, hatched with saltglands that are relatively large, similar to those of adults or to those of some marine birds. It has been argued that there is an endogenous rhythmicity in the size and activity of adult waterbird saltglands (reviewed in Gutiérrez 2014). If stilts also hatch with large saltglands, and their size and activity are under endogenous control, independent of the environmental salinity, this could explain why the extra-renal salt-secreting structure showed similar mass across habitats.

We found no evidence for salt stress, since growth rate, fledgling body mass, hematocrit, plasma concentration of sodium and chlorine ions, and Hsp70 did not vary according treatment. These data suggest that stilt chicks ingest very little water while feeding, at least when they feed on saline and hypersaline water. Mahoney (1985) and Mahoney and Jehl (1985) suggested that adult shorebirds such as American avocets and wilson's phalaropes *Phalaropus tricolor*, as well as other waterbird species as eared grebes *Podiceps nigricollis*, rely strongly on behavioural adjustments and anatomical adaptations of the feeding apparatus (bill, tongue, and oral cavity) to avoid ingesting large amounts of saline water. The head-shaking behavior of stilts' chicks increased significantly with increasing salinity, suggesting that this behavior was highly effective in reducing the intake of ions that would have to be excreted otherwise. Additionally, bill morphology and oral cavity can have a significant role in reducing this intake of ions. Many shorebird species, including stilts, phalaropes and small-sized calidrids, possess needle-shaped bills, which allow them to pick prey cleanly from the water column (Mahoney & Jehl 1985; Estrella, Masero & Perez-Hurtado 2007), to spurt water from their bills when feeding in shallow water (Fellows 2013), and to use 'surface tension transport' (Rubega 2007, Estrella *et al.* 2007). These feeding mechanisms could minimize salt ingestion, as they include the disposal of the transported salt water (Masero 2002; Verkuil *et al.* 2003). The morphology of oral cavity, with palatal papillae, also could promote lateral water drainage (see details in Mahoney and Jehl, 1985). In contrast, other species such as red knots *Calidris canutus* which have relatively large saltglands, did not feed extensively on brine shrimp probably because their thick bills do not enable them to feed on this prey without consuming hypersaline water (Masero 2002).

Hannam *et al.* (2003) examined experimentally the effects of a range of salinities (freshwater, brackish, saline, and hypersaline water) on the growth, behavior and survival of captive-reared chicks of the American avocet. Although they found that hypersaline-reared chicks increased significantly their head-shaking behaviour, they also reported negative effects of increasing salinity, resulting in a significant body mass loss and dehydration of avocet chicks. In our experiment, chicks fed on hypo-osmotic prey items with a large amount of body (osmotically-free). It is important to note that in the study by Hannam *et al.* (2003) provided food consisted of an artificial mixture of small-grain trout chow (pelleted food), egg yolk, coarsely ground oyster shell, and vitamin and mineral supplements. The water content of this food type probably was significantly lower than the body water of natural hypo-osmotic prey found in hypersaline habitats (> 78%; see Mahoney and Jehl 1985). In hypersaline habitats, hypo-osmotic invertebrate prey probably provide most or all of the water that shorebirds need (Mahoney & Jehl 1985). The contrasting results of the study by Hannam *et al.* (2003) and those found in our experiment highlight the importance of the availability of hypo-osmotic prey with high content in body water (osmotically free) for waterbirds inhabiting hypersaline habitats.

Obviously, the fact that shorebird chicks cope successfully with saline and hypersaline water is not surprising, given the large number of waterbirds that use natural hypersaline lakes as breeding habitats. Today, a large number of shorebirds and other waterbird groups rely on saltpans as breeding habitats. Saltpans are hypersaline anthropogenic habitats used to obtain salt by seawater evaporation, and worldwide many key breeding sites for waterbirds are saltpans. Previous findings (Hannan *et al.* 2003) could question the role of this and other hypersaline habitats for the development of precocial chicks of several shorebirds such as kentish *Charadrius alexandrinus* and snowy *Charadrius nivosus* plovers, pied avocets or black-winged Stilts, but we showed that these species could raise their chicks successfully in hypersaline habitats when hypo-osmotic prey is available.

Acknowledgments

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Chapter V

A non-lethal biopsy technique for sampling subcutaneous adipose tissue of small and medium-sized birds

Abstract

Analysis of fatty acids from adipose tissue can provide important information about the physiological and nutritional condition of birds. However, non-lethal biopsy procedures and their potential negative effects on small and medium-sized birds have not been adequately assessed. We developed a biopsy procedure for collecting small amounts of adipose tissue in the furcular area of small and medium-sized birds (13 – 62 g) without adverse effects. The biopsy procedure was performed on dunlins *Calidris alpina*, a medium-sized migratory shorebird, and small hybrid songbirds european goldfinch (*Carduelis carduelis*) × atlantic canary (*Serinus canaria*). The biopsy involved making a skin incision 2-3 mm long on one side of the furcular region to collect 2-16 mg of adipose tissue. All birds were kept in captivity and monitored for two weeks after biopsies to examine potential effects of the procedure on body mass, visible fat deposition, time for wound healing, hematocrit levels, total white blood cell counts, and heterophil: lymphocyte ratios. Visible scars were apparent for 10 days for all hybrids and 6 days for dunlins, with no evidence of infection or abnormal scar tissue formation. Body mass in songbirds did not differ before and after the biopsy, but dunlins increased body mass and visible fat deposition after biopsy. The latter had no apparent short-term negative effects in the suit of physiological parameters. The collection of adipose tissue in the furcular region was performed only for birds with fat scores ≥ 2, and this adipose tissue never represented more than 0.07 % of the bird´s body mass, so we recommended both values to prevent any possible unknown negative effects. Our non-lethal biopsy is relatively simple to perform, and we recommend it as an alternative to lethal methods for sampling adipose tissue in studies of wild and captive birds.

Key words: ecophysiology, fat stores, passerines, shorebirds, technical procedures

Introduction

Fat is the major form of energy storage in birds (Griminger 1986, Walsberg 1988), and adequate fat stores are important during periods of high energetic demands or food scarcity. Fatty acids in stored fat can provide a record of dietary intake over a period of weeks to months (Wang *et al.* 2007). Moreover, fatty acids pass intact from food to storage tissue, allowing evaluation of spatial and temporal variation in diet and trophic links (Käkelä *et al.* 2005, Iverson *et al.* 2007, Williams and Buck 2010), and quantitative diet estimates from predators and prey (Iverson *et al.* 2007). Other investigators have used fat stores to examine the bioaccumulation of pesticide residues (Enderson & Berger 1968), plastic chemicals (Tanaka *et al.* 2013), and carotenoid levels (Møller *et al.* 2000, Bortolotti *et al.* 2003).

For small (10-25 g) and medium-sized (30-65 g) birds, stored fat can be sampled from carcasses (Seewagen 2008) or sacrificed birds (Egeler and Williams 2000) or can be obtained using non-lethal methods such as biopsy procedures. Although several investigators (Käkelä *et al.* 2009, Wang *et al.* 2010, Owen *et al.* 2013) have sampled adipose tissues of seabirds by non-destructive biopsy, only Owen *et al.* (2010) reported details about sampling adipose from the synsacral region of adult and nestling black-legged kittiwakes *Rissa tridactyla*, common murres *Uria aalge*, and northern fulmars *Fulmarus glacialis* (the smallest individuals sampled were ~ 250-g common murre chicks). These authors suggested that smaller birds (< 250 g) could be biopsied using a similar method, but small birds may have little fat in the synsacral region (Blem 1976). Egeler *et al.* (2003) used a similar biopsy procedure to sample adipose tissue from the furcular cavity of medium-sized birds (western sandpipers, *Calidris mauri*; body mass range = 24-27 g), but did not describe the technique in detail or provide information about wound healing and bird condition.

Owen *et al.* (2010) assessed the healing process for black-legged Kittiwakes, but the time interval between the biopsy procedure and recapture of birds to assess body mass change (3-20 days) and healing of biopsy wounds (3-50 days) varied considerably. Although such general evaluation may be adequate for large birds, a closer evaluation of wound healing and bird health is important during the first days after the biopsy for small and medium-sized birds, given their greater susceptibility to problems (stress, hypothermia or blood loss) during handling and biopsy procedures (Whittow 1999). For a more complete evaluation of bird health over time, other physiological parameters such as the hematocrit, total white blood cell (WBC) counts, and heterophil/lymphocyte (H/L) ratios should also be considered (Whittow 1999).

We describe a method for obtaining samples of adipose tissue from the furcular region of small and medium-sized (13 – 62 g) birds. We provide a detailed description of the method, including handling position, topical anesthetic application, sample collection procedures, and healing of the incision. Our method was evaluated in terms of processing time, feasibility of use in the field, and effects on several physiological indices of bird health.

Methods

A biopsy technique was used to collect fat samples from dunlins *Calidris alpina*, medium-sized migratory shorebirds, and a small hybrid songbird european goldfinch (*Carduelis carduelis*) × atlantic canary (*Serinus canaria*); hereafter hybrid). Adult dunlins (body mass range = 40.2 – 57.1 g; Table 5) were captured using mist-nets during October 2013 at Samouco saltpans, Tagus estuary (38º44’N, 8º59’W), Portugal. Adult hybrid songbirds (body mass range = 13.4 – 21.9 g; Table 5) were obtained during October 2013 from the Biological Park of Gaia (Gaia, Portugal). Dunlins (*N* = 20) were measured and

Figure 26. Dunlins in one outdoor aviary.

banded, then transported to two outdoor aviaries (2 x 2 x 2 m each; 10 birds per cage; Fig. 26) and provided with ad libitum access to water and food (fly larvae *Protophormia* sp. and commercial food pellets [Dibaq-Diproteg]). Hybrid passerines (N = 28) were measured and color-banded and kept indoors in six cages (1.0 x 0.5 x 0.5 m each; four to five birds per cage) with ad libitum access to water and food (Versele-Laga Prestige canary seed mix and Orlux Gold patee canary supplement).

Dunlins and hybrids were allowed to acclimatize for two weeks before starting the 16-day experiment. Birds were divided into an experimental group (collection of adipose tissue and blood samples) and a control group (collection of blood samples only). Each group included 10 dunlins and 14 hybrids with similar body mass (dunlins: experimental group = 47.2 ± 4.9 [SE] g, control group = 48.2 ± 6.2 g, t_{18} = 0.4, P = 0.47; hybrids: experimental group = 16.2 ± 2.18 g, control group = 15.9 ± 1.4 g, t_{26} = 0.5, P = 0.13). Experimental and control groups were subjected to the same temperatures (outdoor aviaries = 16.7 ± 0.2 [SE] °C; indoor cages = 17.0 ± 0.3 °C) and light cycle, spent the same amount of time in captivity, and were provided with food and water ad libitum. Once the experiment was completed, dunlins were released at the capture site and hybrids returned to the facilities at the Biological Park of Gaia.

Adipose tissue sampling

One of the most important and accessible places where *birds* (e.g. migratory songbirds and shorebirds) deposit *fat* is the furcular area (Blem 1976), a hollow between the breast muscle and neck (Fig. 27a). After reviewing the literature on methods to collect visible fat stores in birds and discussions with veterinarians (F. Martinho and E. Chaher), we decided to perform the biopsies on a side of this furcular area to collect adipose tissue from the visible fat stores. Owen *et al.* (2010) performed the biopsy in the synsacral region. However, songbirds and shorebirds accumulate fat in the synsacral region only at a late stage of fattening (Prater 1975, Kaiser 1993), which could limit collection of fat samples. By choosing the furcular area, we could collect fat samples with a smaller and shallower incision (see below). Fat scores (0 = no fat to 8 = fat layer covering completely the under and ventral sides of the bird; Kaiser 1993) of dunlins in the experimental group ranged from 2 to 5 at the time of the biopsies. The mean amount of adipose tissue collected was 9.02 ± 1.18 [SE] mg (range = 4 - 16 mg) from dunlins and 6.31 ± 0.67 mg (range = 2 - 11 mg) from hybrids. The maximum amount of adipose tissue collected represented 0.03% and 0.07% of dunlin and hybrid's body mass, respectively. Fat samples were collected as part of a study on the accumulation of fatty acids in small to medium-sized birds. Two people were required to conduct the biopsies (conducted outside to simulate field conditions), one to handle and restrain the bird and the other to make the incisions, collect fat samples, and glue the incisions. Hybrids were held in one hand, with the neck between the index and middle fingers and the tarsi held in place with index and middle fingers and the thumb from the other hand. Dunlins were placed dorsoventrally on a soft cloth on a table, with the tarsi and wings held with one hand and the head with the other hand (and with the head covered by a soft cloth). In this position, the furcular region was cleaned and disinfected using cotton soaked in antiseptic solution (Chlorhexidine gluconate 4g % 100 ml, AGA, Prior Velho, Portugal). The wet feathers were then moved laterally to expose the skin. The subcutaneous yellow fat tissue was clearly visible through the skin, allowing us to select an exposed area without visible capillaries (Fig. 27a). With a swab, we applied a topical anaesthetic gel (Tetracaine hydrochloride 7.5 mg/g, B. Braun, Bethlehem, PA) to the selected area, and birds were then placed in either cotton bags (hybrids) or cardboard boxes (dunlins) for 5 - 10 min. Birds were then removed from bags or boxes and the effectiveness of the local anesthetic was tested by pinching the skin with forceps to insure that birds showed no pain response. A 1.5 – 3.4-mm-long and 0.5-1-mm-deep incision was then made in the skin using the bevel of a 30-gauge needle (Microlance BD, Becton Dickinson, Franklin Lakes, NJ) (Fig. 27b). A small piece of adipose tissue was gently pulled through the incision using a sterile forceps with atraumatic tips. A sterile scissor was used to cut the sample under the forceps and the sample was retained in the forceps (Fig. 27c); the rest of the adipose tissue then fell back into place. When fat showed poor consistency and could not be grasped with forceps, we used a syringe with 16-gauge needle to collect adipose tissue by suction.

Incisions rarely bled (three of 48 birds) and, when they did, it was always in small amounts (< 0.05 ml), in those cases we applied light pressure using cotton soaked in antiseptic solution to aid the

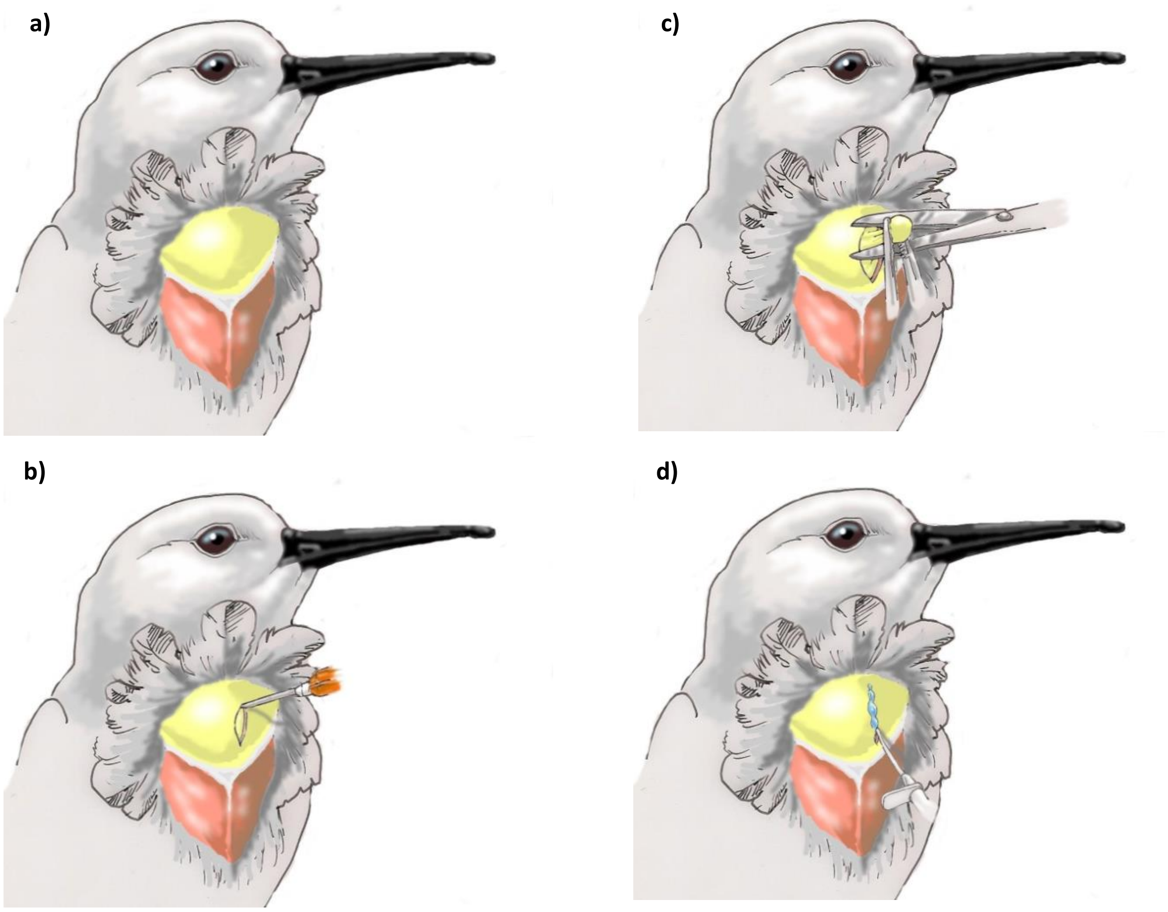

Figure 27. The first step in an adipose tissue biopsy on wader is to restrain the bird: a) after laterally moving the feathers from the breast area, the subcutaneous fat is clear and an area without visible capillaries is selected; b) a small skin incision is made with the bevel of a needle; c) a piece of adipose tissue is pulled through the incision with sterile forceps and cut with scissors or, alternatively, a syringe can be used to aspirate adipose tissue with poor consistency; d) the margins of the incision area are aligned back together and a thin layer of tissue glue is applied over the incision. The incision size in panels b and c is exaggerated to ease interpretation.

coagulation process. Because the biopsy procedure was not performed in a sterile environment, we disinfected the area after fat extraction using the antiseptic solution described above and used sterile cotton-tipped applicators to clean and dry the wound before closing. The incision was closed using one stylet to push the skin back together to align the incision edges, and applying a thin layer of veterinary tissue adhesive (N-butyl cyanoacrylate 100 %, 3M, Vetbond, 3M, St. Paul, MN) over the entire length of the incision (Fig. 27d). The adhesive dried in < 30 sec (Vanholder *et al.* 1993), sealing the incision, and the stylet was quickly removed to prevent it from sticking to the skin. Birds were then allowed to recover from the handling stress inside a bag or box (~ 10 min) before being released into cages and aviaries. Between each biopsy, we sterilized tweezers and scissors by cleaning with cotton soaked in ethanol (70%) and passing them through the flame of a Bunsen burner. Birds in the control group were handled similarly, but without the incision.

We noted how long each procedure took, and any indication of bird discomfort, stress, or injury. Discomfort and stress were directly evaluated through an increase of respiratory rate, open-mouth breathing, and behavioral clues such as closed eyes and non-responsiveness to external stimuli (e. g., lack of response to sounds and handling; Spotswood *et al.* 2012). Every two days after the procedure, we weighed (± 0.1 g) and assessed fat scores (0 – 8; Kaiser 1993) of all experimental and control birds. For the experimental group, we also evaluated skin healing by measuring the length of the scar with a digital caliper (± 0.1 mm) and by visually scoring the wounds using categories described by Owen *et al.* (2010), including: (1) not yet healed or poorly healed (e.g., wound edges not completely joined together and absence or small amount of granulation tissue formation), (2) completely closed, but still visible, or (3) completely healed and no longer visible. Birds were always scored at the same time of day and in the same place with the same light conditions.

Blood samples (<100 µl) were collected from brachial veins, just before the biopsy or control procedures (0 d) and at the end of the experiment (16 d) to determine total WBC counts, H/L ratios, and hematocrits. The hematocrit is the relative volume of red blood cells in total blood and can be used to assess the presence of anemia or hemoconcentration, usually caused by dehydration (Clark *et al.* 2009). The total WBC count is a general measure of the status of the immune system, and an estimated count as described in Clark *et al.* (2009) was made. High values can indicate either an absence of immune system suppression or elevated immune cell numbers in response to infection (Johnstone, Reina & Lill 2012). H/L ratio is a general stress indicator in avian studies, such as fasting and food deprivation and injuries (Maxwell 1993, Ruiz *et al.* 2002, Gross and Siegel 2013). For example, in case of injuries, the H/L ratio increases due to an increase in heterophils (phagocytizing cells responsible for inflammation; Siegel 1980, Klasing 1991).

For each bird, a small drop of blood was collected from the brachial vein, and placed on a slide to make a blood smear for identifying and counting white blood cells. Blood smears were fixed and stained with a commercial stain kit (Diff-Quik Hemacolor, Merck KGaA, Darmstadt, Germany). Hematocrits were determined by centrifuging blood samples for 10 min at 11,800 rpm, and measuring the ratio of packed red blood cells to whole blood volume.

Statistical analysis

We used a generalized linear mixed model (GLMM) fitted with Restricted Maximum Likelihood (REML) to assess the effect of treatment (fixed factor with two levels: with and without biopsy), time period (fixed factor with two levels: before and after treatment), and the interaction between both factors on body mass. We selected the Gaussian family for all of our GLMMs with “identity” as a link function. Bird identity and sampling day were entered as random factors to control for possible intrinsic differences between individuals and avoid possible differences between days, respectively. WBC counts, H/L indices, and hematocrits were analyzed similarly with GLMMs, but using only bird identity as a random factor. All statistical procedures were performed in the R environment (R Core Team 2013). Analyses were conducted using several functions within different R packages (psych, doBy, plyr, MASS, lme4, and lmerTest). All values are presented as means ± SE.

Results

We collected adipose tissue from birds either using scissors or tweezers or, for four hybrids, using a syringe to collect fat by suction. Total handling time required for biopsies was similar for dunlins (mean = 3.90 ± 0.28 min) and hybrids (mean = 4.36 ± 0.68 min; one-way ANOVA: $F_{1,8}$ = 0.2, P = 0.65), and biopsies took on average of 2.23 ± 0.28 min (range = 1.25 - 3.59 min). No birds showed signs of stress either during or after the biopsy procedure above that observed with control birds.

We found no significant effects of treatment or time period on body mass, hematocrits, and WBC counts for either dunlins or hybrids (all $P \geq 0.06$). Similarly, the interaction between treatment and time period was not significant for any variable for either dunlins or hybrids (all $P \geq 0.32$). For dunlins (Z = 2.6, P = 0.02) and hybrids (Z = 2.8, P = 0.01) in both treatment groups, H/L ratios decreased significantly after biopsies (Table 5). In addition, for hybrids in both treatment groups, WBC counts decreased significantly after the biopsy ($Z = 2.4$, $P = 0.02$; Table 5). We found that fat scores of migratory Dunlins increased significantly after biopsies ($Z = 5.3$, $P < 0.001$) and no significant differences were found between treatments ($Z = 0.7$, $P = 0.51$).

Three days after biopsies, 10% of the Dunlin incisions had not yet healed (score 1). After 10 days, 50% of the incisions were closed, but still visible (scored 2), and 50% were completely healed (score 3). For hybrids, 64% of the incisions were not healed (score 1) after three days, but 22% of the incisions were closed after 10 days (score 2) and 78% were completely healed (score 3). After 10 days, only three hybrids had visible scars (0.9-1.2 mm; Fig. 28 B). For dunlins, 15 days were required for most incisions to heal, and only one bird had a visible scar (2 mm; Fig. 28 A).

Discussion

We used a non-lethal biopsy technique to sample adipose tissue from the furcular region of small and medium-sized birds (13-62 g), with no apparent negative effects on bird condition. Biopsy incisions took 10-15 days to completely heal. Owen *et al.* (2010) reported that incisions in the synsacral region of larger species of birds (adult and nestling black-legged Kittiwakes, common murres, and northern fulmars) healed completely within six days

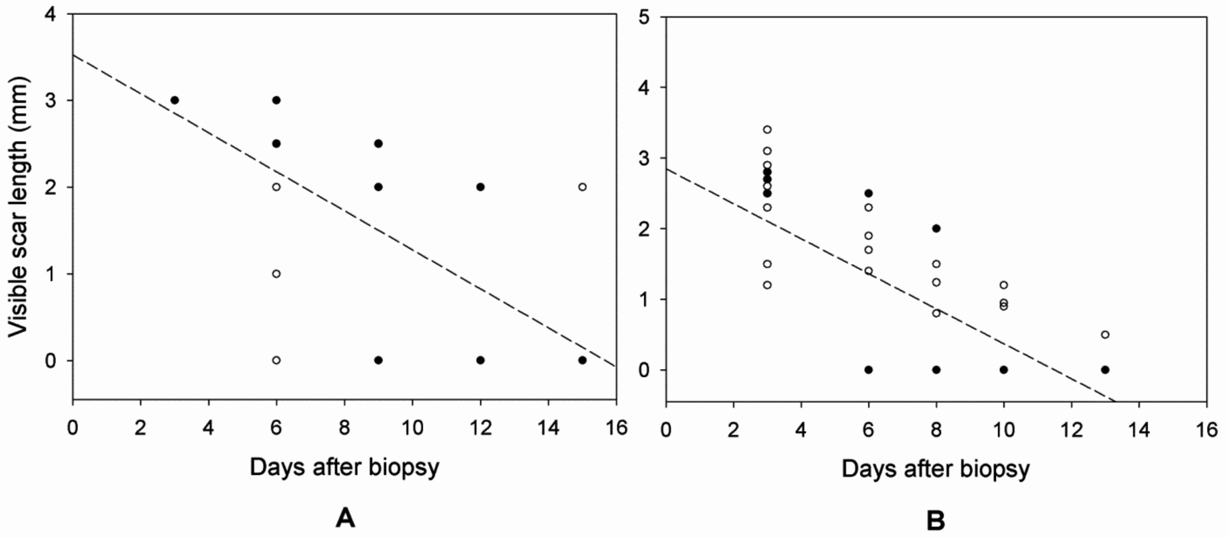

Figure 28. Variation in the length of incision scars after biopsies of dunlins (A) and hybrid songbirds (B). Lines were fitted with least squares regression. Scars were measured every two days during the healing process: from (1) wound not yet healed or poorly healed if wound edges not completely joined together or formation of small amount of granulation tissue to (3) wound completely closed, but still visible. Filled circle indicate more than one bird.

of fat sampling. Differences in wound-healing time between our study and Owen *et al.* (2010) may be due to differences in healing rates between species, in the ratio of incision size to bird mass (larger in our study), and between healing rates in the synsacral and furcular regions. Our method could also be used with larger birds, but, for species where the furcular region is thickly feathered (e.g., seabirds), adipose sampling may be easier in the synsacral region (Owen *et al.* 2010).

The limiting factor for collecting adipose tissue using our method would be the amount of fat stored. Because birds usually accumulate more fat in the furcular region (Blem 1976), accessing to fat deposits in this area is easier than in the synsacral region. Therefore, for small birds, we recommend biopsies to collect adipose tissue in the furcular region, but only for birds with a fat scores ≥ 2 (Kaiser 1993); birds with a lower score would have little fat available for sampling.

Table 5. Mean (± SE) body mass (g), hematocrit, total white blood cell (WBC) counts in 10,000 red blood cells, heterophil/lymphocyte (H/L) ratios, and fat scores (only in migratory dunlins; Kaiser 1993) for dunlins and hybrid songbirds in both treatment groups.

Dunlins	**Body mass (g)**	**Hematocrit (%)**	**WBC (number of cells)**	**H/L ratio**	**Fat score (0-8)**
Before biopsy					
Biopsy (*N* = 10)	45.5 ± 0.7	53.2 ± 1.0	3.7 ± 0.4	14.80 ± 1.67	3.9 ± 0.53
No biopsy (*N* = 10)	47.2 ± 1.1	53.9 ± 2.4	3.2 ± 0.1	9.90 ± 4.33	4.5 ± 0.60
16 days after biopsy					
Biopsy (*N* = 10)	46.1 ± 0.6	52.1 ± 1.1	3.9 ± 1.1	0.21 ± 0.06	4.6 ± 0.48
No biopsy (*N* = 10)	48.2 ± 1.0	53.6 ± 0.5	3.6 ± 0.7	0.24 ± 0.09	5.0 ± 0.67
Hybrid songbirds					
Before biopsy					
Biopsy (*N* = 14)	16.6 ± 0.3	56.9 ± 1.1	10.3 ± 1.2	0.32 ± 0.11	
No biopsy (*N* = 14)	16.3 ± 0.2	56.5 ± 1.4	10.7 ± 0.9	0.36 ± 0.06	
16 days after biopsy					
Biopsy (*N* = 14)	16.4 ± 0.2	47.8 ± 0.8	7.9 ± 0.5	0.15 ± 0.02	
No biopsy (*N* = 14)	16.3 ± 0.1	47.9 ± 0.6	7.9 ± 0.4	0.16 ± 0.02	

Non-destructive biopsy techniques used in other studies with large seabird species were used to collect adipose tissue samples ranging from 50 to 300 mg (Iverson *et al.* 2007, Wang *et al.* 2007, Williams *et al.* 2008, Käkelä *et al.* 2009). We collected ~10 mg of adipose tissue, but larger samples could have been collected if required, depending on the amount of stored fat present. Nevertheless, we showed that our biopsy technique had no negative effects when the maximum amount of adipose tissue collected was below 0.07% of the bird's body mass, hence we recommend this maximum value to prevent any possible unknown negative effects.

Our fat sampling technique had no negative effects on body mass and other physiological indices. The fattening rate of migratory dunlins was not affected by the biopsy procedure and fat loads increased over the experimental period. The significant reduction in H/L (dunlins and hybrids) and WBC (hybrids) after biopsies for both control and experimental birds may indicate a reduction in stress levels. The effect of capture, handling, and acclimation to captivity increase the stress levels of wild birds (Wingfield *et al.* 1982), which may lead to higher H/L ratios (Norte *et al.* 2009). This may explain the high values of H/L for dunlins before the biopsy. However, the decrease in H/L ratios for both treatment groups in our study suggests that the biopsies had no detrimental effect.

Finally, hematocrit values were within normal ranges (40 – 60%; Potti 2007).

Our biopsy technique can be performed in the field, and our results suggest that sampling adipose tissue using our technique has only very short-term detrimental effects on birds. According to these results, we recommend the use of this non-lethal biopsy technique to sample the adipose tissue of small and medium-sized birds. However, further studies are needed to understand possible long-term effects of the biopsy procedure on migratory species, i.e. effects on fat deposition rate, stopover duration, productivity and survival.

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General discussion

In the previous chapters of this thesis many aspects of how waders use man-made habitats such as coastal salinas during their annual cycle were addressed, and the specific objectives and conclusions were extended in each study. Below I will highlight the most important findings and the new approaches that came out from those studies, and discuss their implications for the conservation of wader populations on salinas. Finally, I will mention some management approaches anchored on the results of this thesis and in my experience on management and conservation of waterbirds on salinas.

In this thesis, particular attention was given to the factors that are advantageous and constrains for the use of coastal salinas by waders. Our results emphasize the importance that this anthropogenic habitat plays in the conservation of wader populations as alternative foraging areas, high-tide refuge and nesting grounds.

Pros and cons of salinas as foraging habitats for waders

We present the first evidence that supply ponds after dewater could play a substantial role as foraging grounds during a short time window (≤12 days) for migratory waders. Substrate dryness was presumably the major factor affecting the benthic invertebrate community rather than prey depletion due to bird predation after dewater. Therefore leaving a shallow water layer should maintain the water content in the substrate (Granadeiro *et al.* 2006; Kuwae *et al.* 2010), preserving the invertebrate community and extending the potential bird foraging period. This management could be applied to other habitats such as artificial lagoons and aquaculture ponds.

Like in other drawdown studies (Velasquez 1992; Taft *et al.* 2002) waders responded opportunistically forming very dense foraging flocks after sediment exposure. The study pond harboured 15 wader species, and from these, dunlin, ringed plover, sanderling and little stint exceed 10 % of the entire Tagus population. The high proportion of foraging waders during high-tide may indicate that birds did not satisfy their daily energy requirements during the low tide period on the estuarine mudflats, and had to extend their feeding time during high tide at saltpans. This is one of the few studies on feeding behaviour of waders during autumn migration, because most studies were conducted during the winter and spring migration periods (e.g. Moreira

1996; Masero and Pérez-Hurtado 2001; Goss-Custard *et al.* 2006; Martins *et al.* 2013). More studies are needed on the feeding behaviour of waders in the intertidal mudflats during autumn migration. The conservation value of saltpans will increase if several ponds could be dewatered during the migratory periods. This measure should be more relevant if it could be implemented on other high-tide roosts, improving the Tagus estuary as a stopover site in the East Atlantic flyway. Moreover these alternative foraging areas may presently be more important for migratory and also wintering wader populations due to the increase of clam shellfishers in the intertidal areas in the last years, which may cause the loss of foraging habitat (Stillman *et al.* 2001). Shellfishing is a common activity in the Tagus estuary, particularly the traditional hand-raking and bait-diggers. Dias *et al.* (2008) found that the impact of the disturbance caused by the levels of shellfishery during 2005/2006 on the Tagus was relatively small, particularly because the most inaccessible areas of the estuary remain undisturbed. However, the high abundance of the exotic clam *Corbicula fluminea* in the estuary, together with the economic crisis, lead to the spread of thousands of shellfishers through the estuary during low and rising tides in the past years (personal observations). Such potential conflicts between economic and conservation interests in the intertidal areas suggest that saltpans can function as buffer areas against the impact of habitat loss for waterbirds.

Salinity is the major limiting factor for the benthic community in saltpans (Vieira & Amat 1997; Sánchez *et al.* 2006c). However, other factors can influence the benthic fauna such as the presence of submerged plants and or sediment type (Vieira & Amat 1997; Wolfram *et al.* 1999; Weatherhead & James 2001). These factors should contribute to the dominance of one or more species of benthic invertebrates (Evagelopoulos *et al.* 2007), with an impact on the density of the whole benthic community. This dominance seems to explain the differences in the density and biomass of the benthic community when compared with other ponds from saltpans (Masero *et al.* 1999; Pandiyan, Naresh & Nagarajan 2014) or aquaculture (Martínez-Córdova, Porchas & Cortés-Jacinto 2009). The dominance of one or more species should be reflected also on the attractiveness of the ponds as feeding areas for some wader' species, and also in their food intake rate. For instance, the dominance of the polychaete worm *C. capitata* in our experimental pond attracted large numbers of ringed plovers, and this was reflected on their high food intake rate, enabling them to rapidly reach their daily plus fattening energy requirements. Conversely, the low biomass and density of bivalves should explain the reduced number of black-tailed godwits feeding in our study pond and also their reduced food intake rate. However, if some species of waders could achieve their daily biomass requirements and gain mass foraging only or mostly on dewater ponds, why should they expend energy flying to tidal foraging grounds? Two major factors could explain this behaviour: 1) the higher predation risk in saltpans (Rosa *et al.* 2006) than in mudflats; 2) the increment of social interactions during feeding activities resulting from high bird densities (Ens & Goss-Custard 1984) in a limited area such as a pond, which may have forced part of the wader population to move to intertidal mudflats. In future drawdown studies undertaken in salinas, it will be important to quantify the time period that is required for the invertebrate community to reach the initial densities prior to dewater.

Salinity may also have a strong impact on the chicks growth for those species that breed and feed in the saltpans, such as the black-winged stilt. Chicks of this species feed by themselves in small family groups in saltpans, and apparently cope successfully with saline and hypersaline waters during their development, since most of them stay in the same salina until fledging (per. observations). In hypersaline habitats, hypo-osmotic invertebrate prey probably provide most or all of the water that shorebirds need (Mahoney & Jehl 1985). Our experiment showed that feeding on hipo-osmotic prey items in hypersaline water was not a major physiological problem for the black-winged stilt chicks. We expected that the saltglands of chicks from hypersaline habitats should have a higher degree of hypertrophy, as hypertrophy of salt glands in adult waders is strongly influenced by salinity levels (Gutiérrez *et al.* 2012). However, saltglands from both hypersaline and freshwater chicks had similar hypertrophy, and this could partly explain the lack of a positive effect of salinity on the basal metabolic rate of black-winged stilt chicks. In fact, we found no differences in growth rate, body mass, and the suite of physiological measurements among chicks reared in freshwater, saline and hypersaline water, emphasizing the ability of stilt chicks to successfully maintain their ionic and osmotic homeostasis. We found that the head-shaking behaviour increased significantly with increasing salinity, suggesting that this behaviour was determinant in reducing the intake of ions that would have to be excreted. Additionally, bill morphology and oral cavity can have a significant role in reducing the intake of ions. Many shorebird species, including stilts, phalaropes and small-sized calidrids, possess needle-shaped bills, which allow them to pick clean prey from the water column (Mahoney & Jehl 1985; Masero *et al.* 2007), and to use a feeding transport mechanism named 'surface tension transport' (Rubega 1997; Estrella *et al.* 2007). Our findings give strong support to the fact that behavioural responses such as head-shaking play a significant role in the avoidance of salt-loading by wader chicks.

Salinas as roosting sites for waders

Roosting is an important component of the daily time budgets of non-breeding waders, and may comprise 50% or more of their time budget because of the tidal cycle (Burton *et al.* 1996). Habitat modifications and disturbance may result in changes in the attractiveness of roost sites for waders. We found that saltpans lose their value as high-tide roost for waterbirds populations rapidly in the absence of adequate management, even if suspended only for a short period of time. Our results show that after a lack of management small and medium wader populations showed a significant reduction during winter (50% and 38% respectively) and in the migratory periods. Different bird groups recovered differently: large waders reached the initial densities after 2 years, medium waders after 3-6 years, and small waders and autumn migratory medium waders did not recover even after 6 years. The negative trends for this group of birds over the entire Tagus estuary can explain about half of this decline at the Samouco saltpans complex. The other half appears to be due to the birds' decreasing preference for the site after the suspension of management actions. Waders are known to respond opportunistically to the increase in foraging space (Velasquez 1992; Taft *et al.* 2002) or to new high-tide roosts (Huang *et al.* 2012), and several restored sites had similar or higher bird densities and species diversity as in control areas. This agrees with the expectation that waders would rapidly colonize restored sites (Hemesath & Dinsmore 1993; Passell 2000; Armitage *et al.* 2007; Athearn, Takekawa & Shinn 2009). However, it remains unclear why small waders did not return to the same roosting site once management was re-established. The answer may be related with the flight costs of using the same foraging ground which are contiguous to the Samouco saltpans. Rehfisch *et al.* (2003) found that any change in roosting conditions is likely to be less detrimental than the loss of feeding areas. Large waders fly farther in their routine movements between roosts (Rehfisch *et al.* 1996, 2003) or from roosts to foraging areas, and may maintain the previous foraging grounds, roosting in alternative roosts and returning to the Samouco saltpans right after the habitat conditions were improved. Small waders such as dunlin fly short distances (<5km) between roost and foraging grounds (Dias *et al.* 2006a). This could force them to change the foraging area and the roosting site. A constant demand for better roosts is not advantageous for waders due to flight costs, and may also increase the likelihood of predation while in flight (Rehfisch *et al.* 1996). Therefore, small waders might only return to Samouco saltpans when the habitat conditions of the presently used roosts decrease, or as a response to an increment in disturbance at those roosts. The strong roost fidelity of some wader species reinforces the idea that it is crucial to maintain a network of supra-tidal roosts close to the main foraging grounds in the Tagus estuary (Dias *et al.* 2006a; Catry *et al.* 2011) to ensure the conservation of a large and diverse wader community. The maintenance of high quality roosts such as saltpans depends on the implementation of management measures such as the regulation of water levels and saltmarsh overgrowth control.

Salinas as breeding sites for waders

We demonstrated that saltpans are an important alternative breeding habitat for kentish plover. Our long-term study shows that kentish plovers nest success in saltpans was similar to that of natural habitats, and the strong inter-annual fluctuation of nest success was similar to the variability among sites compiled by Norte and Ramos (2004), and also with a number of studies of snowy plover (Wilson-Jacobs & Meslow 1984; Powell 2001; Page *et al.* 2009b; Colwell *et al.* 2010). Therefore, annual and site specificity are likely to overcome the habitat per se in explaining nesting success of plover species. Kentish plovers showed preference to nest in the edge of the paths between ponds in areas with good visbility, but the nest-site characteristics were not correlated with nesting success. However the habitat characteristics may be important for thermal regulation of eggs and incubating birds under very hot conditions (Amat & Masero 2004). For instance, the presence of structures or sparse vegetation should reduce the thermal stress of the incubating plover and can shelter the chicks after hatching (Lorenzo & González 1993). The fact that salinas' paths are well above the ponds' water level ensures the protection of the clutches against flooding, which occurs when nests are located in pond banks or within dry ponds, as a result of water level management during salt production or rainfall. However, it is also important to ensure the attractiveness of the paths by maintaining open areas with reduced vegetation cover, which enable the detection of possible terrestrial predators by incubating birds (Walters 1984).

Predation was the main cause of nest loss in saltpans as already found for natural habitats (Székely 1990; Schulz & Stock 1993; Domínguez & Vidal 2003; Pietrelli & Biondi 2012), and the variability of predators among sites suggests that the breeding location is more important to explain the net effect of predation than habitat type. Infrared-triggered cameras were used to provide information on predator identification and predation timing of kentish plover nests, and confirmed the predation pressure by carrion crows, magpies and red foxes. We showed that when the breeding season of kentish plover was broadly coincident with that of the black-winged stilt, the nesting success of plovers was significantly higher. Kentish plover nest success benefited from the ability of stilts to mob potential predators, which was related to the density of nesting black-winged stilts. Black-winged stilts showed a strong anti-predator behaviour against birds of prey, but were less efficient against carrion crows and magpies (on the ground) and even less so with mammals. Saltpans could play an important role in the conservation of waterbirds breeding populations if both small and large wader species could nest together in large colonies which can have a positive feedback on each other's breeding success. Taken altogether our findings reinforce the importance of saltpans paths as an alternative breeding habitat for plovers, and call upon the need to maintain multi-specific groups of breeding waterbirds in these areas.

Future approaches for research and management of salinas for waders

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Research

The use of fatty acids from fat stored as tools to investigate the physiology and diet is growing in ecological and physiological studies. We described a non-lethal biopsy technique for sampling adipose tissue from the furcular region in small and medium-sized birds, and showed that it has no apparent negative effects on the birds' health (body mass and other physiological indices). This technique allows sampling species of conservation concern, attain large sample sizes, and to re-sample the same individual on different occasions, which obviously are not possible with lethal methods. Currently, we are participating in some studies where this technique is essential: "Dietary regulation of lipid synthesis in black-tailed godwits *Limosa limosa* feeding on distinct diets" and the "Differences on fatty acid composition from fat stores on migratory and wintering waders".

The use of tissue fatty acid signatures (FAS) is quite well-established in studies of marine mammals (Iverson *et al.* 2004; Cooper, Iverson & Heras 2005; Beck *et al.* 2005) and seabird diet (Raclot, Groscolas & Cherel 1998; Dahl *et al.* 2003; Käkelä *et al.* 2005, 2006, 2007). This powerful method provides information about diets over an extended time period, rather than just the most recent meal as with most traditional approaches. The biopsy method that we describe highlights a new approach in the study of the diet composition of other bird groups such as passerines and waders by using fatty acids.

Studies about the effects of salinity in precocial chicks that rely on hypersaline wetlands for feeding are also very scarce. Previous findings (Hannam *et al.* 2003) questioned the role of hypersaline habitats for the development of precocial chicks of several wader species. However, we showed that black-winged stilt could raise their chicks successfully in hypersaline habitats using behavioural responses such as head-shaking to avoid ingesting salt. Studies on other species breeding in salinas, such as the kentish plover, are needed because this specie may use different physiological and behavioural responses to cope with saline and hypersaline water. Future studies should also compare the energy and water content of the chicks' prey between saline and freshwater habitats.

Similarly to other studies (Wiklund 1982; Burger 1984, 1987; Paulson & Erckmann 1985; Mayer & Ryan 1991; Powell 2001), we found strong benefits for the reproductive success of non-mobbing bird species nesting close to mobbing species. Our study focused on the black-winged stilt, but other species with mobbing behaviour breeding in saltpans, such as little terns and least terns (Powell 2001) should be relevant. Thus, further aspects should be analysed regarding the association of kentish plovers with little terns and also in the presence and absence of other mobbing species. Moreover, the presence of nesting mobbing species could benefit the success of precocious chicks from non-mobbing bird species. Our study and other studies call upon more studies of the relationships within the entire shorebird breeding community. Such an holistic perspective will have more realistic conservation implications.

The disturbance in the intertidal foraging grounds could potentially affect waders by keeping them away from the feeding areas (Navedo & Masero 2007; Dias *et al.* 2008), and may affect their roost fidelity and roost preferences (Rehfisch *et al.* 1996, 2003; Colwel *et al.* 2003). Future studies should address the importance of the managed roosting sites by linking the increase of disturbance in the intertidal areas to changes in the abundance and feeding behaviour of waders in related high-tide roosts. Waders are known to respond opportunistically to dewatering experiments (Velasquez 1992; Taft *et al*. 2002 and our study). We demonstrated that some species of waders could reach their daily biomass needs and gain mass foraging only or mostly on dewater ponds. Social interactions during feeding activities, resulting from high bird density in the pond, may have forced part of the wader population to move to intertidal mudflats in low-tide, according to predictions of the ideal-despotic model (Ens & Goss-Custard 1984). Future studies should address the carrying capacity of dewater areas of salinas for waders and this dynamic link to the intertidal areas during migratory periods.

Management

One the most important conclusions of this thesis is that the conservation and management of man-made coastal salinas have major implications for the conservation of waders. The maintenance of inactive salinas as a high quality habitat for waders depends on the implementation of adequate management actions, and in its absence salinas rapidly lose their value. Most efforts for the conservation of Mediterranean salinas have been focussed on the inclusion of these habitats under some form of legal protection (Sadoul *et al.* 1998). However, this effort is insufficient, because even protected, the majority of the salinas remain without regular and adequate management for waterbirds. Most present day salinas are privately owned, and have been converted into aquaculture ponds. Therefore, site-managers of coastal wetlands should establish agreements with the owners to ensure the adequate management of water levels and vegetation overgrowth on dykes, in order to balance the economic interest of salinas and waterbirds conservation. Despite the great value of the salinas as roosting, feeding and breeding habitat, it is not advisable to build new salinas to replace natural habitats, thus the following recommendations are intended for existing ponds whether they are salinas or aquaculture ponds.

Site-managers recognize the impossibility of conservation of all important areas for waders in an estuary, as there are a large variety of constrains given the conflicts with human activities or the reduced conservation budgets. One of the first criteria to decide which are the most important salinas for waders should be the **distance to intertidal foraging grounds** and **alternative roosting sites**. These distances are crucial for waders to use the most profitable foraging grounds and high-tide refuges. The flight energy costs and the predation risk are the major factors in routine movements between roost and foraging grounds or between roosts e.g. after disturbance (Rehfisch *et al.* 1996; Rogers, Piersma & Hassell 2006b). The conservation of a large and diverse wader community is dependent on a network of high quality roosts within the same wetland (Rehfisch *et al.* 1996, 2003; Rogers *et al.* 2006a; Dias *et al.* 2006a; Catry *et al.* 2011). Dias *et al.* (2006a) showed that more than 80% of dunlins forage within 5km from their roost in the Tagus estuary. A study in Wash estuary, England, concluded that the optimal distances between roosts may differ among species, however about 50% of the wader populations would favour refuges 7-10 km apart and 75% refuges 3-6 km apart (Rehfisch *et al.* 1996). Based on the most conservative estimates of ideal distance, and using the least mobile species such as dunlins, we advocate a distance between roosts and between roosts and foraging grounds of about 3km.

Supra-tide **roosting refuges** such as salinas are crucial when the intertidal flats are no longer available to waders as the tide advances, especially in spring tides. During the high-tide period waders congregate in dense flocks at salinas ponds and dykes, and spend most of their time sleeping, preening or feeding. Water depth is the major limiting factor in the selection of different ponds by ground-feeding waterbirds to roost and forage in saltpans. This limitation is directly related with bird size and more specifically to leg length (Isola *et al.* 2000; Ntiamoa-Baidu *et al.* 2008; Ma *et al.* 2009), and small waders such as sandpipers forage in water less than 5cm deep, and large waders such as godwits forage in water up to 15cm depth. The water depth of ponds is determinant to harbour different wader's species (see above). The dry areas in salinas such as dykes or paths between ponds, islets, pond banks or dry ponds are key sites for roosting. The height of dykes allow birds to roost, independently of the pond water level. These structures function as shelters, reducing the thermal stress by protecting birds from wind and cold, which induce the increment of maintenance costs (Wiersma & Piersma 1994; Warnock & Takekawa 1996). The overgrowth of saltmarsh plants in dry areas and also in shallow water ponds is a well-known factor that results in the reduction of roosting and foraging areas. Site-managers could control and reduce the vegetation cover with regular mechanic mowing, flooding the areas for temporarily periods or wash the dry areas with brine solution from saline ponds during the summer if available. Overall, the maintenance of these dry areas with special focus on dykes add value to salinas as refuge for waterbirds. Site-managers should be informed about the predation risk of each roost with especial attention to raptors and human related predators such as dogs and cats. As much as possible the access of human related predators should be reduced. The increasing number of runners, birdwatchers and nature photographers could constitute important focus of disturbance, and the access to roosts should be limited during the high-tide period.

We showed clearly that manipulating water level in a flood pond where a high density of benthic invertebrate occurs dramatically increases the value of salinas as a **foraging habitat** for several species of waterbirds. The management practices during the migratory and also wintering periods at salinas ensure quality feeding conditions for waders, and can contribute significantly to the maintenance of high density of foraging waders on the intertidal mudflats.

We propose a mosaic sequential strategy of dewatering and flooding ponds after an extended flood period or shallow water period, respectively (Fig. 29). This strategy must be implemented in ponds from salinas or aquaculture in different high-tide refuges within a wetland.

To extend the attractiveness of the ponds as foraging areas during a period of 15 days, we recommend maintaining a low water depth (< 5cm) instead of a complete drawdown, because the water content in sediments directly influences prey availability. After this period and taking advantage of fortnight spring tides, ponds could be flooded again (30-40 cm water depth) and the other set of ponds should be dewatered during the low-tide period. Site-managers should ensure water admissions especially in spring and summer months, i. e. during the recruitment period to provide high density

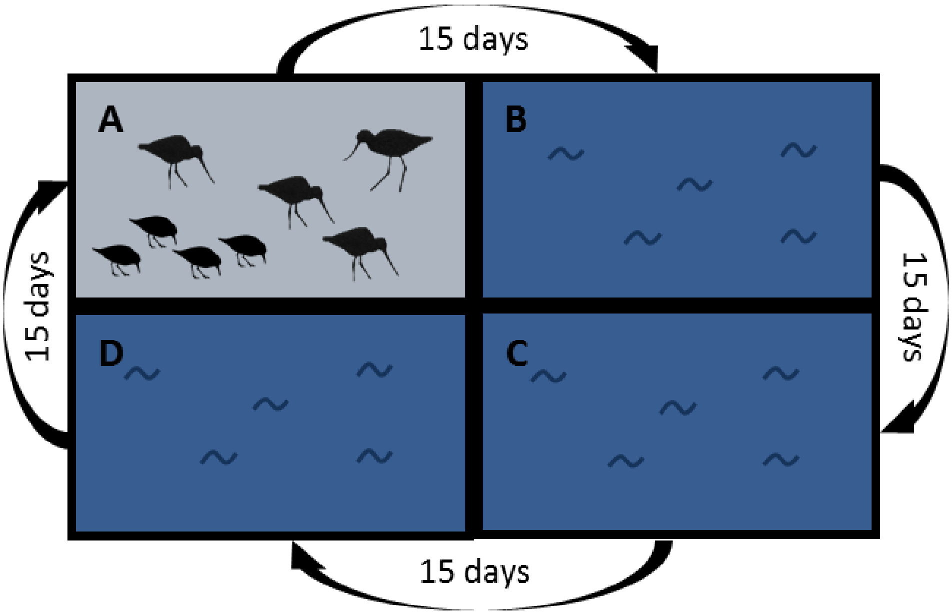

Figure 29. Representation of the mosaic sequential strategy of water management. Waterbirds could feed on the pond A during 15 days and after that pond B will be available as a foraging ground and so on. Taking advantage of spring tides, every 15 days the shallow ponds would be flooded again while the following ponds are drained. For instance, in an area with four ponds available for waterbird conservation, each pond would be available every 45 days.

of benthic invertebrates in the sediment ponds. Depending on wader population size and refuge size, site-managers must provide available areas enough for all wader species to roost and feed during the high-tide period. About one third of the area must be available for waders at any time. In aquaculture ponds the site-manager must give preference to central ponds, which are better in terms of bird safety, with an efective water circulation sistem.

The natural breeding habitats have been lost at an increasing alarming rate, meaning that salinas will be increasingly important as an alternative **nesting habitat** for coastal bird species. However, the attractiveness of salinas as nesting ground is dependent on active water and saltmarsh management. Common ground-nesting waterbirds such as kentish plover, black-winged stilt, avocets or little tern nest in different dry areas of salinas, however site-managers could promote their nesting in the dykes of ponds. Dykes play an important role in the nesting success of ground-nesting birds in salinas in two ways: 1) prevents the risk of flooding resulting from changes in the ponds' water level; 2) promotes the formation of dense colonies which reduces the predation risk. Different species selected nest-sites with different vegetation cover but all of them nest in open areas. Site-managers should provide attractive nesting sites, maintain dykes with sparse vegetation cover. Vegetation, rocks and debris of wood resulting from the maintenance of dyke structures in salinas area important for thermal regulation of eggs and incubating birds, can work as a shelter for adults and chicks after hatching against weather conditions or predation and help to dissimulate the nest-site. The management of these areas must be finished before the beginning of the breeding season, and the management could be reinforced during the breeding season in areas with no nests. To prevent birds from nesting in the pond banks and bottom with unregulated water levels and high risks of nest flooding, such ponds should be flooded (5-10 cm) during the nesting period. The height and verticality of dykes of the evaporation and crystallizer ponds can be deadly traps for unfledged chick which fall into these ponds, therefore ramps should be placed in the corners of the ponds. The suitable and restricted area of dykes promotes the formation of mixed breeding colonies and this association could be essential for the breeding success of different species. We clearly showed that when timid species such as kentish plover nest close to species with strong anti-predator behaviour such as black-winged stilts these experience higher reproductive success than that of conspecifics nesting in areas without such protector species. Therefore site-managers should promote the breeding of large populations of both small and large wader species in the same areas, because it will have a positive feedback on each other's breeding success. Safety during breeding period is related to the risk of predation of nesting birds, chicks or eggs but also to human disturbance of nesting birds and nest stepping. The human activities in salinas during the breeding season must be restricted to the minimum. We showed that carrions crows and red foxes have a strong negative effect in the nesting success of kentish plover. Several strategies could be implemented to reduce the impact of these predators in breeding populations, e.g. electric fences around breeding areas, fences over plover nests, or modifying the predators' behaviour through aversive conditioning.

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