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Conservation biology of wetland birds: breeding ecology, spatial analyses and genetic differentiation

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Conservation biology of wetland birds: breeding ecology, spatial analyses and genetic differentiation

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Contents

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Summary

Conservation biology uses various tools including spatial ecology and molecular ecology to provide better understanding of species that can be used to support and design effective conservation strategies. Many wetland bird species in the Kingdom of Saudi Arabia (KSA) are poorly known, and we lack detailed knowledge about their breeding ecology, spatial distribution and genetic differentiation. The first objective of my PhD research was to investigate the parental behaviour of an understudied endemic shorebird to the Middle East, the Crab Plover *Dromas ardeola* to record parental behaviour at the burrows over a 24-hour period. Since adult males and females look identical, I used molecular markers for sex determination. Molecular sexing was conducted using two different markers applied for 66 Crab Plover blood samples. I demonstrated that both males and females fed chicks, and that females brought food to chicks more frequently than did males (Chapter 2). The second objective was to investigate the breeding distribution of Crab Plovers along the Red Sea coast of Saudi Arabia and to compare the results with the last comprehensive survey conducted in 1996. I showed that the Red Sea coast of Saudi Arabia has approximately 35% of the Arabian breeding population of Crab Plovers. The major threats to this species along the Red Sea coast were also discussed (Chapter 3). The third objective was to model the spatial distribution of 22 wetland bird species along the Red Sea coast of Saudi Arabia using maximum entropy (MaxEnt) based on occurrence data and 10 environmental variables and then to determine sites with high species richness. This analysis identified 17 areas predicted to be suitable for supporting high species richness. I recommended

using this model of areas with high wetland bird species richness as a guide for monitoring and surveys to inform conservation strategies in the Red Sea region of the KSA (Chapter 4). The fourth objective was to use microsatellite markers to investigate the genetics and morphometric differentiations of a wide-spread shorebird species, the Kentish Plover, between islands and mainland sites. The main result of the latter investigation was that breeding populations are genetically and morphometrically differentiated between mainland sites and islands, as well as between different archipelagos. This finding calls for a reconsideration of the current conservation status of this species (Chapter 5). Finally, my PhD research has generated several research lines that warrant further investigation (Chapter 6).

Chapter 1

Introduction

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Behavioural ecology of breeding system

Behavioural ecology aims to explore the ways in which behavioural and morphological characteristics promote the survival and reproductive success of species in different ecological environments, extending to the dynamics of populations, including foraging behaviour, mate choice, fighting strategies and parental investment (Caro 1998, Davies *et al.* 2012).

Breeding systems consist of all the kinds of social behaviours demonstrated by males and females such as courtship, mating and parental care (Reynolds 1996). Understanding drivers of mating systems and parental care is one of the main directions in behavioural ecology, and a large amount of research has focused on these behaviours since the 1970. Mating systems can be simply described based on the number of mates obtained by males and females during the breeding season (Shuster & Wade 2003). For many bird species, mating systems have typically been identified based on behavioural observations (Wink & Dyrcz 1999), although with the advent of DNA fingerprinting, behavioural ecologists are increasingly quantifying genetic mating systems as well (Davies *et al.* 2012). Factors influencing the evolution of mating systems include natural selection, sexual conflict, sexual selection, sex ratios, parental care, food availability, ecological factors such as the spatial and temporal distribution of males and females, and the life histories of each sex (Dias *et al*. 2008).

Birds exhibit a broad range of mating systems including monogamy, polygyny and polyandry (Wink & Dyrcz 1999, Colwell 2010, Davies *et al*. 2012). The most common form of mating system among birds is monogamy (more than 92%; Lack 1968, Ford 1983, Møller 1986). This is an exclusive social pair bond between one male and one female. It can last for a single breeding season or for a lifetime, with both sexes sharing duties of parental care and offspring defence (Davies *et al*. 2012). Davies *et al*. (2012) reported that an important factor influencing the occurrence of monogamy in a population is decreased opportunities for individuals to find mates. The second most common mating system among birds is polygyny (approx. 5% of bird species; Hasselquist & Sherman 2001). This occurs when males mate with multiple females during a single breeding season (Hasselquist & Sherman 2001). A key factor that may influence polygynous mating systems among birds is male territoriality, with males on better quality territories attracting a larger number of females (Davies *et al*. 2012).

Parental care is any form of rearing provided by parents to enhance the rate of offspring survival (Clutton-Brock 1991, Royle *et al.* 2012). Parental care typically includes activities such as preparation of nests or burrows, production of large, heavily-yolked eggs, care of eggs or young inside or outside the parent’s body, provisioning the young with food before and after birth, caring for the offspring after nutritional independence and defence of offspring from predators (Clutton-Brock 1991, Balshine 2012).

Parental care in birds can be divided into six different forms: use of geothermal heat to incubate eggs (found only in Galliformes), brood parasitism, male-only care, female-only care, biparental care and cooperative breeding (Cockburn 2006). Cockburn (2006) reported that among bird species, 81% of care is provided by both parents (biparental), 9% of care is biparental with input from helpers, and 8% of care is provided by the female only. Male-only care occurs in about 1% of bird species. Brood parasitism and the use of geothermal heat to incubate eggs, involving no direct parental care, are rare, occurring in less than 1% of bird species.

Biparental care is most likely to occur when it contributes to the survival and development of offspring, under extreme or stochastic environmental conditions (Lack 1968, Clutton-Brock 1991, Brown *et al*. 2010, AlRashidi *et al*. 2011), or when adult population numbers limit mating opportunities (Martin & Cooke 1987, Balshine-Earn & Earn 1998). In biparental care, both males and females share the care of their offspring. However, despite cooperation between parents, care duties are often not shared equally (Lynn & Wingfield 2003, Schwagmeyer *et al*. 2002, 2005, McGraw *et al*. 2010, Barta *et al*. 2014). Both cooperation and conflict are found in species with biparental care (Harrison *et al*. 2009). Care is generally costly to parents in terms of time and energy, increasing parent mortality and decreasing potential future reproductive output (Clutton-Brock 1991, Székely *et al*. 2006, McGraw *et al*. 2010). Thus, sexual conflict arises since it is in the interest of each parent for the other to invest more and take responsibility for a greater share of the care duties (Arnqvist & Rowe 2005, Székely *et al*. 2006).

The significance of mating system and parental care in biodiversity conservation

Conservation biology is a multidisciplinary science that offers supporting evidence for protecting managing and restoring biodiversity (Soulé 1985, Soulé & Wilcox 1980, Sahney & Benton 2008). The idea of introducing behavioural research into wildlife conservation was first applied in 1974 (Geist & Walther 1974). Many studies since then have reported that understanding animal behaviour can play a significant role in conservation management (Sutherland 1998, Wallace & Buchholz 2001, Blumstein & Fernández-Juricic 2004, Shier 2006) and it is clear that some behavioural activities, such as movement, feeding and mating need to be taken into account in the planning and execution of many conservation programmes (Knight 2001, Buchholz 2007).

Social behaviour and conservation are often linked by demography (Székely *et al.* 2010). A vital demographic factor for understanding population viability is effective population size (N_e) (Anthony & Blumstein 2000). N_e is a fundamental principle in the conservation management of threatened species (Rieman & Allendorf 2001). It is affected by a variety of factors, for example, the exclusion of mating with closely-related individuals, unequal sex ratios, unequal family sizes, unequal generation sizes, overlapping generations, and inbreeding (Falconer 1989, Sutherland 1998, Székely *et al.* 2010). Mating systems can influence N_e since they determine which gametes are transferred to the next generation (Anthony & Blumstein 2000).

Determining the factors that limit population growth is vital to wildlife conservation. For example, if adult males are removed from a monogamous population, levels of population growth are expected to be lower than if the population were polygynous.

Determining the mating system of wild populations is therefore important for understanding the effects of biased sex ratios on population size for species that are in decline, and this may have implications for conservation management (Hummel & Ray 2008).

Predation increases mortality and can ultimately drive populations to extinction (Schoener *et al.* 2001). Understanding the behaviour of predators can play an important role in reducing mortality and based on this understanding, conservation measures can be taken, for example, manipulating the habitat through creating physical barriers for predators (Hinsley *et al.* 1995) or using high-frequency sounds or distasteful chemicals (Sutherland 1998). Additionally, understanding parental responses to predators (e.g. nest defence strategies) is important for evaluating the influence of predation and associated risks for offspring (Cresswell 1997, King *et al*. 1999).

Captive breeding and the reintroduction of endangered species have become important components in the conservation of a diversity of species (Snyder 1996, Beissinger 1997, Fraser 2008). In captive breeding situations, understanding behavioural mechanisms such as mate choice, social structure, and ecological influences on mating, is essential (Grahn *et al.* 1998). Many reintroduction efforts fail due to captive-bred animals lacking the behavioural skills needed to survive in the wild, including foraging behaviour, defence from predation and interspecific interactions (van Heezik *et al.* 1999).

Conservation biology of wetlands

Wetlands are among the most diverse and rich ecosystems on earth as they provide an essential habitat for many kinds of living creatures, such as birds, mammals, reptiles, fish, frogs and insects (Costanza *et al.* 1997, Buckton 2007, BirdLife International 2011). They are typically defined as transitional lands between uplands and fully aquatic environments on which the water table is either at or near the surface of the soil (Wetlands International 2014, Mitsch & Gotteschalk 2008). However, they can include many different kinds of habitat, including "marshes, peatlands, floodplains, rivers and lakes, and coastal areas such as saltmarshes, mangroves, and seagrass beds, areas of marine water the depth of which at low tide does not exceed six metres, also man-made wetlands, such as waste-water treatment ponds and reservoirs" according to the Ramsar Convention Secretariat (2011). Although wetlands only cover about six per cent of the earth's surface (Ramsar Convention Secretariat 2011), they harbour diverse plant and animal communities and provide essential services for humankind.

However, despite the fact that wetlands support considerable biodiversity, they are exposed to anthropogenic alteration (Eldridge 1992) due to their fragility and vulnerability (Hollis *et al.* 1988, Moor 2006). A number of processes have led to wetland ecosystem loss throughout the world, for instance, conversion or drainage for agricultural use, alterations in water regimes, urbanisation, overharvesting and overexploitation of natural resources, pollution, and the introduction of invasive species (Moser *et al.* 1996, Millennium Ecosystem Assessment 2005). As a result, approximately 50 % of the world's wetlands have been lost in the last century (Shine & Klemm 1999), which has given rise to a decline in biodiversity (Polasky *et al.* 2005). The conservation of wetland habitats has therefore become a priority in order to save wetland bird populations and other organisms from decline, which in turn may lead to the protection of wetland ecosystems around the world.

Wetland birds are an important indicator of wetland ecosystem health (Nebel *et al.* 2008). They are a vital component of the wetland environment (Nebel *et al.* 2008) and an important indicator of wetland ecosystem health as they are large organisms that occupy relatively high trophic levels (Furness & Greenwood 1993). Approximately 12% of bird species that depend on wetlands are classified as threatened (BirdLife International 2012).

Determining priorities for conservation requires accurate and reliable information on the distribution of species across wide areas (Hernandez *et al.* 2006). Species distribution models (SDM) provide simple methods for linking species distribution with environmental variables (Guisan & Zimmermann 2000, Guisan & Thuiller 2005), enabling recognition of the correlations between habitat features and species distribution (Guisan & Zimmermann 2000). This ability to model species distributions accurately and generate predictions has become a key tool in conservation planning (Guisan & Thuiller 2005). Species distribution model (SDM) is increasingly being implemented in wildlife management, landscape ecology and conservation biology (Akçakaya & Atwood 1997, Dettmers & Bart 1999). A detailed understanding of the ecological factors that determine geographical distribution of a species is essential for developing an appropriate conservation strategy (Rushton *et al.* 2004). Predictive habitat models, which are dependent on the species needs over large geographical regions, can be applied in variety of ways, for studies of landscape ecology, conservation biology and wildlife management (Dettmers & Bart 1999, Akçakaya & Atwood 1997).

The most effective species distribution models require the selection of key environmental variables. Remote sensing and spatial tools, such as geographic information systems (GIS), can contribute significantly to helping researchers identify appropriate conservation strategies for wetland areas (Pacione 1999). Remote sensing has been used in a variety of ways, for example, identifying and mapping wetland areas (Gluck *et al.* 1996, Kindscher *et al.* 1998, Lowry 2006), monitoring wetland changes (Haack 1996, Dahl 2006) and predicting sea level changes on coastal wetlands (Jensen *et al.* 1993). GIS tools and software are crucial in species distribution modelling (Pearson 2007). The combination of remote sensing data and GIS provides a vital tool for wetland conservation and management (Kashaigili *et al.* 2006).

My research has focused on The Kingdom of Saudi Arabia that has a hot and arid desert climate in most parts of the country but also comprises a variety of wetland types (Newton 1995). Tinley (1994) identified eight wetland systems in Saudi Arabia: coastal, dune field, salt marsh, karst, mountain, geothermal, valley and man-made. In recent years, excessive hunting, irrigation projects, pollution, overgrazing of vegetation and land reclamation have caused major losses and degradation of wetlands in Saudi Arabia (Faizi & Al Wetaid 1997). The IUCN (1984) reported that wetlands were exposed to deterioration, changing the habitat in Saudi Arabia. Sambas & Symens (1993) highlighted decline in wetland habitats in the Gulf area. To protect these important areas, more information on wetland bird abundance and distribution is critical for effective conservation prioritisation and may be gathered using tools such as remote sensing and species distribution modelling.

Molecular ecology and conservation

In this dissertation I use molecular tools to address behavioural ecology and conservation significance of mating systems and parental care. Molecular ecology is the application of molecular genetic methods to addressing ecological problems (Beebee & Rowe 2004, Andrew *et al.* 2013). It is often utilised in species identification, studies of animal behaviour, population genetics and conservation biology (Beebee & Rowe 2004). In recent years, molecular biological techniques have led to great advancements in conservation management (Haig 1998), providing researchers with a better understanding of how to conserve biological diversity most effectively (Beebee & Rowe 2004, Frankham *et al.* 2009).

Molecular ecology research has been applied to several conservation issues. Firstly, it has enhanced our understanding of the negative effects of inbreeding on reproduction and survival, since inbreeding raises extinction risks for a population (Wright *et al.*

2008). Secondly, it provides tools for measuring the loss of genetic diversity. Genetic diversity, species diversity and ecosystem diversity are considered the main factors of biodiversity (Pojar 2000). Understanding the correlation between genetic diversity and population viability is considered one of the fundamental goals of conservation genetics (Beebee & Rowe 2004). Thirdly, molecular ecology provides tools for testing levels of gene flow between fragmented populations (Frankham *et al.* 2009), determining levels of genetic diversity and genetic similarity between populations that are more or less isolated from others (Haig 1998). Identifying patterns of gene flow is considered crucial in evaluating conservation strategies. Fourthly, since the resources assigned for conserving particular species are often prioritized based on taxonomic status, molecular genetic techniques can provide effective tools for defining species and subspecies, and this is crucial to targeting endangered and threatened species, subspecies and populations for conservation action (Haig 1998). Fifthly, al molecular ecology provides tools for determining the nature of genetic differentiation that can advance our understanding of the demographic history of species (Willing *et al.* 2012).

Species' extinction risks are increased by a wide range of factors including habitat loss, over-exploitation, introduced invasive species, pollution, climate change, demographic and ecological variation, genetics and catastrophes (Frankham *et al.* 2003). A combination of multi-disciplinary evaluations that comprise species biology, ecology, demographic life-history, and genetic diversity are needed in order to design effective conservation plans (Frankham 2009).

Thesis objectives

The overall objective of my PhD dissertation was to learn essential tools in behavioural ecology, spatial ecology and molecular ecology to assist conservation of wetland birds in the Kingdom of Saudi Arabia. The specific objectives of my PhD dissertation was as follows:

1. *To investigate sex-related variation in body size and the breeding ecology of Crab Plovers in the KSA.* The Crab Plover *Dromas aredeola* is unique among waders as it breeds in colonies and digs burrows more than two metres long for nesting. Males and females are monomorphic and not easily distinguished (Hockey & Aspinall 1996, Delany *et al*. 2009). Furthermore, the parental behaviour of Crab Plovers has not been previously investigated. My objective was to monitor provisioning behaviour of parents to nesting burrows during the breeding season. Additionally, I used discriminant function analysis (DFA) to detect differences in the morphological traits between male and female Crab Plovers and used molecular markers for sex identification.

2. *To determine the breeding distribution of Crab Plovers across the Red Sea coast of the KSA and to compare the results with previous research.* The breeding distribution of Crab Plovers along the Red Sea of KSA is poorly known and its protection status is poorly described. I have carried out extensive field surveys during multiple breeding seasons, covering 16 islands across the Red Sea coast of KSA.

3. *To identify potentially suitable habitats for wetland birds along the poorly conserved Red Sea coast of the Kingdom of Saudi Arabia (KSA).* Information about habitat

suitability of wetland birds along the Red Sea coast of KSA is lacking. The most recent study by Alrashidi (2011) provided a habitat suitability map for one species, the Kentish Plover *Charadrius alexandrinus*, along the Red Sea coast of Saudi Arabia and the Farasan Islands. My objective was to apply species distribution models for 22 wetland bird species across the Red Sea coast of KSA to identify the relative importance of key sites by determining areas of high species richness as priority areas for future habitat protection. Using these results I propose the formation of conservation strategies for the Red Sea coastal area of the KSA.

4. *To explore the morphological and genetic differentiation of Kentish Plovers between Macaronesian islands, mainland Iberia, and North Africa*. To get training in molecular ecology I carried out a microsatellite analyses of a widely distributed shorebird, the Kentish Plover. Isolation-by-distance and isolation-by-adaptation are factors that may play important roles in genetic and phenotypic variation among populations (Orsini *et al.* 2013). I examined the correlation between genetic and phenotypic differences among populations using Mantel’s tests. I planned this study as a stepping stone to investigate morphological and genetic differentiation of Kentish Plovers in KSA in a future study.

Throughout the course of my PhD research, I have enjoyed the opportunity to learn a wide range of key skills. For instance, learning field techniques for investigating the behavioural ecology of mating systems (Chapters 2 and 3), modelling species distributions and richness using GIS and MaxEnt programs (Chapter 4), and working in a molecular ecology laboratory, utilising conservation genetic methods based on Kentish Plover models (Chapter 5).

The Appendices include a research paper I was invited to co-author (submitted to Ostrich) and my Field Report.

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Chapter 2

Sex differences and breeding ecology of a burrow-breeding shorebird, the Crab Plover, *Dromas ardeola*

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Author´s contributions

MA: study design, data collection, molecular sex-typing, statistical analysis, manuscript preparation

MS: comments on manuscript

MAR: data collection, comments on manuscript

NDR: molecular analysis, comments on manuscript

TS: data collection, comments on manuscript, general editing

Abstract

The Crab Plover *Dromas ardeola* is endemic to the Indian Ocean basin and breeds on islands around the Arabian Peninsula. Unique among shorebirds, it nests in an underground burrow where it lays a single white egg and feeds one chick. We investigated sex-related differences in body size and parental care of this species in Saudi Arabia. Molecular sexing of DNA samples of 66 adult Crab Plovers indicated that 26 were males and 40 were females. Males had significantly longer bill, wing and tarsus lengths than females, confirming previously published reports on sexual size dimorphism in Eritrea. Observations of molecular-sexed adults at four nests showed that both parents fed the chicks; however, females brought food to the nest-burrow more often than males (67.6% of all cases). We found that the temperature inside active nesting burrows was relatively stable at 35.0 ±SE=0.18°C (n = 11 nests) regardless of ambient temperature just outside the burrows. This suggests that burrows serve a purpose in incubation as well as in defence from predation. In the colony, adults were seen to prevent chicks from multiple burrows from leaving the nest when their own parents had left the colony, confirming a helper breeding system. We suggest areas for future investigation to further elucidate the breeding behaviour of this enigmatic and unique burrowing shorebird.

Key words: breeding ecology, chick diet, Crab Plover, molecular sexing, nest burrow temperature, Saudi Arabia

Introduction

The Crab Plover *Dromas ardeola* is a distinctive, medium-sized shorebird (weight: 230–325 g), with black and white plumage, a strong bill and long, black legs (Burton & Burton 2002). Sexual dimorphism occurs in size, though not in plumage, and males are generally larger than females (De Marchi *et al.* 2012). Juvenile Crab Plovers lack the characteristic adult patterns, instead emerging a uniform grey-brown with a smaller bill and shorter legs (Burton & Burton 2002).

The breeding ecology of Crab Plovers is unique among waders. They breed in colonies which are re-established each year on sandbanks (Chiozzi *et al.* 2011), in which they dig nest-burrows more than two metres long (Hockey & Aspinall 1996). The female lays a single large, white egg (in rare cases, two eggs) without any pigmentation (Hockey & Aspinall 1996, Delany *et al.* 2009, Tayefeh *et al.* 2013), a trait typical of species that nest in holes or hollow trees (Burton & Burton 2002, Jennings 2010). Reports suggest that the average incubation period is 33 days (De Marchi *et al.* 2008, Tayefeh *et al.* 2013). After hatching, a Crab Plover chick stays inside its burrow for several days (del Hoyo *et al.* 1996, Hockey & Aspinall 1997) and both parents deliver food (Burton & Burton 2002, Hockey & Aspinall 1996). Chicks fledge at the age of seven weeks (Tayefeh *et al.* 2013), at which time they leave the colony with their parents and migrate together as a family (Delany *et al.* 2009, Hockey & Aspinall 1997). Crab Plovers are partial migrants (Delany *et al.* 2009, del Hoyo *et al.* 1996), and are the only reported waterbird species in which adults continue to provide food for their chicks during post-breeding migration; juveniles remain at least partly dependent on their parents to provide food for several months (Delany *et al.* 2009, De Sanctis *et al.* 2005, Fasola *et al.* 1996).

De Sanctis *et al.* (2005) suggested that the long duration of parental care may be attributed to the low reproductive yield of the parents, which is a consequence of the ecological limitations of the breeding areas; namely extremely hot environments. Their breeding range spans Kuwait, Iran, the United Arab Emirates, Oman, Yemen, Saudi Arabia, Somalia, Eritrea, Sudan and possibly western India, the Lakshadweep and Maldives (Almalki *et al.* 2014, Delany *et al.* 2009, De Marchi *et al.* 2006, Javed *et al.* 2012, Jennings 2010, Shobrak *et al.* 2002, Tayefeh *et al.* 2013) and the breeding season takes place during the hottest time of the year, between April and August (Hockey & Aspinall 1997, PERSGA/GEF 2003).

Recent research suggests that the role of nest-burrows is to protect the eggs against high ambient temperatures (which can reach up to 45°C in the shade) providing near-optimal temperature and humidity for egg development (based on abandoned burrows, a temperature of 35.2°C and a humidity of 60.2% have been estimated; Aspinall & Hockey 1996, De Marchi *et al.* 2008). This minimizes the time required for incubating the eggs (28.3% of the time of the parents; De Marchi *et al.* 2008). When the chicks begin to leave their burrows, they avoid high temperatures and only come out in the early morning or late afternoon (Hockey & Aspinall 1996).

During the breeding season, Crab Plovers commonly forage singly or in loose groups on tidal mudflats or in shallow water (Burton & Burton 2002, Delany *et al*. 2009, del Hoyo *et al.* 1996). These foraging groups typically contain 20 to 30 individuals (Burton & Burton 2002) and foraging takes place during both day and night, in different locations in the intertidal zone (Burton & Burton 2002, Fasola *et al.* 1996). Their diet consists largely of crabs, but also includes other marine animals such as crustaceans, small molluscs and marine worms (del Hoyo *et al.* 1996, Fasola *et al.* 1996). The heavy and powerful bill gives this species the ability to crush larger crabs into pieces to eat, though smaller crabs are often swallowed whole (Soni & Bhuva 2007). De Marchi *et al.* (2012) established that bill size differs between males and females, which might be related to the prey size caught by each sex (Rands 1996).

Our study aimed to further our understanding of sex differences and the breeding ecology of the Crab Plover with four main objectives. First, we measured the body size of males and females on the Farasan Islands in the Red Sea using molecular markers for sex determination. De Marchi *et al.* (2012) applied discriminant function analysis on Dahret Island in the Dahlak Archipelago, Eritrea, and we used the same approach to investigate whether morphological traits differ between the Dahlak Archipelago and the Farasan Archipelago. Second, we assessed the roles of males and females in parental care, by monitoring the delivery of food to chicks in nest-burrows according to the sex of the adults. Third, we determined the type of food items provided by the parents. Fourth, we measured temperatures both inside and outside active nest-burrows during the breeding season. We also investigated whether burrow temperatures differ in different regions.

Methods

Study area

We investigated a colony of Crab Plovers on Humr Island in the Farasan Archipelago of Saudi Arabia (16°47’00”N, 42°00’42”E; Fig. 1) on 47 days between 19May and 15 July

2012. Humr Island, 310 ha, is sandy, largely low and approximately flat with several reef platforms. Vegetation is sparse and includes salt-tolerant plants such as *Halopeplis perfoliata, Zygophyllum album, Zygophyllum simplex and Suaeda monoica*. Mangroves, mainly *Avicennia marina*, exist along a wide area of the Humr Island coast. The weather from May to July is arid and hot with daily temperatures exceeding 50°C in direct sunlight.

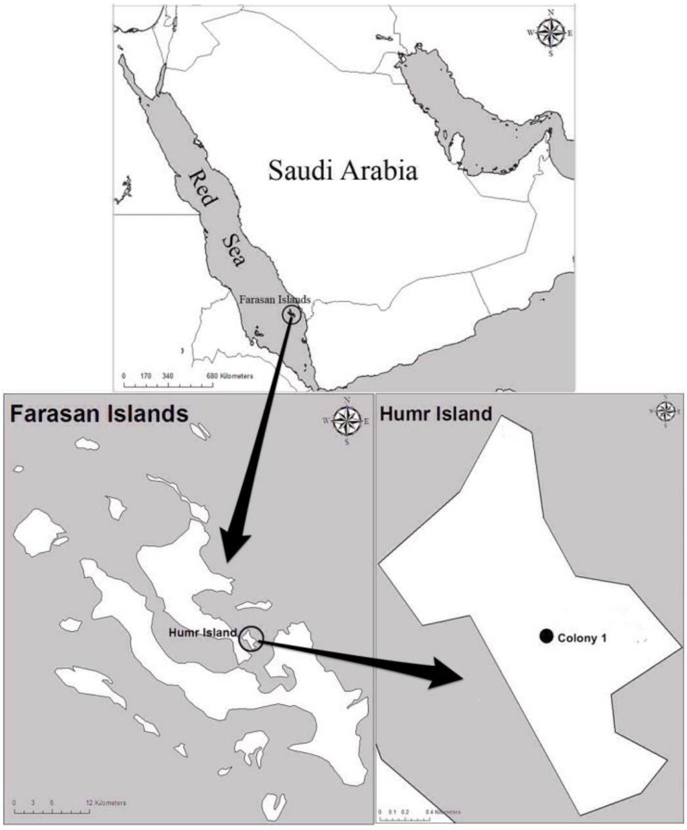

Fig. 1. *Map showing location of Crab Plover breeding colony (black dot) on Humr Island, Red Sea.*

The Crab Plover colony we studied comprised 265 active nest-burrows. We recorded three other bird species breeding on the island: Kentish Plover *Charadrius alexandrinus*, Saunders's Tern Sterna *saundersi* and Sooty Gull *Larus hemprichii*. We also found an abandoned Osprey *Pandion haliaetus* nest.

Trapping, morphometric measurements, behavioural observations and breeding ecology

Crab Plovers are easily disturbed by observers and are therefore difficult to capture, especially around their breeding sites. We captured Crab Plovers using mist-nets during the night. We used torchlight to dazzle the birds and drive them towards the mist nets. Captured birds were removed immediately to reduce the possibility of injury.

Morphometric measurements were taken from 66 adult Crab Plovers captured between 6 June and 15 July. Three measurements (all in mm) were taken from each adult: (1) bill length (the exposed culmen) was measured with calipers from the edge of the feathers on the top of the bill to the bill tip; (2) the flattened and straightened length of the right wing was measured using a ruler from the carpal joint to the tip of the longest primary; and (3) the length of the right tarsus was measured with calipers from the notch on the knee to the distal end of the tarsometatarsus. Weight (in grams) was also measured, using a 600 g Pesola spring balance. However, we excluded weight measurements from morphometric analyses to determine differences between the sexes since weight may vary considerably between individuals and over time (even within days) and therefore they may not be good predictors of body size (van de Pol *et al.* 2009). All birds were ringed on the tibia with single metal rings provided by the Saudi Wildlife Authority and one–three coloured plastic rings for individual identification. Blood samples (25–50 μl) were taken from the brachial vein of adults and stored in Queen’s lysis buffer (Székely *et al.* 2006) for molecular sex-typing.

To mark nest-burrows, we used numbered plastic spoons placed near the burrow entrances. We used the presence of fresh tracks at a nest-burrow entrance to distinguish inhabited burrows from uninhabited ones (De Marchi *et al.* 2006). To extract the eggs from the burrows for brief examination, we used a tool constructed by attaching a spoon to the end of a 1.5 m stick. The eggs were extracted from burrows at the beginning of the breeding season and were accessible at this stage since they were not positioned at the far end of the curved burrows. We collected and measured four eggs; two in June 2011 from the Albatain Islands, which are located about 10 km northwest of Al Qunfudhah city, and two from Humr Island in 2012. As the breeding area was very fragile, we used sand-shoes – analogous to snow-shoes – for walking around the colony in order to avoid destroying burrows (Fig. 2).

Fig. 2. *Sand-shoes, analogous to snow-shoes, designed for walking around the colony in order to prevent damage to burrows.*

A mobile hide was used for making behavioural observations to avoid disturbing the Crab Plovers. The hide was located about 30 m from the colony. Observations of bird behaviour were made during the day, and activities at the nest-burrows were also recorded using Bushnell Trophy Cam 270p HD (model119466 IR) and Reconyx (SC950 HyperFire Security IR) cameras. It was difficult to determine by direct observation which nest-burrows belonged to the individually-marked birds as the burrows were close to one another and the birds were highly mobile within the colony.

Therefore, the Bushnell camera was set up to record an image every minute to determine which nest-burrows the ringed birds were using. In this way, six burrows were identified as being inhabited by one ringed and one unringed parent (two contained eggs and four contained chicks). To determine the behaviour of parents at the nest-burrows, the Reconyx camera was positioned about 1 m from a nest-burrow entrance and set to record one image every five seconds for 24 hours and in several cases for 48 hours. The cameras were operated from the beginning of June until mid-July 2012.

Ground temperatures and temperatures inside active nest burrows were recorded using iButtons (model no. DS1922LF50), with an accuracy of ±0.5°C between -10°C and +65°C, as tested by the manufacturer. Ground temperatures were measured using iButtons placed on the surface of the ground in the colony. We recorded nest-burrow temperatures using iButtons placed approximately 100 cm inside 11 active nest burrows for 24 hours at 30-second intervals from late June to mid-July 2012. The devices were glued to a small wooden stick and inserted through the wall of the burrow to keep the iButton data logger inside the nest-burrow. The birds did not appear to be disturbed by them.

The types of food provided for chicks and the feeding frequency throughout the day were determined by deploying the Reconyx camera for 24 hours at each of four nest burrows. We also collected some items discarded by the Crab Plovers from around the nest-burrow entrances. Prey size was estimated in two ways. First, based on the photographs, we evaluated prey size by comparison with the size of Crab Plover bills (approx. 5.5 cm ±SE=0.05; n = 42). Second, we directly measured discarded food items that we found in the colony around the nest-burrows.

DNA extraction and sex determination

DNA extraction from 66 blood samples was carried out using an ammonium acetate method (Nicholls *et al*. 2000, protocol online at *http://www.shef.ac.uk/nbaf-s/protocols_list*). DNA concentration was evaluated using a Nanodrop ND8000 spectrophotometer. Sex determination in birds is usually based on amplification (through polymerase chain reaction - PCR) of fragments of a Z and W gene that differ in size, such that males (ZZ) and females (ZW) are easily distinguishable by the observation of the differently sized amplicons. For each sample, PCR amplification was conducted using two primer pairs, P2/P8 (Griffiths *et al*. 1998) and Z-002A F/Z-002A R (Dawson 2007) in a single duplex reaction with fluorescent dye labelled forward primers (HEX and 6FAM respectively). Using two different markers prevents errors resulting from the misinterpretation of sex-typing data due to allelic dropout or Z polymorphism (Dawson *et al.* 2001, dos Remedios *et al*. 2010, Toouli *et al*. 2000).

PCRs were performed in 2-μl reactions including 10-15 ng dry DNA, 0.2 μM of each primer (combined in 1 ul of low TE buffer) and 1 μl QIAGEN Multiplex PCR MasterMix, with a layer of mineral oil to prevent evaporation. PCR amplification was conducted on a DNA Engine Tetrad 2 Peltier Thermal Cycler using the following conditions: 15 min at 95ºC, followed by 35 cycles of 30 s at 94 ºC, 90 s at 52 ºC, 60 s at 72 ºC, and a final cycle of 30 min at 60 ºC. Amplicons were visualized on an ABI 3730 48-well capillary Sequencer, with GeneScan 500 ROX size standard. Alleles were scored using GeneMapper software version 3.7 (Applied Biosystems).

Statistical analyses

To test whether there is a significant difference in the amount of food delivered by males and females we used Wilcoxon’s test.

Discriminant function analysis (DFA) was used to evaluate sexual size dimorphism. In this, outliers were checked using box plots. Missing values (22.1% of the total data set) were evaluated using the Expectation Maximization (EM) method (Strauss *et al.* 2003) since DFA cannot be applied to samples containing missing values. We applied EM for males and females whose sex was identified via molecular sexing. The Shapiro-Wilk test was applied to detect the normality of each variable, and all variables were normally distributed ($P>0.05$). In order to test for differences in body size between males and females, MANOVA was applied using bill length, wing length and tarsus length as dependent variables and sex as an independent variable. In order to identify the significance of sex differences for each dependent variable, t-tests were applied. Correlation tests (Pearson correlation) were applied to examine correlations between each two variable combinations since DFA supposes predictors should not be highly correlated with each other. We found that none of the three variables were highly correlated with each other (Pearson $r<0.5$). A leave-one-out cross validation test was applied to check the accuracy of prediction. To identify the variable that differed most between males and females, DFA was performed on the three morphological characteristics using the package MASS, implemented in R (version 2.15.1) based on molecularly sexed individuals for which all three measurements were available (26 males and 40 females). The three morphological measurements from these birds were grouped together using DFA to generate a predictive function formula that can be applied to discriminate the sexes. Finally, a cut-off value was identified, with individuals with a higher value being classified as male, and individuals with a lesser value being classified as female.

Results

Timing of breeding and egg laying

Crab Plovers started to dig their nest-burrows in mid-May 2012 and egg-laying was initiated at the end of May. The average egg length and width measurements were 64.3 ±SE=0.80 mm and 45.1 ±SE=0.47 mm respectively. Egg hatching appears to be synchronized, and the eggs started to hatch at the end of June. When the eggs hatched, the parents would throw the eggshell fragments outside the nest-burrow. From the eggshell fragments recorded at the end of June, we estimated that at least 64 eggs laid in the colony had hatched. At the beginning of July, new eggshell fragments were found outside the nest-burrows, but due to strong winds, we could not estimate the numbers accurately. The parents started to bring food to their chicks at the beginning of July.

Incubation temperature

The lowest and highest ground temperatures in the colony were recorded as 28.15°C and 55.56°C, respectively. The average temperature inside 11 active nest-burrows was 35.0 ±SE=0.18 °C (n = 11 nest-burrows). The temperature inside the nest-burrows was almost constant from midnight to midnight but the outside temperature varied from around 30°C at night to 50°C in the middle of the day (Fig. 3).

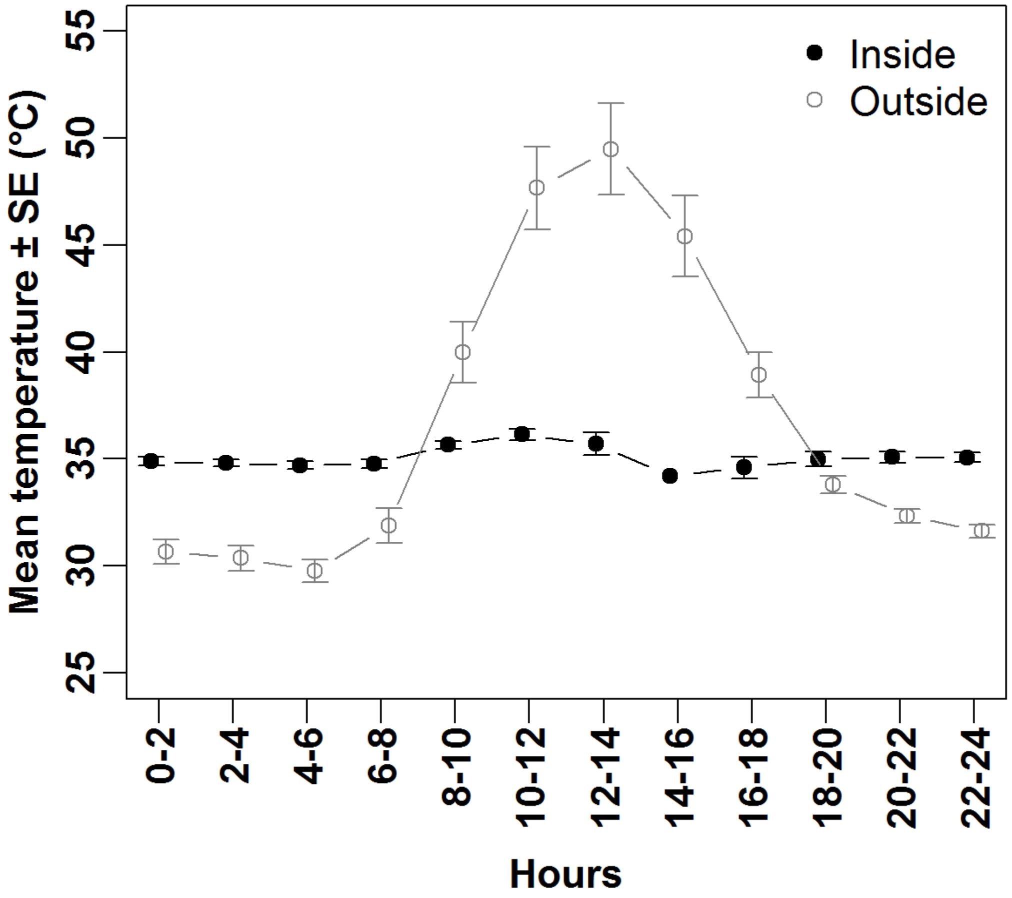

Fig. 3. *Ambient temperature (±SE) outside seven Crab Plover nestburrows on Humr Island, Saudi Arabia, and temperature at about one metre inside 11 active nest-burrows from midnight to midnight in two-hour periods during June 2012.*

Molecular sexing and sexual size dimorphism

Sixty-six adult Crab Plovers captured on Humr Island were sexed using molecular techniques and found to comprise 26 males and 40 females (Table 1). Although no samples were available from individuals of previously known sex, all samples were amplified with both molecular markers and the results based on P2/P8 and Z-002A markers were consistent for all 66 samples.

Table 1: *Data collected on Crab Plovers caught on Humr Island, Saudi Arabia, during May–July 2012 and sexed using molecular techniques.*

	Number of sexed	Bill measured	Wing measured	Tarsus measured	Weight measured
Male	26	15	25	19	22
Female	40	27	38	33	39
Total	66	42	63	52	61

MANOVA of the birds' bill, wing and tarsus lengths indicated significant morphological differences between males and females (F1, 64 = 10.5, P<0.0005; Table 2). T-tests revealed that the bill, wing and tarsus lengths of males significantly exceeded those of females, bill lengths being the most significantly different and tarsus lengths the least (Table 2). The results of direct DFA also showed that bill length is the most reliable single predictor of sex, whereas wing length and tarsus length are not so effective (Table 3). The coefficients of the three linear discriminant models were: (bill length x 0.480237862) + (wing length x 0.02213118) + (tarsus length x 0.028304745). The overall discriminatory power of the model was high (83.3% of individuals were correctly classified). Using this discriminant model to classify the 66 individuals resulted in misclassification of 7 of 36 females (19%) and 4 of 19 males (21%).

Table 2: *Body size parameters of adult Crab Plovers caught on Humr Island, Saudi Arabia, during May–July 2012, after estimating missing values using the expectation maximization (EM) method (SE = Standard Error). Note that as all these parameters are measures of size, it is appropriate to apply the Bonferroni correction. Therefore the null hypothesis should only be rejected if p <0.05/3 = 0.0166.*

	Male	Female	p value, t test
	Mean ±SE (N)	Mean ±SE (N)	
Bill length (mm)	58.10 ±0.47 (26)	54.07 ±0.27 (40)	<0.0005
Wing length (mm)	213.51 ±0.75 (26)	209.72 ±0.82 (40)	0.001
Tarsus length (mm)	98.06 ±0.58 (26)	96.04 ±0.54 (40)	0.0139

Table 3: *Classification accuracy using discriminant function analysis (number/total (and %) correctly assigned) based on measurements of single morphological characteristics in males, females and in all birds from a sample of 66 Crab Plovers (26 males and 40 females) caught on Humr island, Saudi Arabia, during May–July 2012 and sexed using molecular techniques.*

Predictor of sex	Males	Females	All birds
Bill Length	23/26 (88.5%)	34/40 (85.0%)	58/66 (87.9%)
Wing Length	19/26 (73.1%)	21/40 (52.5%)	42/66 (63.6%)
Tarsus Length	17/26 (65.4%)	27/40 (67.5%)	45/66 (68.2%)

Brood care

At the beginning of the breeding season, we observed several juvenile Crab Plovers at the colony on Humr Island, but we found no evidence that they took part in nesting activities as helpers or otherwise.

During chick-rearing, only one parent usually attended the nest-burrow entrance to provide food, although in a small number of cases both parents were present at the same time. In addition, there were many occasions when both parents were away from the nest-burrow and most adults left the colony at midday when ambient temperatures were highest.

Based on observations of individuals of known sex at four nests, the number of visits by the female and by the male were respectively 12 and 8 (60% by the female), 15 and 7 (68.2%), 15 and 6 (71.4%) and 17 and 7 (70.8%). This difference is not statistically significantly different from equal feeding rates (Wilcoxon's test, $p = 0.25$), probably due to the small sample size. After hatching, the parents usually remained outside the nest-burrows and fed their chicks from the nest-burrow entrances. We also observed cases when one adult guarded several nest burrows and the single adult prevented chicks to come out from the burrows.

Composition of food fed to chicks

Altogether 86 prey items were identified from Reconyx Camera photos while being fed to chicks by adults at four nests; these included crabs, fishes, prawns and worms (Table 4, Fig. 4). The remains of 23 other food items discarded around the nest-burrow entrances were also collected and measured the majority (17/23, 74%) of these were fish (Table 4). The photos showed that the parents brought only one prey item per feeding visit. On average each chick was fed 21.5 (±SE=0.96) times per day. Food was delivered during the day and night, but based on four nests, provisioning was higher by day than at night (Fig. 5).

Nest-burrow structure

At the end of the breeding season we dug up three used nest burrows and found that the burrows extended 70 to 90 cm below ground level, were about 2 m long, and included a bend so that in some cases the direction of the end of the burrow was opposite to that of the entrance. The nest chamber was at the end of the burrow (Fig. 6).

Table 4: *Type and size of food items delivered to Crab Plover chicks on Humr Island, Saudi Arabia, during June–July 2012 identified using camera-trap photos at four nests. In addition, measurements are given of the remains of some prey items that were collected from around the nest burrow entrances.*

	Prey identified from photos			Prey remains collected from burrow entrances	
Prey type	% of prey items	Prey sizes (cm ± SE)	Number	Prey sizes (cm ±SE)	Number
Crab	53.5	3.8 ± 0.13	46	3.25 ± 0.25	2
Fish	23.3	5.7 ± 0.20	20	6.1 ± 0.33	17
Prawn	3.5	5.5 ± 1.3	3	5.1 ± 1.6	4
Worm	2.3	Not measured	2		0
Unknown	17.4	4.1 ± 0.28	15		0
Total	100		86		23

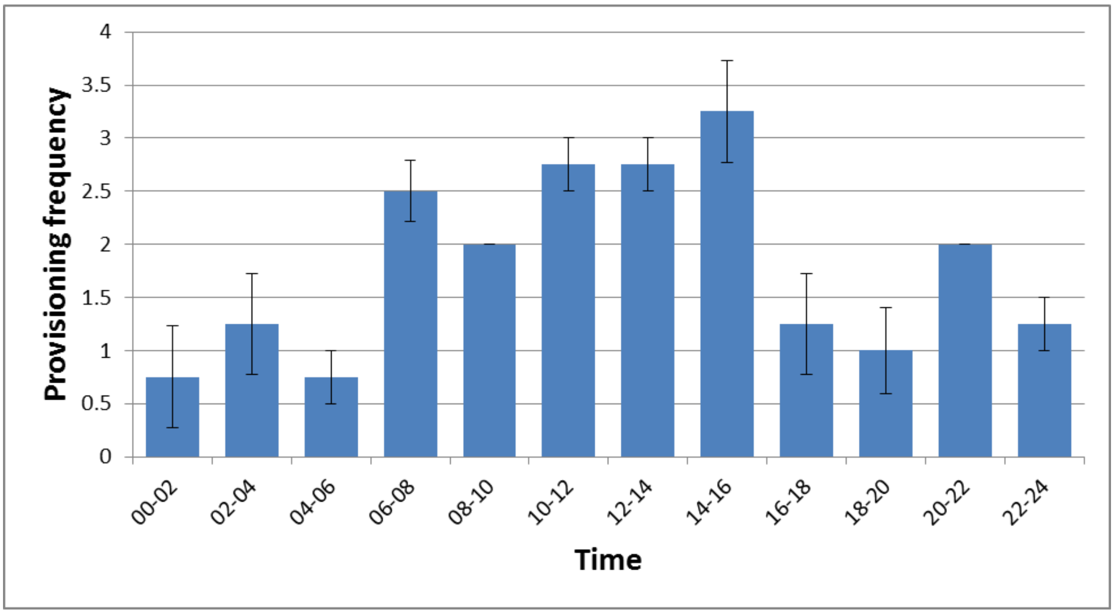

Fig. 5: *Provisioning frequency (number of feeding visits by adults per nest per two-hour period ±SE) from midnight to midnight at four Crab Plover nest-burrows on Humr Island, Saudi Arabia, during June-July 2012.*

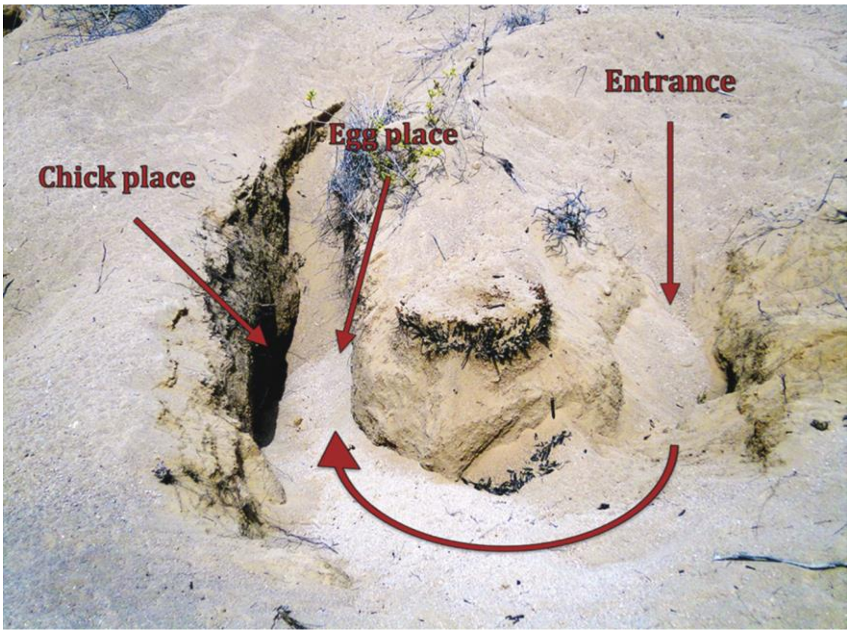

Fig. 6. *Excavation revealed that from the entrance, the burrow sloped downwards and turned 180° before coming to the nest chamber.*

Discussion

Our study yielded several important results. To our knowledge, this is the first study to identify differences in food provisioning by male and female Crab Plovers identified by molecular sex-typing. Using the data gathered from four nests we found females brought food to their chicks more frequently than males. In about 81% of bird species, both males and females contribute to the care of offspring; however, parental investment is often not shared equally between the parents (Cockburn 2006, Olson *et al.* 2008).

We also found, through analyses of sexual size dimorphism in molecularly sexed Crab Plovers, that males are larger than females in bill length, wing length and tarsus length and that bill length is the best predictor of sex. This finding is consistent with that of De Marchi *et al.* (2012) who found that males are larger than females for all morphological measurements they studied. However, they found that the best characters for distinguishing male and female Crab Plovers were head-bill length, bill length, bill depth, wing chord and weight, whereas tarsus length was a poor predictor.

Our results indicate that the main primary items fed of Crab Plover chick are (in descending order of importance) crabs, fishes, prawns and worms. This is consistent with the results of Aspinall (2010) who reported that about 95% of full-grown Crab Plovers depend on crabs, but chicks are occasionally fed fish and molluscs. Morris (1992) found that in Abu Dhabi, parent Crab Plovers mainly fed their young with crabs, and in some cases fish, but very rarely molluscs. Therefore, we can conclude that the Crab Plover’s main prey is crabs. Indeed Aspinall & Hockey (1996) suggested that the distribution of Crab Plovers is restricted to tropical and subtropical areas due to the abundance of crabs. However, the tidal hypothesis cannot be tested in Farasan Islands because no publicly available tidal table seem to exist.

Based on four nests, we found that the rate of delivery of prey to the chick was roughly twice as high by day than at night (Fig. 5). De Marchi *et al.* (2015) suggested that the foraging times of Crab Plovers are related to the tidal rhythm; similarly Geering *et al.* (2007) found that feeding activities by the majority of shorebird species are driven by tidal rhythm. However, the tidal range in the Red Sea is extremely small (Edwards 1987), so we were unable to evaluate the effect of tide on the provisioning of chicks in our study population.

On several occasions we observed Crab Plover adults guarded several nest burrows and single adult prevented chicks to go out from nest-burrows while their parents were away from the colony. These findings suggest that there may be a cooperative care system operating among Crab Plovers. The present findings seem to be consistent with other research which suggested the existence of helpers at Crab Plover colonies. Helpers may increase vigilance and thus protect the Crab Plover chicks from potential risks (Hockey & Aspinall 1997, Aspinall 2010). Covas *et al.* (2008) indicated that helpers can positively influence reproductive performance in many ways; for example, food provisioning or parental care can enhance the condition and survival of chicks. We identified several juvenile birds at the Crab Plover colony on Humr Island at the beginning of the breeding season. However, we have no evidence that these juveniles shared the care of chicks with the adults.

One possible benefit of colonial breeding in birds is enhancing their defence against predators, yet conversely, breeding in a colony also raises the visibility of the group to predators (Ashbrook *et al.* 2008, Urfi 2003). Nest defence can be considered a parental strategy to increase fitness by decreasing the probability that a predator will target their offspring (Redmond *et al.* 2009). However, the Crab Plovers we observed did not defend their colony against the attendance or attacks of Sooty Gulls. Furthermore, when we visited the colony, we noticed that all the Crab Plovers would leave the colony and move to neighbouring sites. Therefore, nesting in burrows may help the parents to protect their young without the need to interact with predators, thus avoiding any physical risk associated with an active defence. If offspring are well-protected underground, Sooty Gulls might not depredate the Crab Plover chicks but instead collect their discarded food.

The average temperature inside 11 active Crab Plover nest-burrows was relatively stable at around 35.0°C regardless of the ambient temperature outside. This finding is consistent with that of De Marchi *et al.* (2008), who found the average temperature inside nest-burrows at 100–200 cm from the entrance to be 35.2°C. Conway & Martin (2000) reported that the optimal temperature for egg development for the majority of bird species is between 36 and 40.5ºC. De Marchi *et al.* (2008) suggested that the most obvious purpose of nesting in burrows is to protect the eggs from high temperatures. As a consequence, parents have to spend very little time incubating their eggs (De Marchi *et al.* 2008, De Marchi *et al.* 2014).

Conclusions

The demographic and behavioural data collected on Crab Plovers breeding on Humr Island, Saudi Arabia, in this study revealed several key findings: (1) the most reliable morphological trait indicating the sex of Crab Plovers in our study population was bill length; (2) both males and females provided food to the chicks but provisioning was carried out more often by females than males; (3) this study has gone some way towards enhancing our understanding of daynight cycles in chick feeding routines, with higher provisioning rates during the daytime than at night; and (4) the temperature inside active nest-burrows was rather stable and did not change significantly with above-ground temperature.

In future, cannon-netting could be used to catch a larger number of birds in the colony or at their feeding sites. While this study has provided valuable insights into the breeding ecology of a highly specialised, unique shorebird, several questions still remain. In particular, we suggest future studies should concentrate on: (1) investigating whether the Crab Plover can incubate and rear two eggs and chicks at the same time; (2) studying mate fidelity in Crab Plovers; (3) studying the nest architecture of the Crab Plover; and (4) investigating the identity and function of the helpers at Crab Plover colonies.

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Chapter 3

Breeding distribution and conservation of Crab Plover (*Dromas ardeola*) in Saudi Arabia (Aves: Charadriiformes)

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Author´s contributions

MA: study design, data collection, manuscript preparation

MAR: comments on manuscript

MS: comments on manuscript

TS: comments on manuscript, general editing

Abstract

We surveyed Crab Plovers, a species which is endemic to the Arabian Peninsula, in 2011, 2012 and 2013 in coastal and off shore areas of Red Sea in Saudi Arabia, and compared the data with the survey carried out in 1996. We found two new colonies which comprise together about 539 pairs. Altogether 1380 pairs of Crab Plover breed in the Red Sea of Saudi Arabia in five colonies, representing approximately 35% of the Arabian breeding population. Major threats to the Crab Plover are human disturbance, egg collection and introduced animals (e.g. cats, rats and snakes) that may feed upon eggs and chicks.

Keywords: Crab Plover, conservation status, threats, human disturbance, Saudi Arabia.

Introduction

Crab Plover breeding sites are restricted to islands around the Arabian Peninsula, although they probably also breeds in Western India, in the Laccadive Islands and the Maldives (Delany *et al.* 2009). Jennings (2010) reported about 4000 pairs of Crab Plover breeding in approximately 20 colonies around the Arabian Peninsula, mostly in Kuwait, the United Arab Emirates, Oman, Yemen and Saudi Arabia. In recent years, 30 Crab Plover colonies have been discovered in Eritrea, comprising an estimated 5000–6000 pairs in total (De Marchi *et al.* 2006). In addition, four colonies of Crab Plover were reported from Iran in 2011, the largest of which, on Dara Island, had 3527 nests (Tayefeh *et al.* 2011). Breeding has also been recorded in Sudan and on islets off Northern Somalia (Shobrak *et al.* 2002; Delany *et al.* 2009).

We surveyed Crab Plovers in the Red Sea region of Saudi Arabia for two reasons. First, the latest comprehensive survey was in 1996, and these data required updating. Second, there is a gap between the estimated number of breeding pairs (14,000 – 15,000, Aspinall 1996, as cited in Javed *et al.* 2012), and the estimated number of non- breeding birds 60,000–80,000 individuals (De Marchi *et al.* 2006), and thus former surveys may have overlooked some of the breeding sites in the Red Sea region.

Methods

The surveys were carried out between May and June 2011, in May and July 2012, and in May and June 2013, and covered 16 islands. Five of these islands belong to the Farasan Islands, two to the Albatain Islands, seven are situated in Umluj and two in the

Al Wajh Archipelago. The total length of the Red Sea coastline of Saudi Arabia is approximately 1840 km, of which our survey covered about 1300 km (PERSGA/GEF 2003). The climate is arid and hot, particularly during summer. Average rainfall is less than 70 mm per year (PERSGA/GEF 2003). Boats were used to visit the islands, and the location of each colony was recorded using a handheld Global Positioning System (GPS) device. In each colony, Crab Plover population was estimated from the number of active burrows. The presence or absence of fresh tracks at a burrow entrance was used to determine whether it was active (De Marchi *et al.* 2006). We also recorded the number of Crab Plover present in or around each colony (Table 1).

During the surveys we recorded evidences of predation, including egg harvesting by fishermen, broken eggs and dead chicks. To identify potential predators, a Bushnell Trophy Cam 270p HD (model 119466) camera was set up at 40 nests for 12 days on the Humr Island, Farasan Islands (Table 1). The camera recorded one image every minute for 24 hours per day. The nest cameras recorded evidence of Sooty Gulls inside the colony. We also recorded tracks of animals such as snakes and rats.

Results

Breeding population

We found Crab Plover colonies on four islands (Figure 1, Table 1). In addition, fishermen told us of a colony on Abu Tok Island (Farasan Islands) although we were unable to verify this. Crab Plovers usually establish new colonies around old colonies. However, in small islands with insufficient area for new colonies, such as the Albatain Islands, new colonies may be established on nearby islands. Based on the number of active burrows, we estimate the total number of Crab Plovers in the Red Sea of Saudi Arabia as 1380 pairs (Table 1).

Population development

Table 2 compares results of the current surveys with those of previous surveys. The comparison reveals that the numbers of Crab Plover has tended to increase at each breeding site, including Al Sheick Marbat (2011: 46 burrows; 2013: 79 burrows), Umm Ar Rak (2007: 140-150 burrows; 2011: 624 burrows) and Mandhar Island (2007: 50 burrows; 2012: 138 burrows). However, Al Sheick Marbat and Umm Ar Rak were surveyed in early of the breeding season, thus may be the number of burrows is substantially higher than 46 and 150.

Threats

Sooty Gulls *Larus hemprichii*, large omnivorous gulls from the region, visit Crab Plover colonies, although we did not record them predating eggs, chicks or adults. Using nest cameras, we noticed that Sooty Gulls visit Crab Plover colonies in the early morning and in the evening, although surprisingly, Crab Plovers did not defend their colony from the gulls. In several cases when Sooty Gulls arrived at a colony, Crab Plovers left the colony and watched the gulls from a nearby location.

Snake tracks were seen on Mandhar and Humr Islands, and snakes may feed on Crab Plover eggs. Fishermen reported that they have found snakes inside Crab Plover burrows.

Two broken eggs were found on Humr Island, and rat tracks were observed around one colony suggesting that suggest that rats may impact on breeding success. We found six dead chicks, although none of these chicks showed visible signs of predation.

Local fishermen collect eggs, although there has been no attempt to quantify the magnitude of this threat. We found a tool that was used to gather eggs of Crab Plover at the colony on Mandhar Island. According to the fishermen, at full moon the eggs are at the entrance of the burrows and thus easy to collect.

Discussion

Data on breeding Crab Plovers have been collected inconsistently (Jennings 2010). In the Red Sea of Saudi Arabia, a few surveys have been carried out to study distribution and abundance of summer breeding seabirds and discovered a number of Crab Plover colonies. For instance, Newton & Al Suhaibany (1996) found only two colonies of Crab Plovers along the Red Sea coast of Saudi Arabia, Gregory & Goldspink (1996) documented two active colonies, AlRashidi (2007) reported three colonies, and Shobrak & Aloufi (2013) found two colonies. AlRashidi (2007) documented a colony of Crab Plover on Dushik Island (Farasan), but the current study did not find any colony on this island. Here we report colonies from five islands with the 1,380 pairs in the area our surveys covered, representing about 35% of the known Arabian breeding population.

Table 1. *The number of individuals and active burrows of Crab Plover on the Red Sea coastline of Saudi Arabia. No. (bur.) = number of burrows. 0 = site visited, but no Crab Plover or burrows was found.*

	Coordinates	**Date**	**No. (ind.)**	**No. (bur.)**	**Remarks**
Al Sheick Marbat	25°52'N 36°36'E	18.v.2013	119	79	2 colony sites near the new colony
Birrim	25°39'N 36°30'E	18.v.2013	0	0	
Umm Sahar	24°56'N 37°10'E	14.v.2013	0	0	
Mulayhah	24°59'N 37°08'E	14.v.2013	0	0	
Al Munqalib	25°09'N 37°08'E	14.v.2013	0	0	
Attaweel (Al Fawaida)	25°11'N 37°10'E	14.v.2013	0	0	
Jizayah	25°12'N 37°10'E	14.v.2013	0	0	
Umm Al Malik	25°14'N 37°08'E	14.v.2013	0	0	
Umm Juluf	25°09'N 37°09'E	14.v.2013	0	0	
Albatain Islands: Umm Ar Rak	19°16'N 40°59'E	11.vii.2011	480	624	Colony on small island; new colony next to the old one.
Albatain Islands: Umm Al Quronatayn	19°15'N 40°58'E	21.v.2012	23	28	
Farasan Islands / Dushik	16° 39'N 41°52'E	14.v.2011	0	0	
Farasan Islands / Mandhar (2 colonies)	16°57'N 41°48'E	15.v.2011	14	10	Colony next to an abandoned colony site
	16°57'N 41°48'E	24.v.2012	198	138	Two abandoned colonies near the new colony
Farasan Islands / Abu Shawk	17°00'N 41°46'E	25.v.2012	0	0	
Farasan Islands / Ar Rasib	17°00'N 41°47'E	25.v.2012	0	0	
Farasan Islands / Humr: Colony 1	16°47'N 42°00'E	26.v.2012	174	265	Five abandoned colonies
Farasan Islands / Humr: Colony 2	16°46'N 42°00'E	26.v.2012	0	13	Abandoned
Farasan Islands / Humr: Colony 3	16°47'N 42°00'E	31.v.2012	166	274	One abandoned colony
Total			**1174**	**1431**	

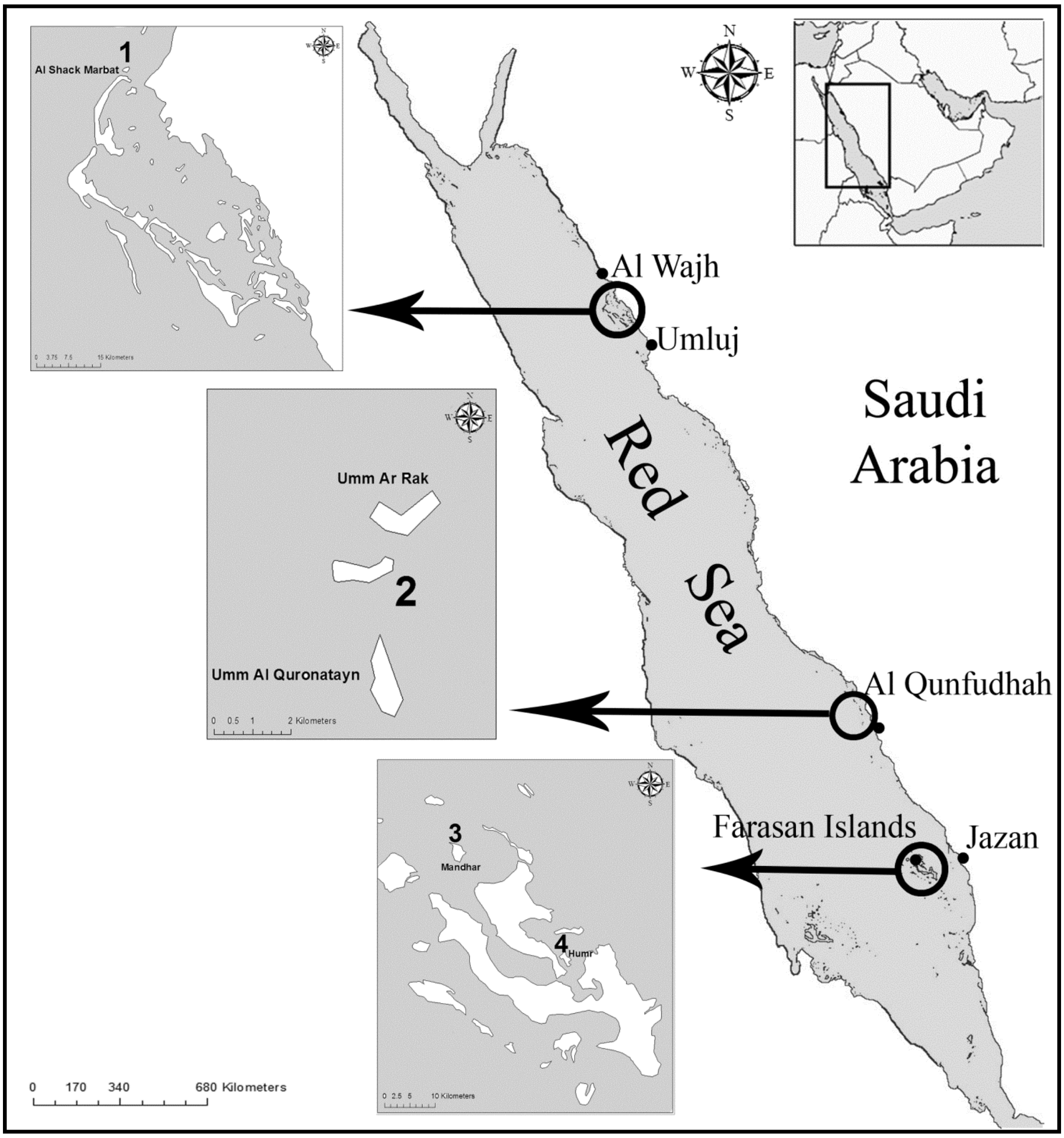

Fig 1. *Breeding colonies of Crab Plover in the Red Sea region of Saudi Arabia.*

Our population estimates are based on the numbers of active nests. Nest cameras noted that the male and female rarely remain inside the nest at the same time. Therefore, the number of birds observed in and around the colony may not reflect real population size. This finding is supported by the conclusion of De Marchi *et al.* (2006), who suggest that population estimates based on active nests provides a more reliable number than counting the Crab Plovers around the colony.

Table 2. *Population assessment of the Crab Plover in Saudi Arabia: Comparison between the current study and previous surveys. Sources: 1995: Gregory & Goldspink (1996), 1996: Newton & al Suhaibany (1996), 2007: AlRashidi (2007), 2010-2011: Shobrak & Aloufi (2013), 2011-2013: this study. (0) = not visited or no birds/colonies found; (?) = visited in May1996, but no indication of breeding activity. NoC = number of colonies, NoN = number of nests. * = deserted.*

		1995		1996		2007		2010-2011		2011– 2013	
Sites	Coordinates	NoC	NoN	NoC	NoN	NoC	NoN	NoC	NoN	NoC	NoN
AlSheick Marbat	25°52’N 36 36’E	0	0	0	0	0	0	1	46	1	79
Madarah	25°36’N 36°55’E	0	0	1	100	0	0	0	0	0	0
Attaweel	25°11’N 37°10’E	0	0	0	0	0	0	1	26	0	0
Umm Ar Rak	19°16’N 40°59’E	0	0	0	0	1	140-150	0	0	1	624
Dushik	16°39’N 41°52’E	0	0	0	0	1	40	0	0	0	0

Mandhar	16°57’N 41°48’E	1	?	0	0	1	50	0	0	1	138
Murain	16°22’N 42°17’E	1	180	1	0*	0	0	0	0	0	0
Humr	16°47’N 42°00’E	0	0	0	0	0	0	0	0	3	552

Principal requirements for a successful establishment of colonies are sandy islands suitable for burrowing, a predator-free nesting area and abundant food (Aspinall & Hockey 1997). Newton & Al Suhaibany (1996) reported colony extinctions on some Farasan Islands thought to be driven by human disturbance and/or predators. The survey of Dushik Island in 2011 found snake and cat tracks, and one proposition is that Crab Plovers have deserted this island as a consequence of snake and cat presence. Newton & Al Suhaibany (1996) reported that cats caused Crab Plover colony desertion on Murain Island.

Implications for conservation

Although the distribution of the Crab Plover is restricted to islands around the Arabian Peninsula, it is currently not listed as threatened (IUCN 2013). However, potential threats include predation, human exploitation, egg collection, tourism activities, and oil pollution and habitat loss (Del Hoyo *et al.* 1996, Hockey & Aspinall 1996, De Marchi *et al.* 2006). Further field assessments are urgently needed on other islands in the Red Sea region which we were not able to survey. Detailed monitoring of several islands are also needed to provide vital data whether predation and disturbance reduce reproductive success. Presumably, Crab Plovers are long-living birds, and thus demographic consequences of breeding failures would go unnoticed for several years. Therefore, establishing key aspects of their demography (e.g., reproductive success, maturation, juvenile and adult survival) and the causes of these demographic components are long overdue.

Introduced mammals are one of the most serious problems threatening bird populations on islands (Courchamp *et al.* 2003, Russell & Le Corre 2009). In Abu Tok Island the fisherman do not collect Crab Plover eggs because they fear snakes that live in Crab

Plover burrows. We were unable to identify the snake species, but it should be either Farasan Island Racer *Coluber insulanus*, Burton's Carpet Viper *Echis coloratus* or the Arabian Horned Viper *Cerastes gasperettii*. White-tailed Mongooses *Ichneumia albicauda* are native mammalian predators in the Farasan Islands; although no mongoose tracks were recorded at Crab Plover colonies. Mongoose can swim and if they reach islands a short distance from mainland, e.g. Humr Island, they could quickly wipe out full colonies. Therefore, there is an urgent need to monitor mongoose, rats and snakes on islands that are important breeding sites for Crab Plover and other waterbirds, seabirds and shorebirds.

Egg collection is a traditional activity in the Red Sea region, and is still practiced despite its known negative impact on the bird populations (PERSGA/GEF 2003). Egg collection is illegal according to Saudi legislation. The effect of egg collections on Crab Plovers and other islands nesting birds (e.g. White-cheeked Tern *Sterna repressa*, Saunders's Tern *Sterna saundersi*, and White-eyed Gull *Larus leucophthalmus*) warrants attention. The fishermen interviewed were knowledgeable about the location of colonies and the laying period of the Crab Plover. They collect fresh eggs early in the breeding season, which are boiled. Whilst checking the burrows and collecting eggs, many burrows are destroyed potentially smashing eggs. Egg collection appears to be common in Farasan Islands, but not so much in Albatian Islands and Al Wajh Archipelago. The impact of egg collection on population trend has not been assessed.

Recently, tourism and recreational activities are becoming increasingly widespread along the Red Sea coast of Saudi Arabia. Crab Plovers are easily disturbed; the birds depart the colony when people walk near the colony, and the birds only come back after the people leave the vicinity of the colony. Therefore, regular visits, whether by fishermen or passers-by during the breeding season may lead to colony desertion, and immense loss of reproductive effort. Therefore, with increasing tourism in Saudi Arabia, disturbance may pose a serious threat to shy birds such as the Crab Plover.

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Chapter 4

Modelling the distribution of wetland birds on the Red Sea coast in the Kingdom of Saudi Arabia

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Author´s contributions

MA: study design, spatial analysis, manuscript preparation

MAR: data collection, comments on manuscript

MO: study design, comments on manuscript

MS: comments on manuscript

TS: comments on manuscript, general editing

Abstract

Species distribution modelling is useful for addressing knowledge gaps for poorly studied geographical areas by identifying potentially suitable habitats for species across landscapes. This study was undertaken to identify areas containing wetland habitats to support actions aimed at the conservation of wetland bird species on the Red Sea coast of the Kingdom of Saudi Arabia (KSA). We used a maximum entropy approach to build habitat suitability models for 22 wetland bird species (gulls, shorebirds, and terns). Ten variables relating to topography, habitat, latitude, coast complexity, man-made structures, and human settlements were used to produce individual habitat suitability models for each of the bird species. The areas under the curve (AUC) for the final model were 0.9013 and 0.879 for the training and testing data, respectively, and the jackknife analyses suggested that the models generally performed well. Using the modelled distributions of the 22 species along the Red Sea coast, the analysis suggested 17 core areas where the habitat and landscape configuration were suitable for supporting high species richness. Of these 17 sites, one is already protected, and there is a need to protect the remaining sites. The use of these models to inform conservation strategies in the Red Sea region of the KSA is discussed.

Keywords: wetland birds, Red Sea coast, species distribution model, MaxEnt.

Introduction

The Red Sea region has diverse coastal and marine habitats. These include mangroves, mudflats, marshes, sand dunes, sand plains, rocky shores, coastal reefs, and marine islands (PERSGA/GEF 2003). The region is particularly important for corals, marine invertebrates, and turtles (AbuZinada *et al.* 2002).

However, the size of the region and limited opportunities for survey and census fieldwork, mean that the fauna of the Red Sea coast remains relatively poorly studied.

Previous surveys have suggested that the region is important for a variety of bird taxa, including Palaearctic migrants and winter residents (PERSGA/GEF 2004). On the Red Sea coast and the Gulf of Aden, 17 seabird species have been recorded as breeding, including endemic species such as White-eyed Gull (*Larus leucophthalmus*), a subspecies of the Red-billed Tropicbird (*Phaeton aethereus indicus*) and the Brown Noddy (*Anous stolidus plumbeigularus*). Furthermore, some species endemic to the northwest Indian Ocean and important sub-populations breed in the region such as Jouanin’s Petrel (*Bulweria fallax*), Sooty Gull (*Larus hemprichii*), Swift Tern (*Sterna bergii velox*), and White-cheeked Tern (*Sterna repressa*) (PERSGA/GEF 2004). This region includes Socotra Cormorant (*Phalacrocorax nigrogularis*) that is classified as ‘threatened’, with three further species classified as ‘near threatened’ on the IUCN Red List (IUCN 2012): Jouanin’s Petrel, Persian Shearwater (*Puffinus persicus*), and White-eyed Gull that are classified as being ‘near threatened’ on the IUCN Red List (PERSGA/GEF 2004). Furthermore, AlRashidi *et al.* (2011) found that some sites of the Red Sea coast are highly suitable for breeding and wintering Kentish Plover (*Charadrius alexandrinus*).

Despite its relative isolation, the Red Sea coast is subject to growing human pressures. In particular there has been an issue with coastal development and pollution (PERSGA/GEF 2004). Moreover, the taking of seabird eggs is a potential problem in the region and needs regulation and/or sustainable take agreements (Newton 2006).

Within the Red Sea area, research and monitoring was one of seven priority ‘enabling’ actions identified in the 2004 PERSGA report (PERSGA/GEF 2004). Understanding the complex ecological relationships between species distribution, environmental structure and human pressure is of high utility for the development of: (i) Integrated Coastal Zone Management (ICZM) frameworks, (ii) the identification of protected sites and networks, and (iii) effective evaluation of conservation activities. The present project is one of only a small number of research initiatives that have so far been undertaken in the region.

Despite the need for greater understanding species-habitat relationships, modelling wetland birds in the Red Sea region can be problematic because of the lack of range and gradients of the landscape’s physical features AlRashidi *et al.* (2011).

Recent field monitoring efforts at key seabird sites and greater availability of high resolution data from the region now make it possible to develop species distribution models (SDMs). Recent testing of SDMs using biological data (including birds) has shown that robust biologically relevant models can be developed from the integration of ‘presence only’ observations of species occurrence with measurements of environmental characteristics. These models predict the actual or potential distribution of a species (Elith & Leathwick 2009), and provide an understanding of the underlying species-habitat relationships (Guisan & Zimmermann, 2000, Franklin, 2009). SDMs have also been used for estimating the effects of climate change (Buckland *et al.* 1996, Austin *et al.* 1996, Thomas *et al.* 2004), estimating population size (Long *et al.* 2008), understanding the correlation between distribution and abundance (Milsom *et al.* 2000, Ledee *et al.* 2008), conservation planning and forecasting (Rushton *et al*. 2004, Phillips & Dudík 2008), and species distribution monitoring (Rodriguez *et al.* 2007).

Species distribution models require detailed information about the environment of the study area, and species occurrence data. This can be either presence-only data or presence- absence data (Graham *et al.* 2008). In practice, ‘true’ absence data are rarely available, because they require a considerable amount of time and effort to collect and it can be difficult to ensure that there are no false-negatives in the data. The vast majority of datasets for species distribution modelling are therefore presence-only datasets (Phillips *et al.* 2006), and this is the case for the present study.

To address the current lack of understanding about factors driving the abundance and distribution of shorebirds along the Red Sea coast of the Kingdom of Saudi Arabia (KSA), we have utilised available data from an extensive coastal bird survey (AlRashidi *et al.* 2011), to attempt the first species distribution modelling in the region. The core aim of this research was therefore to give guidance for the development of conservation strategies for the Red Sea coastal area of the KSA by providing a better understanding of the distribution of 22 wetland bird species along the Red Sea coast. Therefore, this will help guide future surveys and monitoring of wetland bird species in this region. We also targeted to quantify the relative importance of key sites by determining areas of high species richness.

Methods

Environmental variables for the model

The Red Sea coastline of the KSA extends 1,840 kilometres from the border with Jordan in the northern Gulf of Aqaba region (29° 30’ N) to the border with Yemen in the southern Red Sea region (16° 22’ N) (PERSGA/GEF 2003). This coastline has an arid climate with temperatures reaching up to 50 °C in summer. The average rainfall is less than 70 mm per year (PERSGA/GEF 2003).

We utilised the wetland bird species data collected by AlRashidi *et al.* (2011) between 2 July and 10 August 2008 (Fig. 1). The data were gathered from 98 randomly selected sites located within 1 km of the sea along the west coast of Saudi Arabia including the Farasan Islands. 35 bird species have been recorded along the Red Sea coast, and here we focus on 22 of these species, for which more than 14 records were available (Table 1).

Ten environmental variables were selected for the species distribution modelling: vegetation cover, soil moisture, distance to main cities (big cities (these cities were Jeddah, Jazan, Yanbu and Rabigh)), distance to cities (small cities (these small cities were like Al Wajh, Umluj, Al Lith, and Al Qunfudhah)), distance to roads, brightness, latitude, coast complexity, elevation, and slope. Information about vegetation cover, soil moisture, brightness, and elevation was derived from 21 Landsat 7 satellite images and rendered into GIS format (below) by online tools associated with the Global Land Cover Facility (AlRashidi *et al.* 2011).

The tasseled cap transformation was introduced by Kauth & Thomas (1976). It is an appropriate tool for improving spectral data and deriving important environmental information (Crist & Cicone 1984). A tasselled cap transformation with coefficients for the Landsat ETM+ sensor was used (Huang *et al.* 1998) to produce three rasters: tasselled cap greenness shows the existence and density of green vegetation; tasselled cap moistness, which describes the amount of soil moisture; and tasseled cap brightness, shows variations in soil structures. Finally, all transformed images were rescaled such that pixels took digital number values from 0 to 255 (see AlRashidi *et al.* 2011, Long *et al.* 2008).

Elevation data were derived from the Shuttle Radar Topography Mission (SRTM). Tiles of SRTM data corresponding to the 21 WRS-2 scenes of Landsat data used were downloaded from the Global Landcover Facility (http://www.landcover.org). These were then mosaiced and clipped in the same way as the satellite images were. The resolution of this dataset was 90 m, but in order to overlay all layers of environmental data exactly, we resampled the SRTM to 30 m pixel size to produce the final elevation map (see AlRashidi *et al*. 2011, Long *et al.* 2008).

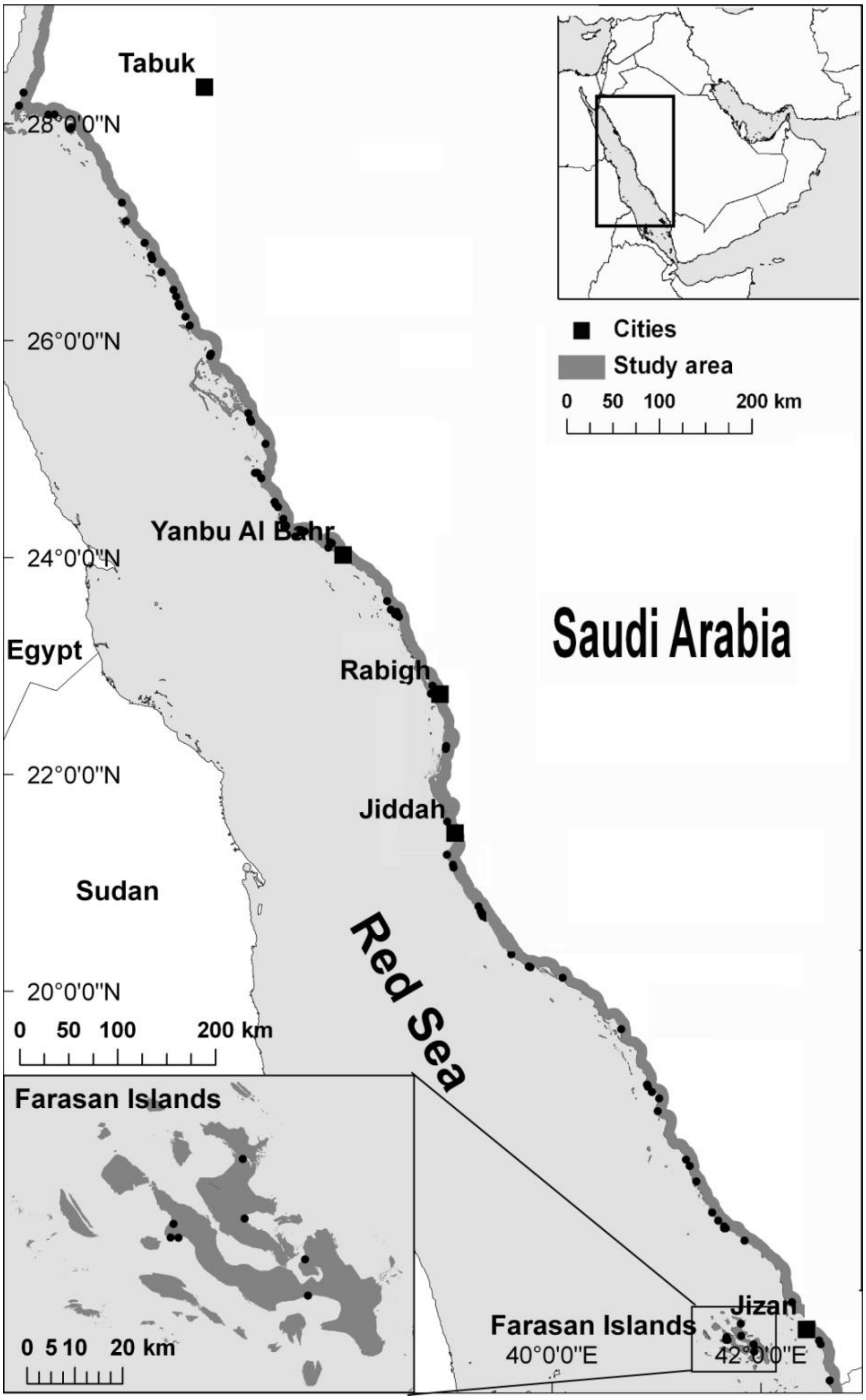


Fig 1. *Map showing the study area on the west coast of Saudi Arabia (shaded dark grey); black dots represent starting points of 98 randomly selected sites (AlRashidi et al., 2011)*

Table 1. *The 22 wetland bird species included in distribution modelling. Note:'LC' is Least Concern;'NT' is Near Threatened.*

No	Species	Scientific name	Threat status (IUCN 2012)	Population trend (IUCN 2012)
1	Eurasian Spoonbill	*Platalea leucorodia*	LC	unknown
2	Intermediate Egret	*Mesophoyx intermedia*	LC	decreasing
3	Western Reef Heron	*Egretta gularis*	LC	stable
4	Pink-backed Pelican	*Pelecanus rufescens*	LC	stable
5	Eurasian Oystercatcher	*Haematopus ostralegus*	LC	decreasing
6	Crab Plover	*Dromas ardeola*	LC	stable
7	Kentish Plover	*Charadrius alexandrinus*	LC	decreasing
8	Lesser Sand Plover	*Charadrius mongolus*	LC	unknown
9	Greater Sand Plover	*Charadrius leschenaultii*	LC	Unknown
10	Whimbrel	*Numenius phaeopus*	LC	decreasing
11	Eurasian Curlew	*Numenius arquata*	NT	decreasing
12	Redshank	*Tringa totanus*	LC	unknown
13	Marsh Sandpiper	*Tringa stagnatilis*	LC	decreasing

14	Terek Sandpiper	*Xenus cinereus*	LC	stable
15	Ruddy Turnstone	*Arenaria interpres*	LC	decreasing
16	White-eyed Gull	*Larus leucophthalmus*	NT	stable
17	Sooty Gull	*Larus hemprichii*	LC	decreasing
18	Caspian Tern	*Sterna caspia*	LC	increasing
19	Swift Tern	*Sterna bergii*	LC	stable
20	Lesser Crested Tern	*Sterna bengalensis*	LC	stable
21	Saunders's Tern	*Sterna saundersi*	LC	decreasing
22	White-cheeked Tern	*Sterna repressa*	LC	decreasing

As a proxy measure of human impact, we made a data layer showing the distance to the nearest main cities, cities and roads. A point shapefile containing all buildings on the west coast of Saudi Arabia was projected to UTM 37N and clipped to the study area. The source of these data was http://www.gospatial.com. Three distance-to-feature rasters were created to measure the euclidean distance between all cells within the study area to the nearest main cities, cities and roads. We then converted the data to raster format in which each cell took as its value the distance (km) to the nearest main cities, cities and roads (see AlRashidi *et al.* 2011).

To investigate the effect of latitude, we added latitude coordinate data in ArcGIS as a layer with decimal degrees format. We then converted the data to raster format. After that, raster was clipped to the study area to generate the latitude layer that matches all layers of environmental data exactly. We then converted raster to ASCII.

To investigate the effect of coastal complexity (the physical shape attributes of each coastal pixel), a fishnet layer was created to split the coastline into 1 km^2. Next, we calculated the geometric intersection of the feature classes and feature layers. We then converted the data to raster format. Finally, this raster was clipped to the study area to generate the coast complexity map that matches all layers of environmental data exactly. We then converted raster to ASCII.

Species data

The species occurrence data were entered into ArcGIS version 10, re-projected to UTM in the same coordinate system, and then rasterised. All environmental variables (described above) were converted to raster layers within ArcGIS, and modified to fit the same geographical boundary and cell size. Finally, we converted the environmental layers from raster format to ASCII and exported them to the modelling program.

Species distribution modelling

All analyses were implemented using MaxEnt software version 3.3.3e (Phillips *et al.* 2006). MaxEnt uses presence-only data to predict the likelihood distributions of maximum entropy as the basis for forecasts of potential distributions of species (Young *et al.* 2009).

The models were estimates of the maximum and the most uniform spread of a species across a study area, based on environmental constraints. An internal regularisation technique is used to control overfitting data (Phillips *et al.* 2006). The output of MaxEnt was a raster map of the same resolution as the input data; each cell represented the relative suitability of an area for a species to reside there. The value of each cell is re-scaled from 0 to 1, with zero being the lowest and 1 being the highest probability of suitability (Phillips *et al.* 2006).

MaxEnt’s jackknife options were used to investigate the relative importance of the environmental variables. The gain is a measure of the probability of the samples; which specifically maximizes the likelihood of the presence samples with reference to the background data. Thus, the higher gain value denoting a better fit of model (Phillips *et al.* 2006). For each candidate model set/species, ten replicate models were run and model averages were taken across the set in order to display the distribution of wetland birds as a species group. Sufficient data were available to allow replicate models to be run with a split 50% training data and 50% test data. This permits an evaluation of the model’s performance.

To produce an indication of the distribution of wetland birds as an assemblage of wetland bird species and to estimate the proportion of suitable habitats along the Red Sea coast, Maxent was run with a single model based on all of the 22 bird species to give “*All birds*” model, where presence equal the presence of any species. After that, the Maxent map of *“All birds*”, was imported into Arc GIS and cells with a habitat suitability probability of <0.7 were given the value 0 (not suitable) and those with a probability <1 given a value of 1 (likely to be suitable habitat). The proportions of suitable and unsuitable habitat cells were then calculated.

Evaluation of the models

The predictive performance of the models was tested using receiver-operating characteristics (ROC). The area under the curve (AUC) was then used as a measure of model performance; the value of AUC was scaled from 0 to 1, where 1 is optimal performance, 0.5 is no better than random, and less than 0.5 is worse than random (Elith *et al.* 2006, Pearce & Ferrier 2000).

22 species models were used to build the species richness map. Maximum training sensitivity plus specificity was used to determine the threshold value necessary to predict unsuitable and suitable habitats for each species model. According to Jiménez-Valverde & Lobo (2007), the resulting threshold generally achieves high prediction accuracy. In order to apply the maximum training sensitivity plus the specificity threshold to each species, the MaxEnt average ASCIIs were converted to rasters in ArcGIS. Then the threshold value was assigned to each model by reclassifying raster values to 0 for unsuitable habitat (< threshold) and 1 for potentially suitable habitat (≥ threshold). Then, the threshold-validated distribution models of each species were combined in one model using raster calculator in ArcGIS. After that, raster appeared in the display looking with a different colour scheme allow us to determine the areas of high species richness across Saudi Arabia’s Red Sea coast.

Results

Individual species models

The performance was generally high for all individual species models. The mean AUC ranged from 0.532 and 0.990. The key significant variables for the 22 wetland-bird species distribution models are summarised in Table 2.

Table 2. *Summary statistics of the 22 wetland bird species on the Red Sea coast of Saudi Arabia including species occurrence data, variable with highest gain (from jackknife test of variable importance), percent contribution of top two contributors to each model (from the table of relative contributions of the environmental variables), and the mean test AUC for each model*

Species	Occurrence records	Variable with highest gain	The first important variable	Percent contribution	The second important variable	Percent contribution	Mean test AUC
Eurasian Spoonbill	17	Distance to cities	Distance to cities	53.6	Latitude	23.1	0.895
Intermediate Egret	30	Distance to main cities	Distance to main cities	32	Elevation	23.4	0.774
Western Reef Heron	47	Coast complexity	Latitude	31.7	Distance to road	25.7	0.853
Pink-backed Pelican	14	Distance to cities	Coast complexity	36.6	Distance to cities	36.1	0.900
Eurasian Oystercatcher	14	Distance to cities	soil moisture	38.5	Distance to cities	35.8	0.619
Crab Plover	190	Coast complexity	Coast complexity	28.4	Distance to main cities	23.7	0.914
Kentish Plover	766	Distance to main cities	Distance to main cities	27.3	Coast complexity	26.5	0.896
Lesser Sand Plover	210	Coast complexity	Coast complexity	31.8	Distance to main cities	30.2	0.906
Greater sand plover	139	Coast complexity	Coast complexity	29.9	Distance to road	23.5	0.914
Whimbrel	50	Distance to road	Distance to road	54.2	Distance to cities	18.5	0.820
Eurasian Curlew	51	Distance to road	soil moisture	25.1	Coast complexity	19.8	0.881
Redshank	119	Coast complexity	Coast complexity	30.2	Distance to road	23	0.890
Marsh Sandpiper	33	Distance to road	Distance to road	30	Latitude	29.6	0.836

Terek Sandpiper	21	Distance to road	Distance to road	70.1	soil moisture	9.5	0.729
Ruddy Turnstone	59	Coast complexity	Coast complexity	30.9	Distance to cities	24.4	0.791
White-eyed Gull	20	Latitude	latitude	33.1	Distance to cities	21.6	0.990
Sooty Gull	73	Coast complexity	Distance to main cities	24.8	Distance to cities	18.9	0.819
Caspian Tern	26	Distance to road	Distance to road	49.3	Coast complexity	13.1	0.853
Swift Tern	15	Distance to cities	Distance to cities	37.2	Distance to road	34	0.723
Lesser Crested Tern	25	Slope	Slope	29.3	Latitude	25.2	0.532
Saunders's Tern	19	Distance to road	Distance to road	24.5	Distance to cities	22.3	0.878
White-cheeked Tern	16	vegetation cover	vegetation cover	33.1	soil moisture	21.6	0.612

All birds

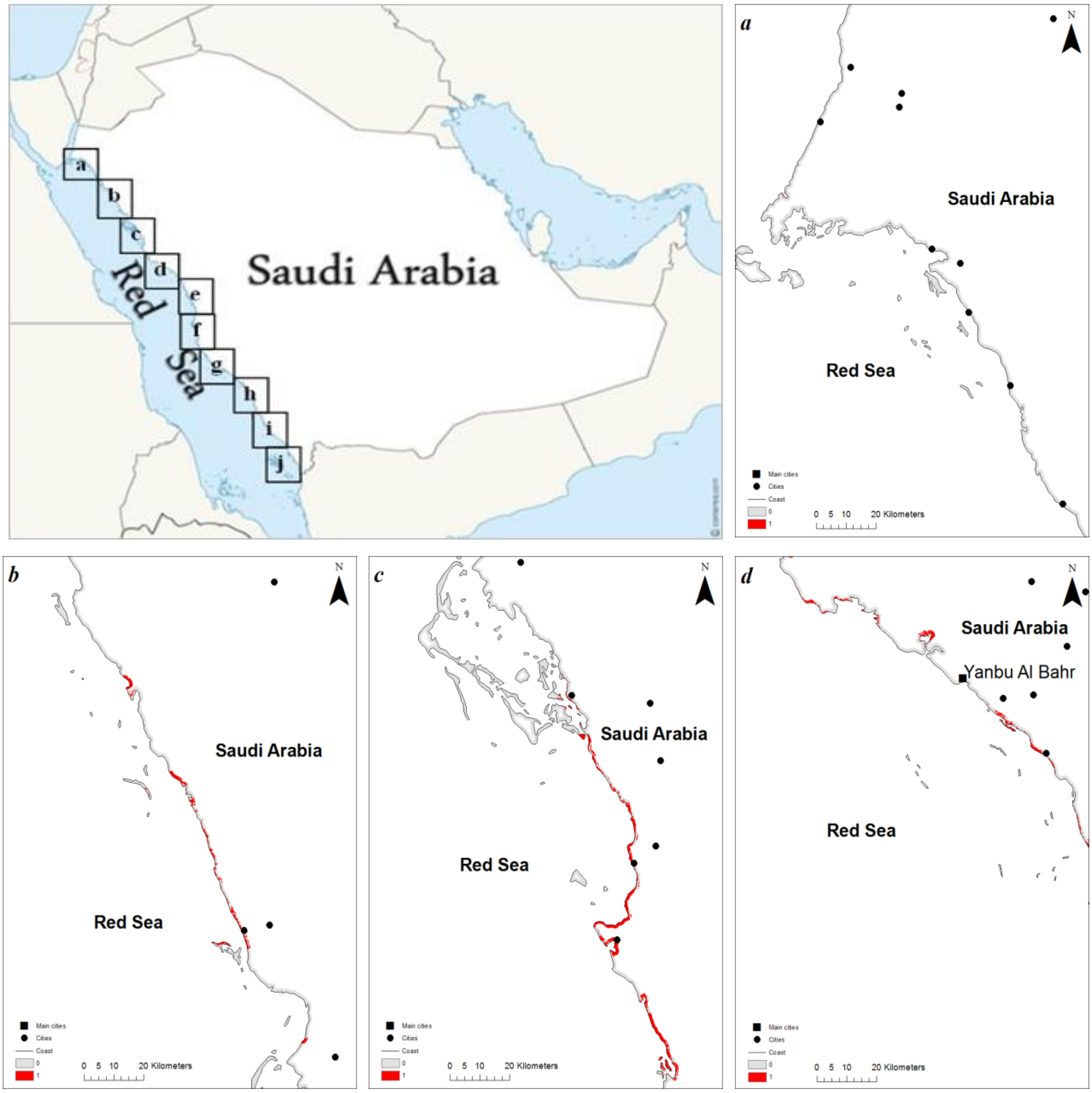
The species distribution model generated when using all bird species occurrence data showed that the most suitable habitats for wetland birds were predicted to be in the middle and southern parts of the Red Sea coast (Fig. 2). The results of the jackknife test (Fig. 3) revealed that coast complexity is the key variable in explaining the distribution of all birds. This variable introduced a higher gain compared to the other variables and would likely be highly influential if withdrawn from the model. Models using slope alone do not perform well and thus would be the least transferable. The next two variables that contributed the most to the model are distance to main cities and distance to roads.

An examination of the model response curves to key environmental variables indicated that the greater the value of the distance to main cities and soil moisture, the greater the value of the potential habitat suitability, while the greater the value of the coast complexity, distance to roads, distance to cities and elevation variables, the smaller the value of the potential habitat suitability.

The "*All birds*" model suggested that approximately 17% of the Red Sea coastal habitat is suitable for wetland bird species.

Model validation

The "*All birds*" model performed well in predicting the presence of all birds when evaluated using a ROC plot (AUC mean = 0.901 and 0.879 for the training and testing data, respectively). This result indicated that in the final model, a cell predicted as suitable habitat at any threshold of suitability would be more suitable than a randomly selected cell in the study area at least 87% of the time.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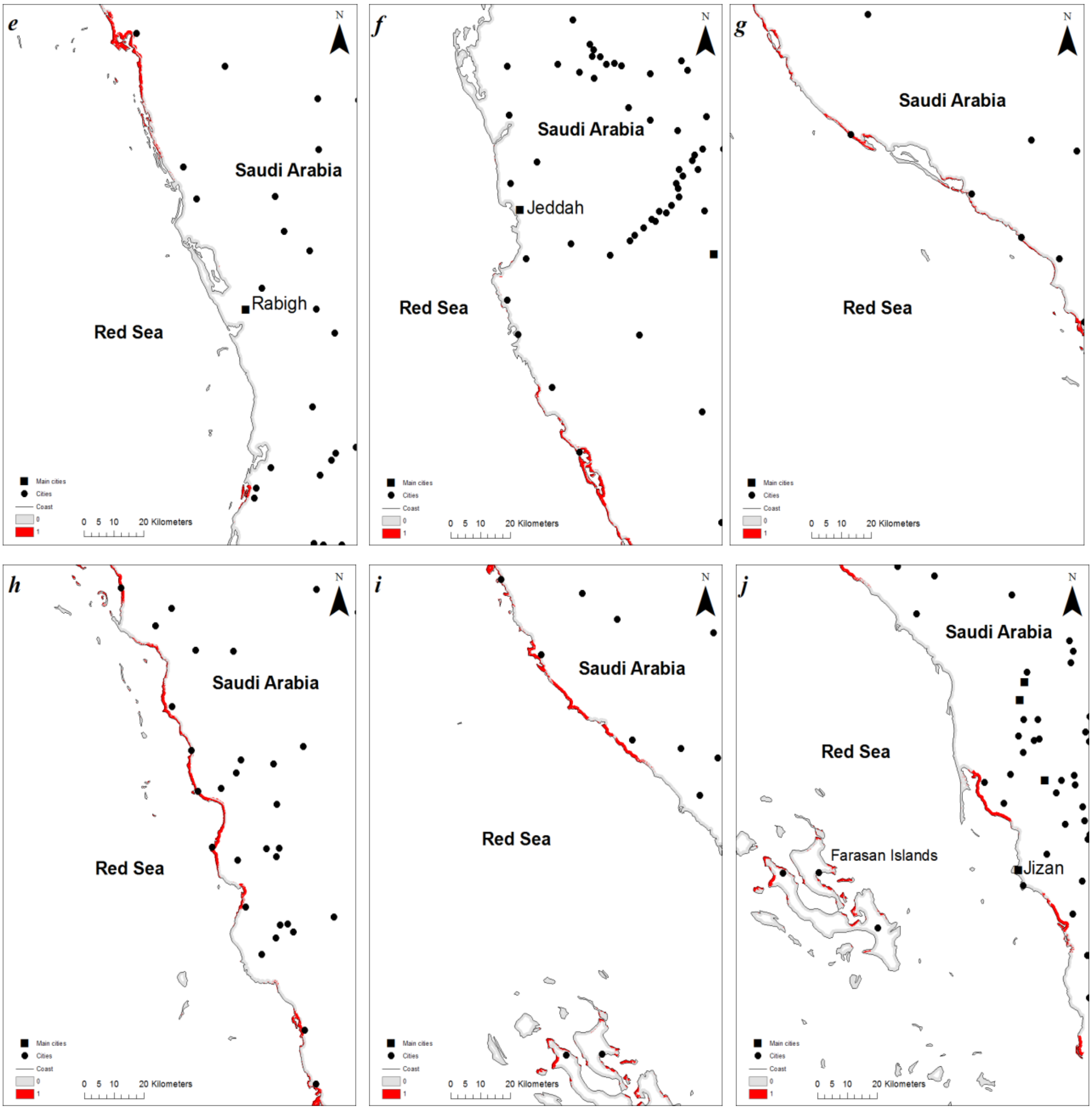
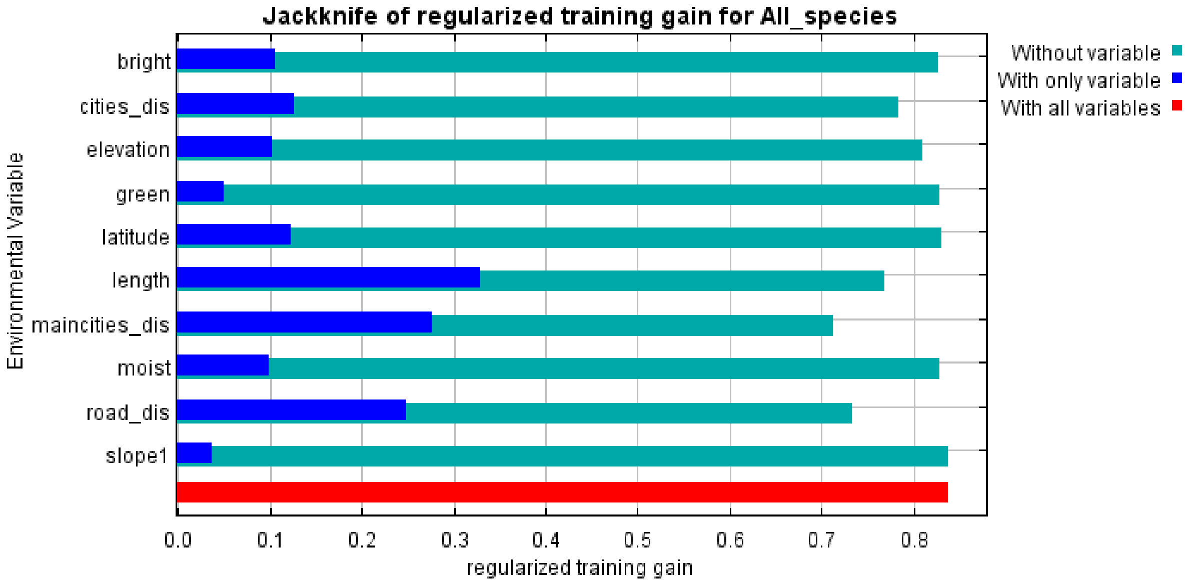


Fig 2. *Maps showing the predicted potential suitable habitats for "All birds" (50% training data and 50% test data); using Maximum training sensitivity plus specificity threshold; red line represent potential habitat suitability for "All birds". The square black dots represent the main cities and circle black dots represent the cities.*

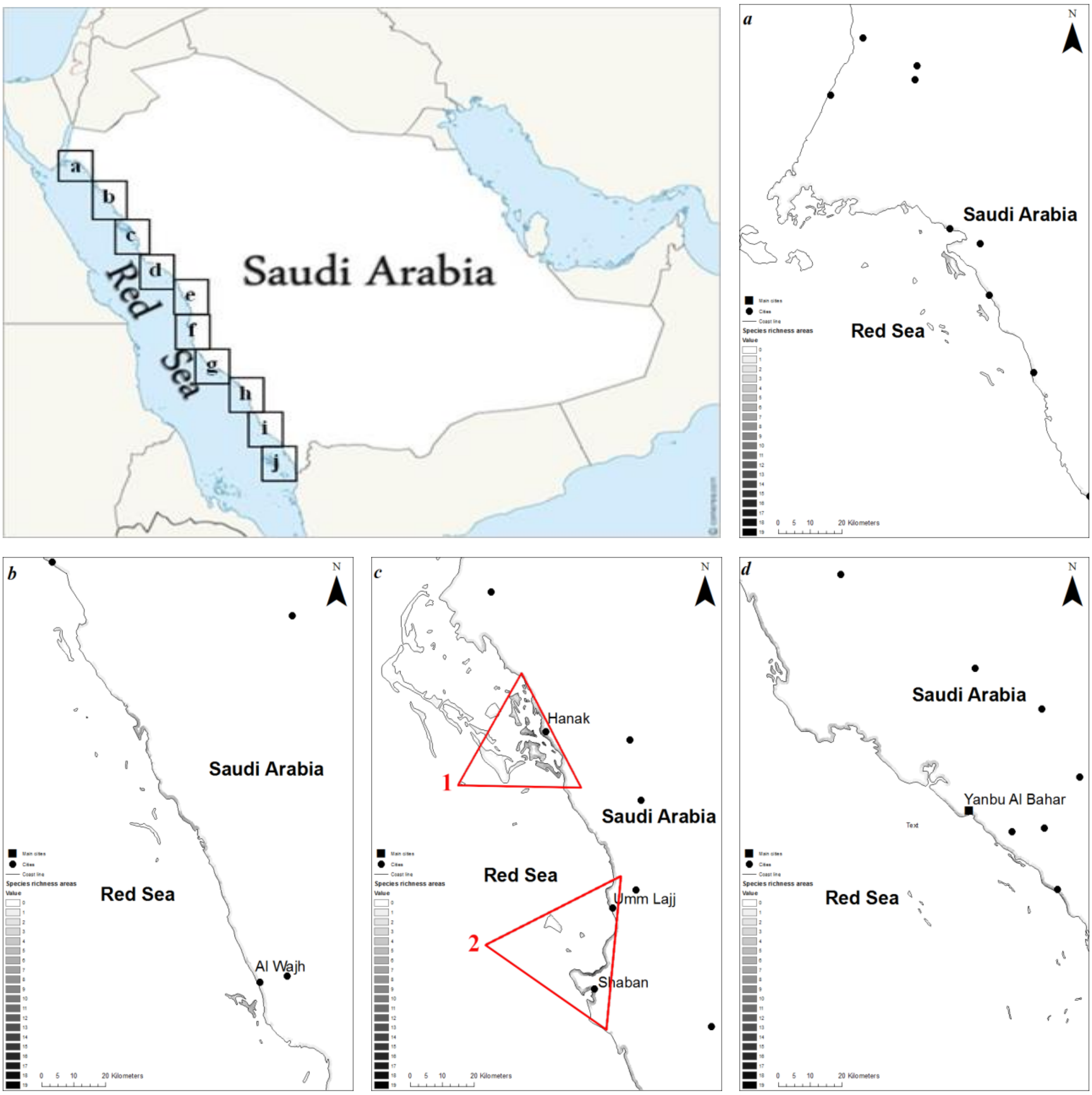


Variable	Percent contribution	Permutation importance
maincities_dis	23	17.3
length	22.3	31.3
road_dis	19.9	21
cities_dis	11.8	6.9
elevation	7.6	9.5
moist	5.6	1.4
bright	4.8	0.9
latitude	3	8.8
green	1.7	2.7
slope1	0.2	0.2

Fig 3. *Results of jackknife evaluations of relative importance of predictor variables and table gives estimates of relative contributions of the environmental variables for "All birds" using Maxent model. Note: 'bright' is brightness; 'cities_dis' is distance to cities; 'green' is vegetation cover, 'length' is coast complexity, 'maincities_dis' is distance to main cities, 'moist' is soil moisture, 'road_dis' is distance to road.*

Species richness areas

The areas of high species richness for wetland birds in the Red Sea coastal area are illustrated in the map shown in *Figure 4*, which reveals that 17 areas may represent potentially suitable habitats for a large number of wetland bird species. Furthermore, the map illustrates that areas of the Red Sea coast were predicted as suitable habitat for up to 19 species, particularly in the middle and southern parts of the Red Sea coast. In contrast, the northern part of the Red Sea coast contains only two areas of high species richness. The areas of high species richness appear fragmented in places and cover broad distances along the study area. The majority of endemic and near-threatened species occurs in all 17 areas, except the White-eyed Gull, which occurs only in two areas located in the northern part of the Red Sea coast (Table 3).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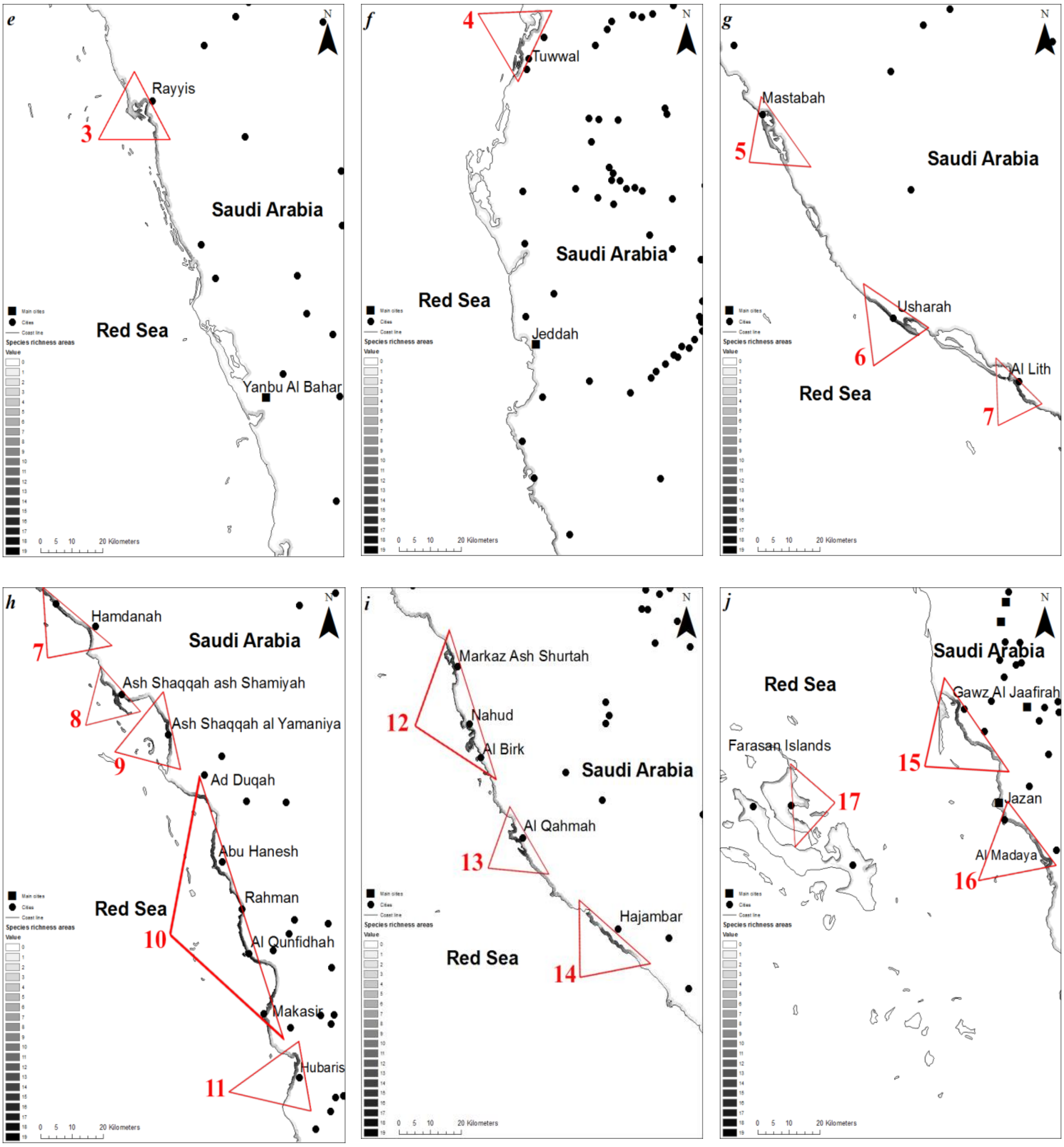

Fig 4. *Maps showing the 17 final predicted areas of high species richness for wetland birds in the Red Sea coast of Saudi Arabia. The red triangles indicate the potential areas of high species richness. The square black dots represent the main cities and circle black dots represent the cities.*

Table 3. *Showing all 17 locations that are considered potentially suitable habitats for wetland birds along the Red Sea coast of Saudi Arabia, the locations of the endemic and near-threatened species that occurs in all 17 sites and the total species numbers that occur in each potential suitable habitat*

No.	Sites	Species						Number of species occur in potential suitable habitats
		Crab Plover	Eurasian Curlew	White-eyed Gull	Sooty Gull	Saunders’s Tern	White-cheeked Tern	
		Endemic LC	NT	Endemic NT	Endemic LC	Endemic LC	Endemic LC	
1	Hanak and the islands around it	√	√	√	√	X	√	18
2	From Noth Umm Lujj to Shaban	√	√	√	√	X	√	16
3	Rayyis	√	√	√	√	√	√	16
4	Thuwwal	X	√	X	√	√	√	18
5	Mastabah		√	X	√	√	√	17
6	Usharah	X	√	X	√	√	√	18
7	From Alith to Hamdanah	√	√	X	√	√	X	19
8	Ash Shaqqah Ash Shammiyah	√	√	X	√	√	X	19
9	Ash Shaqqah Al Yamaniya	√	√	X	√	√	X	19
10	East Ad Duqah 7km to Makasir	√	√	X	√	√	X	19

11	Hubaris	√	√	X	√	√	X	18
12	From Markaz Ash Shurtah to Al Birk	√	√	X	√	√	√	17
13	Al Qahmah	√	√	X	√	√	X	17
14	West Hajambar 3 km	√	√	X	√	√	X	17
15	Qawz Al Jaafirah	√	√	X	√	√	X	16
16	From Jazan to At Tahiriyah	√	√	X	√	√	X	15
17	The East shoreline of Al Segied Island in Farasan Islands	√	X	X	X	X	√	11

Discussion

This study provides the first predicted potential habitat suitability maps for 22 wetland bird species along the Red Sea coast in the KSA. Although we built some models with occurrences data less than 20 locations, MaxEnt proved to give a robust performance in practice compared to other methods and remains effective despite a small sample size (Elith *et al.* 2006, Baldwin 2009). The results demonstrated distance to cities, distance to roads and coast complexity variables influence negatively quite a few wetland bird species. These effects may be mediated through human activities (e.g. disturbance, hunting and pollution), and the risk of predation by introduced cats, dogs and crows in the vicinity of settlements (AlRashidi *et al.* 2011). Furthermore, Findlay & Houlahan

(1997) found that there is highly negative correlation between bird species richness and road within around 1 km of a wetland. Many studies have indicated that some bird species avoid selecting habitats near to roads (Bollinger & Gavin 2004, Carrascal *et al.* 2006, Gavashelishil & McGrady 2006). Roads have highly influence on birds in different directions such as, direct mortality, indirect mortality, habitat fragmentation, isolation and disturbance (Jacobson 2005, Findlay & Bourdages 2000).

Several wetland bird species that have particular importance in terms of conservation status are present on the Red Sea coast. These species include Crab Plovers (*Dromas ardeola),* White-eyed Gulls, Sooty Gulls, and White-cheeked Terns (PERSGA/GEF 2003). Due to their importance in regional conservation strategies, these specific species will be discussed below in light of the potential species distribution models.

Crab Plover. Crab Plovers are known to utilise areas of sandy and muddy coastline on islands, intertidal sandflats and mudflats, estuaries, lagoons, and bare coral reefs (Burton & Burton 2002), and breed on sandy islands or extensive coastal sandy banks (BirdLife International 2013). Examining the habitat suitability model the distance to main cities and soil moisture have a positive influence on the distribution of Crab Plovers, whereas, coast complexity, distance to cities and distance to roads have a negative influence on their distribution.

White-eyed Gull. White-eyed Gulls are known to utilise coastal areas and breed on island shorelines on exposed rock and sand flats (del Hoyo *et al.* 1996). Although some populations in Egypt have been shown to get their food from drifting litter and harbours (Baha El Din 1999), the species mostly feeds at sea (PERSGA/GEF 2003). Examining the habitat suitability model the latitude has a positive influence on the distribution of

White-eyed Gulls, whereas the distance to cities, vegetation cover and slope have a negative influence on their distribution.

Sooty Gulls. Sooty Gulls which are known to utilise areas of along coast and at islands and breed on coastal and inshore islands near sea level which are sparsely vegetated, rocky and sandy (Cramp & Simmons 1983, PERSGA/GEF 2004). Furthermore, sometimes they feed in mudflats and rest in harbours and unusually seen inland (del Hoyo *et al.* 1996). Examining the habitat suitability model the distance to main cities, distance to roads and latitude have a positive influence on the distribution of Sooty Gulls, whereas the coast complexity and distance to cities have a negative influence on their distribution.

White-cheeked Tern. White-cheeked Terns are known to utilise coastal areas and along inshore bodies of water. They nest on rock, sand, gravel, and coral islands (del Hoyo *et al.* 1996), as well as on the exposed sandflats and sparsely vegetated open ground of sand dunes and above shorelines' high-water marks (Snow & Perrins 1998). Examining the habitat suitability model the soil moisture and latitude have a positive influence on the distribution of White-cheeked Terns, whereas the vegetation cover and distance to main cities have a negative influence on their distribution. Saudi Arabia is generally an arid country with very high temperatures in summer. It receives about 70 mm of precipitation annually. Therefore, these factors led to decreased vegetation cover (Darfaoui & Al Assiri 2011).

Kentish Plover. It is encouraging to compare our model results for the Kentish Plover with those reported by AlRashidi *et al.* (2011). He used a Generalised Linear Model (GLM) with four habitat variables elevation, distance to settlements, vegetation cover and soil moisture for predicting potential suitable habitat for Kentish Plover. In particular, AlRashidi *et al.* (2011) reported that the most suitable habitat for Kentish Plovers on the Farasan Islands is located on the northern and eastern shores; this result is consistent with the findings of the present study. Furthermore, AlRashidi *et al.* (2011) found the most suitable continental habitat for the Kentish Plover species to be located in two concentrated areas along the Red Sea coast. The first area is located between Yanbu Al Bahr and Jiddah, and lies approximately 100 km south of Jiddah. The second area is located near the city of Jazan. However, our study predicted the most suitable continental habitat patches for the Kentish Plover to be located along the Red Sea coast south of the cities Hanak and Jazan.

Mapping areas of high species richness

This study provided the first predicted potential habitat richness map for wetland bird species along the Red Sea coast of Saudi Arabia. Mapping species richness and distributions has become a key strategy in conservation planning (Cardillo 1999, Graham & Hijmans 2006). Identifying species richness areas that include endemic species will provide conservation agencies and practitioners with the information needed to develop and optimise conservation strategies and actions (Bojórquez-Tapia *et al.* 1995).

Each of the 17 areas of high wetland bird species richness that were identified in this study contains different levels of endemic species. We already know that wetland birds and their habitats along the Red Sea coast face many threats. Therefore, a survey of these 17 areas should be undertaken in the future in order to investigate the actual presence of wetland birds in these areas and thereby to determine the most significant of these 17 areas for conservation purposes.

Potential Sources of error

Several studies have indicated that environmental variables, such as habitat structure and composition, play important roles in improving the accuracy of bird habitat descriptions (Dettmers & Bart 1999). The advantage of species modelling techniques is that they can be used to estimate the potential conservation value of both surveyed and under-surveyed areas (Maddock & Du Plessis 1999). However, Osborne *et al.* (2001) pointed out two potential issues when building predictive models for species distribution over large regions. First, there may be problems related to the consistency of predictor variables over large geographic spaces, which is particularly apparent in variables derived from remote sensing because of the inherent variability of angular surface reflectance (Stoms 1992). The second issue concerns the variations in habitat selection among individual birds.

Implications for conservation

The conservation of wetland bird habitats has become a global conservation priority (BirdLife International 2011). The species distribution model presented in this study can be used to underpin future conservation planning in the Red Sea region (Guisan & Thuiller 2005). The “*All birds*” model in this study indicated that approximately 17% of the Red Sea coastal habitat is suitable for wetland bird species.

In Saudi Arabia, there are 15 protected areas; only two of these (the Farasan Islands and the Umm Al Qamarie Islands) are located along the shores of the Red Sea (Saudi Wildlife Commission 2011). Thus, we recommend that future monitoring and surveys should be carried out to cover all 17 areas of high wetland bird species richness along the Red Sea coast in order to determine the most significant coastal areas for wetland bird species and therefore, incorporate them into the protected areas system in the future. These areas should include both nesting and foraging sites for wetland bird species. Furthermore, we strongly recommend that establishing new protected areas along the Red Sea coast line are becoming priority to protect in particular the remain suitable habitat areas for threatened and endemic wetland birds.

Conclusions

This study has produced the first predicted potential habitat distribution maps for wetland bird species along the Red Sea coast of the Kingdom of Saudi Arabia. It has revealed that there are 17 important areas of species richness for wetland birds along the Red Sea coast of the KSA. Understanding the correlation between species occurrence and environmental variables will help both researchers and policy makers to implement appropriate conservation plans in terms of wetland bird species. The modelling presented here has the potential to form the basis for conservation strategy of wetland birds in the Red Sea coastal region of Saudi Arabia, and it is hoped that this study will guide future field surveys and conservation programs in the Red Sea coast areas of Saudi Arabia.

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Chapter 5

Kentish Plover *Charadrius alexandrinus* populations are morphologically and genetically differentiated across Macaronesia

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Manuscript

Author´s contributions

MA: molecular analysis, statistical analysis, manuscript preparation

CK: study design, comments on manuscript

MCC-I: comments on manuscript

AK: data collection

PL: data collection

AV: data collection

TS: comments on manuscript, general editing

Abstract

Macaronesia, a group of archipelagos (Azores, Madeira, Canary Island and Cape Verde) in the Atlantic Ocean, is one of the global biodiversity hot-spots although for species that occur throughout these archipelagos the extent of genetic and morphological differentiation between the archipelagos and mainland Iberia and Africa is often unknown. Here we investigate phenotypic and genetic differentiation of Kentish Plovers *Charadrius alexandrinus*, the most common breeding shorebird of Macaronesia. We show that different archipelagos harbour genetically and morphologically different breeding populations. Our results suggest that although these populations are far from being a species-level endemism, they deserve conservation attention given their uniqueness in terms of genetic variation and morphology. Recent loss in suitable breeding sites in Canary Islands and Azores put substantial pressure on the existing plover populations. Further studies of the conservation status and threat to these populations are needed along with a comprehensive conservation action plan to halt population decline and facilitate recovery.

***Keywords*:** Kentish Plover, phenotypic divergence, gene flow, Macaronesian Islands.

Introduction

Divergence of populations may arise by limited dispersal and gradual genetic drift across populations (isolation by distance), adaptation to local environments preventing gene flow between populations (isolation by adaptation), or colonization history and founder effects (isolation by colonization, reviewed by Orsini *et al.* 2013). Given the geographic isolation that may reduce exchange of migrants, island populations are more prone to speciation than mainland populations (Adler 1992), and have therefore been important study systems in which to investigate fundamental ecological and evolutionary processes such as population divergence, adaptive radiation and speciation (Schluter 2000, Whittaker *et al.* 2006, Grant & Grant 2014).

Macaronesia is a collection of archipelagos in the North Atlantic Ocean off the coast of Europe and Africa. It includes Azores, Madeira, Selvagens, Canary Islands and Cape Verde. The Macaronesian Islands are an excellent study system to investigate the evolution and radiation of various taxa including plants, reptiles and birds given their substantial variation in distances from mainland and between members of the various archipelagos, and the variations in geological age of different islands Geldmacher *et al.* 2001, (Illera *et al.* 2007, Whittaker & Fernandez-Palacios 2007). The islands of Macaronesia have a unique biogeography and given their high habitat diversity they are home to a rich endemic biota (Illera *et al*, 2012, Vasconcelos *et al.* 2013), and are considered one of the global Endemic Bird Areas (Stattersfield *et al.* 1998). Much of the avifauna of the Macaronesian Islands exhibits genetic differentiation between islands or archipelagos, suggesting that there is limited gene flow between islands providing the opportunity for population differentiation and eventually new species to arise (Pestano *et al.* 2000, Dietzen *et al.* 2003, Kvist *et al.* 2005, Päckert *et al*. 2006, Illera *et al.* 2007).

The unique flora and fauna of these islands, however, are threatened by various processes. Logging of forests for timber and firewood, clearing vegetation for grazing and agriculture, and the introduced exotic plants and animals by humans threaten the endemic plants and animals (Martín *et al.* 2010, Vasconcelos *et al.* 2013). Poaching and illegal killing of marine turtles, and bycatch of dolphins and sharks put additional threats on coastal and marine species (Dutra & Koenen 2014). Additionally, the conversion of coastal dunes into settlements, hotels and holiday resorts has displaced much of the native vegetation, and all-year disturbance by humans and their pets on the beaches disrupt normal behaviour of birds including breeding. As a result, many of the endemic species of the islands are seriously endangered or extinct (Hazevoet 1995, Martín *et al.* 2010).

The Kentish Plover *Charadrius alexandrinus* is a wide-spread Eurasian shorebird species (Amat 2003) that inhabits North Africa, Europe and Central and Eastern Asia (del Hoyo *et al.* 1996). A previous study of genetic differentiation between different Kentish Plover populations (Küpper *et al.* 2012) showed that although mainland breeders were not differentiated over a large area spanning from Morocco to Eastern China, relatively modest distances over sea between mainland and island breeding sites (e.g., 40 km) produce detectable genetic differentiation. Here we extend this study by focusing on genetic and morphological differentiation between Kentish Plovers that breed on Macaronesian Islands and the ones that breed on mainland North Africa and Iberia.

The objectives of this study were therefore to (i) test for morphological differentiation among Kentish Plover populations across Macaronesia region, (ii) test for genetic differentiation among Kentish Plover populations across Macaronesia region, and (iii) investigate whether morphological and genetic differentiation correlate to each other as well as to geographic distance. Clarifying the morphological and genetic differentiation between these populations is warranted for two reasons. First, morphological and genetic differentiation between different archipelagos and mainland would imply that conservation and management of their breeding site and protection of the remaining habitats are important given the uniqueness of these populations. Second, the breeding Kentish Plover populations in some of these archipelagos (e.g., Canary Islands) are rapidly declining (Lorenzo & Barone 2013), and it is not yet clear whether gene flow between different archipelagos would be able to replenish these declines.

Methods

Sample collection

Morphometric measurements and blood samples were collected between 1994 and 2013 during the breeding season. Birds were caught at four archipelagos: i) Cape Verde (Boa Vista and Maio), ii) Azores (Santa Maria), iii) Canary Islands (Fuerteventura), and iv) Madeira (Porto Santo). In addition, we collected samples using consistent methodologies from mainland populations that included Portugal (Samouco, Fuseta, Atalaia Salt Pans, and Brito Salt Pans), and Morocco (Oued Gharifa Salt Pans, Table 1, Figure 1). In Fuseta, Atalaia Salt Pans, and Brito Salt Pans only morphological data were collected. Adult plovers were caught using mist-nets or funnel traps whilst they incubated the nest or attended the chicks (Székely *et al.* 2008). All birds were ringed with uniquely numbered metal rings, and three traits were measured for each adult: 1) body mass (to the nearest 0.1 gram); 2) right wing length (to the nearest mm), flattened and straightened from the carpal joint to the tip of the longest primary feather; 3) right tarsus (to the nearest 0.1 mm), from the notch of the knee to the tarsus bone ends.

Approximately 25 to 50 µl of blood were taken from the brachial vein. From broods of which the parents were not caught we also sampled single chicks usually caught shortly after hatching and took 25 µl from the tarsal vein for DNA analyses (Székely *et al.* 2008). The blood samples were stored in Queen's Lysis Buffer (Seutin *et al.* 1991) or 95% ethanol until DNA extraction in the laboratory.

Table 1. *Sampling details of Kentish Plovers used in genetic and morphometric analyses. Note: NA = no data available.*

Site		**Population**	**Latitude**	**Longitude**	Morphometric analyses		Genetic analyses
					Male	Female	
Islands	Cape Verde	Boa Vista	16°04.98'N	022°54.00'W	4	7	11
	Cape Verde	Maio	15°09.00'N	023°13.02'W	305	381	25
	Azores	Santa Maria	36°58.02'N	025°09.00'W	43	51	25
	Canary	Fuerteventura	28°43.98'N	013°55.98'W	12	14	11
	Madeira	Porto Santo	33°01.002'N	016°22.02'W	NA	NA	2
Mainland	Portugal	Samouco	38°43.98'N	008°58.98'W	NA	3	25
		Fuseta	37° 2.00'N	7° 44.00'W	34	38	NA
		Atalaia Salt pans	38°44.00'N	8°58.00'W	21	21	NA
		Brito Salt pans	38°44.00'N	8°58.00'W	14	14	NA
	Morocco	Oued Gharifa salt pans	35°30.00'N	006°24.00'W	69	76	25

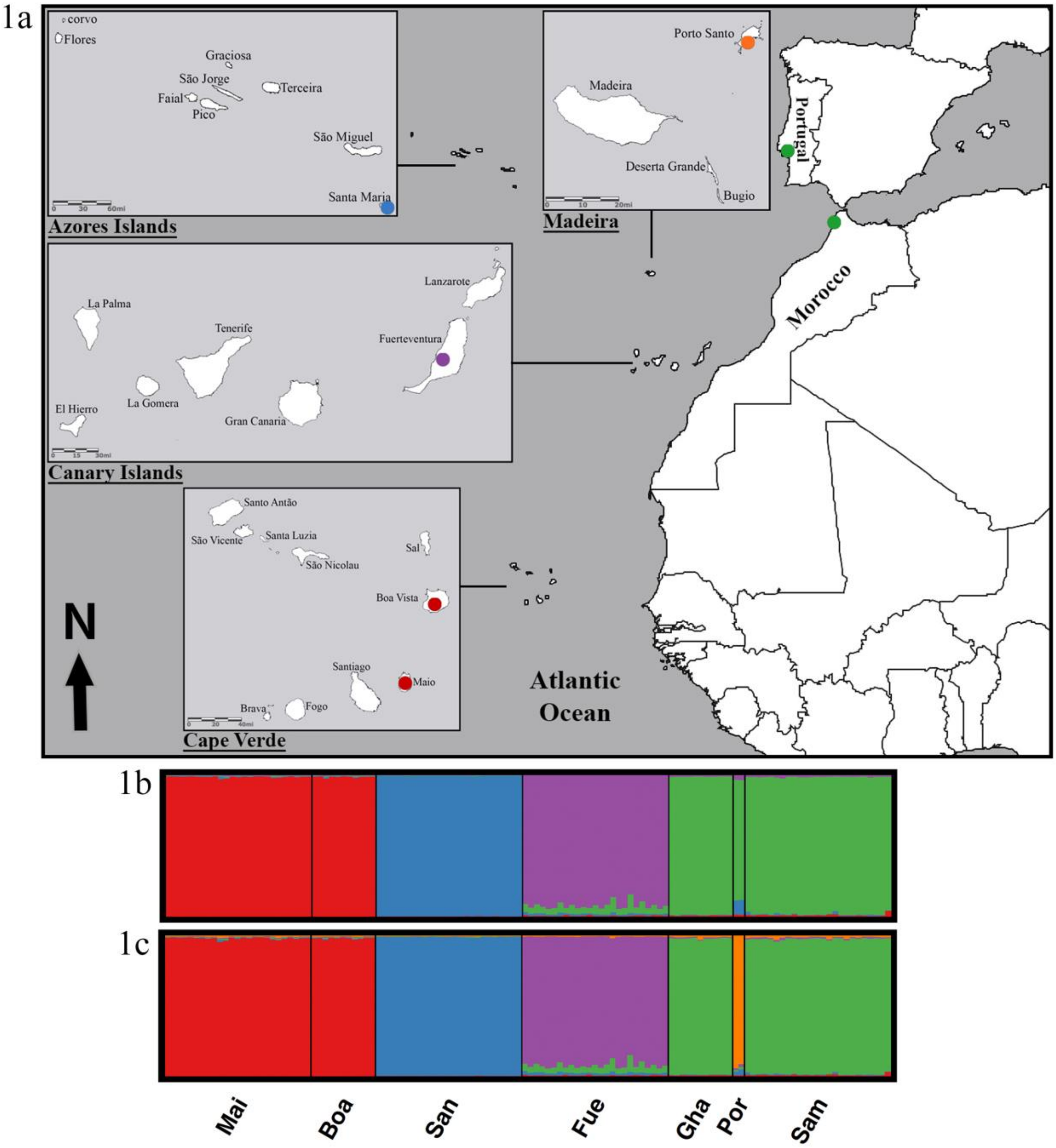


Fig 1. ***a*** *Geographic locations of seven Kentish Plover breeding populations.* ***b*** *Bar plot displaying the genetic identity of individual samples produced by applying STRUCTURE 2.3.4 without location prior (K = 4).* ***c*** *Bar plot displaying the genetic identity of individual samples produced by applying STRUCTURE 2.3.4 with location prior (K = 5). Note: ‘Boa is Boa Vista; ‘Mai is Maio; ‘San is Santa Maria; ‘Fue is Fuerteventura; ‘Por is Porto Santo; ‘Sam is Samouco; ‘Gha is Oued Gharifa.*

Morphological differences between populations

Body mass, wing length and tarsus length between sex and populations were analysed using two-way ANOVAs and Tukey HSD tests. The homogeneity of variance for the morphometric data for each sex was tested with Bartlett's test (Snedecor & Cochran 1989), and normality was assessed with a Shapiro-Wilk test (Shapiro & Wilk 1965). Both tests suggested that the morphometric data had homogeneous variances and were not different from normal distribution (all $P > 0.05$). Since adult Kentish Plovers are sexually dimorphic (Küpper *et al.* 2009), we also compared morphological measurements of males and females separately.

To assess morphological differentiation we calculated pairwise phenotypic distances (P_{ST}) between breeding locations for males and females separately. The P_{ST} index can be interpreted similarly to the commonly used F_{ST} index obtained from neutral genetic markers (Saint-Laurent *et al.* 2003; Raeymaekers *et al.* 2007). P_{ST} values of each trait were calculated separately for males and females between all population pairs using one-way ANOVA. The degree of phenotypic difference between populations was assessed as $P_{ST} = \sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$, where σ^2_{GB} is the variance of intra-populations and σ^2_{GW} is the variance within populations for a phenotype character. Phenotypic variance components were computed following Sokal & Rohlf (1995).

DNA extraction and microsatellite amplification

Laboratory work was carried out at National Environment Research Council Biomolecular Analysis Facility Sheffield (NBAF-S). DNA was extracted from 124 blood samples using an ammonium acetate method (Nicholls *et al.* 2000, see the protocol at http://www.shef.ac.uk/nbaf-s/protocols_list). DNA quantity and purity was assessed using a Nanodrop ND 8000 spectrophotometer. For all samples, PCR amplification was carried out using 20 microsatellite primers grouped into four multiplex reactions based on non-overlapping fluorescent dyes for different fragments (for full details see Küpper *et al.* 2012, excluding marker C204 which amplified the same locus as Calex-14). Automated fragment analyses was performed using an ABI 3730 capillary sequencer, and genotypes were scored using GeneMapper software version 3.7 (Applied Biosystems, Foster City, CA, USA).

Microsatellite analyses

We used ARLEQUIN version 3.01 (Excoffier *et al.* 2005) to compute indices of genetic variation both within and among populations including mean number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E). Pairwise F_{ST} values between population were used to quantify the degree of population genetic differentiation, and to estimate the inbreeding coefficient value (F_{IS}). The Bayesian clustering software STRUCTURE, version 2.3.4 (Pritchard *et al.* 2000), was used to determine population structure. We run two sets of models: i) without location prior as in Küpper *et al.* (2012) and ii) with location prior grouping samples according to archipelago or country. Using the location prior has been shown to identify meaningful genetic structure when the amount of available genetic data (samples or markers) is low (Hubisz *et al.* 2009). The analyses aimed to assign an individual's likelihood of belonging to a certain genetic cluster (K) based on the admixture model with correlated allele frequencies (Falush *et al.* 2003). For each approach, 15 independent simulations with K values ranging from

1 to 7 were performed for 500,000 generations with a burn in of 50,000 generations and the five runs with the lowest Ln probability were discarded. We then assessed the assignment probabilities, logged likelihoods and, delta K (Evanno *et al.* 2005) using STRUCTURE HARVESTER (Earl & Von Holdt 2012) to identify the most appropriate value of K. Results of the retained ten runs for each K were summarised using CLUMPP (Jakobsson & Rosenberg 2007) and visualised with DISTRUCT (Rosenberg 2004).

Non-parametric Spearman's correlation was conducted to examine the degree of correlation between the sample size and number of alleles.

Relationships between genetic, phenotypic and geographical distances

To test for the relationship between genetic, phenotypic and geographic distances, we performed Mantel tests (Mantel 1967) using matrices of pairwise F_{ST}, P_{ST}, and geographical distances (log km). Mantel test to compare geographic and genetic distances was conducted in ARLEQUIN (n = 10,000 permutations) to test for isolation-by-distance patterns of the genetic data using a matrix of F_{ST} values and the log geographic distance matrix between breeding sites. All other Mantel tests were performed using the package ade4 in R (version 2.15.1). We used Bonferroni correction to calculate P value thresholds to account for multiple testing with the three pairwise comparisons involved (corrected P value of 0.05/3 = 0.017, Table 6). Morphological differentiation between male and female was tested using Mann-Whitney U test.

Results

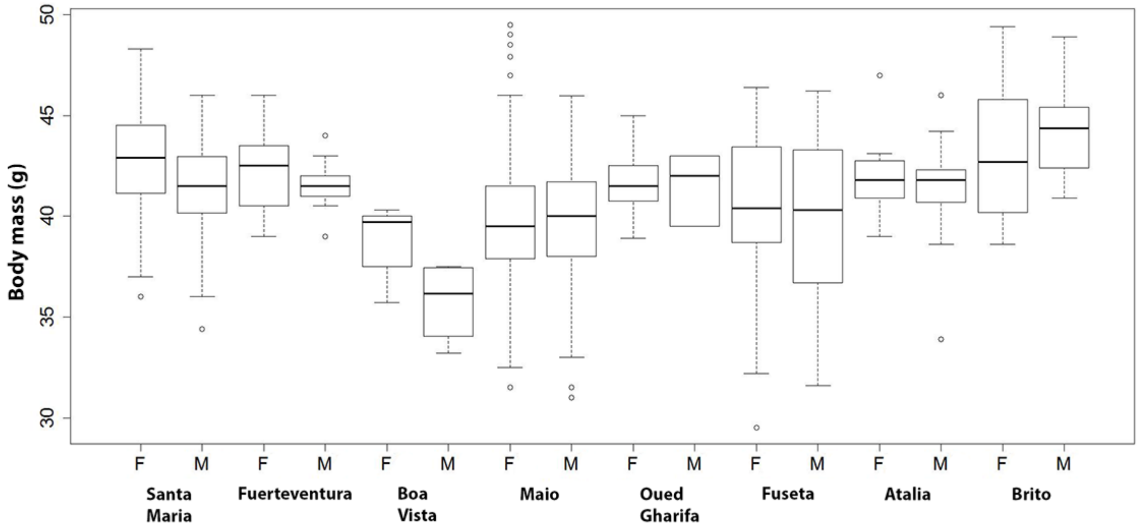
Morphological differentiation

Body mass, wing length and tarsus length were significantly different between populations (Table 2, Figure 2). Male plovers had longer tarsi than females (males: 29.99 ± 0.07 mm [SE], females: 29.29 ± 0.06), although body mass and wing length did not differ between sexes (Table 2). Sex differences were consistent between populations as indicated by the non-significant interaction term between sex and population (Table 2).

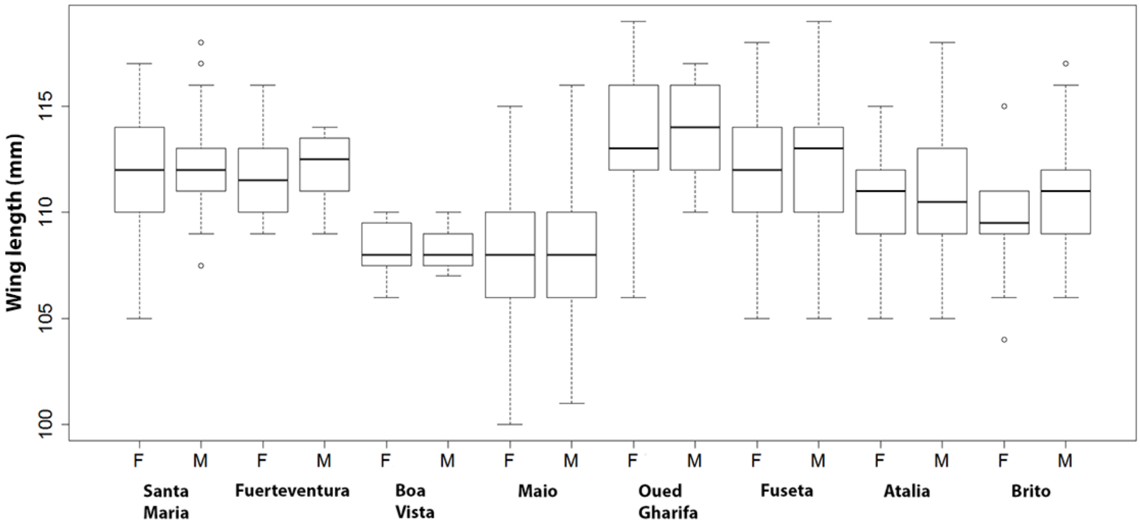
Table 2. *Comparison of body mass, wing length and tarsus length of male and female Kentish Plovers in different populations (two-way ANOVAs).*

Factor (df)	Body mass F(*P*)	Wing length F(*P*)	Tarsus length F(*P*)
Sex (1)	0.05 (0.83)	2.8 (0.09)	89.24 (< 0.0001)
Population (7)	19.64 (< 0.0001)	44.44 (< 0.0001)	58.99 (< 0.0001)
Sex x population (7)	1.34 (0.23)	0.35 (0.93)	1.04 (0.40)
No. of individuals	964	709	945

(a)


Body mass (g)
30
35
40
45
50
F
M
F
M
F
M
F
M
F
M
F
M
F
M
F
M
Santa Maria
Fuerteventura
Boa Vista
Maio
Oued Gharifa
Fuseta
Atalia
Brito

(b)


Wing length (mm)
100
105
110
115
F
M
F
M
F
M
F
M
F
M
F
M
F
M
F
M
Santa Maria
Fuerteventura
Boa Vista
Maio
Oued Gharifa
Fuseta
Atalia
Brito

(c)

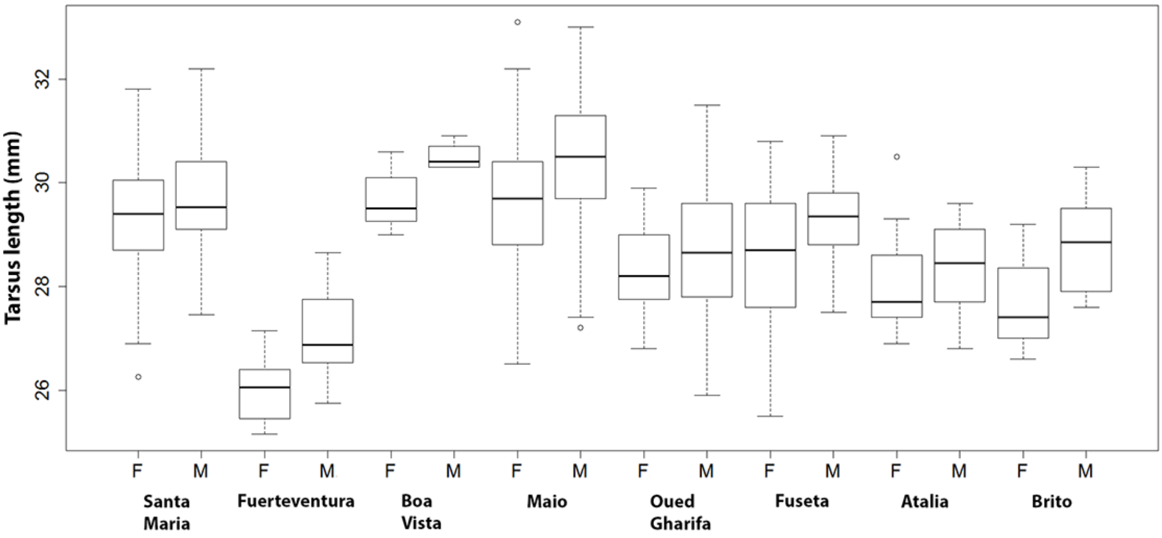


Fig 2. *Sample boxplots display the variations in body mass, wing length and tarsus length of male (M) and female (F) Kentish Plovers in Macaronesia. (a) body mass, (b) wing length, (c) tarsus length.*

Male wing length, tarsus length and body mass were most similar between Boa Vista, Morocco and Fuseta as indicated by the low P_{ST} values (Table 3), whereas the least similar ones were between Fuerteventura and Oued Gharifa. Female wing length, tarsus length and body mass were most similar between Fuerteventura, Fuseta and Oued Gharifa, whereas the least similar ones were between Brito and Morocco (Table 3).

Table 3. *Pairwise morphological differentiation (P_{ST}) for male and female Kentish Plovers. Males are above the diagonal, females below (NS = not significant, * $P < 0.05$, ** < 0.01, *** < 0.001). All significant values were derived from one-way ANOVA. Note: 'Mai' is Maio; 'Boa' is Boa Vista; 'San' is Santa Maria; 'Fue' is Fuerteventura; 'Gha' is Gharifa; 'Ata' is Atalaia; 'Fus' is Fuseta; 'Bri' is Brito.*

a) Body mass

Population	Mai	Boa	San	Fue	Gha	Ata	Fus	Bri
Mai		-0.07^{NS}	-0.001^{NS}	-0.02^{NS}	-0.04^{NS}	-0.01^{NS}	0.12***	-0.01^{NS}
Boa	-0.02^{NS}		-0.01^{NS}	-0.08^{NS}	-0.12^{NS}	-0.08^{NS}	-0.01^{NS}	0.12^{NS}
San	-0.005^{NS}	0.08^{NS}		-0.02^{NS}	-0.02^{NS}	0.04^{NS}	-0.003^{NS}	-0.01^{NS}
Fue	0.006^{NS}	0.09^{NS}	0.09*		0.58**	0.0002^{NS}	-0.02^{NS}	-0.02^{NS}
Gha	-0.04^{NS}	0.13^{NS}	0.19*	0.07^{NS}		-0.03^{NS}	-0.04^{NS}	0.0005^{NS}
Ata	-0.004^{NS}	-0.05^{NS}	0.0006^{NS}	-0.03^{NS}	-0.05^{NS}		-0.007^{NS}	-0.03^{NS}
Fus	-0.004^{NS}	0.05^{NS}	0.04*	-0.06^{NS}	0.03^{NS}	-0.01^{NS}		-0.03^{NS}
Bri	-0.007^{NS}	0.04^{NS}	0.07^{NS}	0.10^{NS}	0.41*	-0.02^{NS}	-0.02^{NS}	

a) Wing length

Population	Mai	Boa	San	Fue	Gha	Ata	Fus	Bri
Mai		-0.06^{NS}	-0.001^{NS}	0.01^{NS}	-0.02^{NS}	0.002^{NS}	0.03*	-0.02^{NS}
Boa	0.01^{NS}		0.38^{NS}	0.09^{NS}	-0.03^{NS}	0.20^{NS}	-0.07^{NS}	-0.06^{NS}
San	-0.01^{NS}	-0.03^{NS}		-0.02^{NS}	0.01^{NS}	0.005^{NS}	0.002^{NS}	0.04^{NS}
Fue	-0.02^{NS}	-0.03^{NS}	-0.02^{NS}		0.01^{NS}	-0.03^{NS}	0.01^{NS}	0.06^{NS}
Gha	0.05^{NS}	-0.02^{NS}	0.01^{NS}	-0.04^{NS}		-0.03^{NS}	0.03^{NS}	0.10^{NS}
Ata	0.001^{NS}	0.31*	-0.02^{NS}	-0.03^{NS}	-0.04^{NS}		0.01^{NS}	-0.02^{NS}
Fus	-0.003^{NS}	0.04^{NS}	-0.01^{NS}	-0.02^{NS}	-0.02^{NS}	-0.02^{NS}		-0.01^{NS}
Bri	0.12*	0.06^{NS}	-0.003^{NS}	-0.02^{NS}	0.07^{NS}	-0.03^{NS}	-0.02^{NS}	

a) Tarsus length

Population	Mai	Boa	San	Fue	Gha	Ata	Fus	Bri
Mai		0.04^{NS}	0.03*	-0.02^{NS}	0.05^{NS}	-0.01^{NS}	-0.01^{NS}	-0.01^{NS}
Boa	0.05^{NS}		-0.07^{NS}	-0.04^{NS}	-0.08^{NS}	-0.08^{NS}	-0.04^{NS}	-0.08^{NS}
San	0.004^{NS}	-0.02^{NS}		0.07^{NS}	0.02^{NS}	0.11*	0.003^{NS}	-0.02^{NS}
Fue	-0.01^{NS}	-0.05^{NS}	-0.02^{NS}		0.002^{NS}	0.03^{NS}	-0.03^{NS}	0.002^{NS}
Gha	0.04^{NS}	-0.06^{NS}	-0.01^{NS}	-0.04^{NS}		-0.04^{NS}	-0.001^{NS}	-0.04^{NS}
Ata	0.03^{NS}	-0.05^{NS}	-0.01^{NS}	0.001^{NS}	-0.02^{NS}		-0.005^{NS}	-0.02^{NS}
Fus	0.01^{NS}	0.07^{NS}	-0.01^{NS}	-0.02^{NS}	0.02^{NS}	-0.02^{NS}		-0.02^{NS}
Bri	0.01^{NS}	-0.05^{NS}	0.06^{NS}	0.007^{NS}	0.002^{NS}	-0.03^{NS}	0.002^{NS}	

Genetic diversity and population differentiation

The lowest number of alleles were found in Madeira (2.31 ± 0.60, Porto Santo) whereas the highest were found in mainland Portugal (9.45 ± 3.85, Samouco, Table 4). Using Spearman correlation, we detected a none significant correlation between sample size and number of alleles (Spearman's rho correlation: rs = 0.60, P = 0.15). No evidence of inbreeding was found in any of these populations as indicated by non-significant F_{IS} values (Table 4).

Table 4. *Genetic diversity of Kentish Plovers in Macaronesia (mean ± SE). Note: 'Boa, CV' is Boa Vista, Cape Verde; 'Mai, CV' is Maio, Cpe Verde; 'San, Az' is Santa Maria, Azores; 'Fue, CIs' is Fuerteventura, Canary Islands; 'Por, Mad' is Porto Santo, Madeira; 'Sam, Por' is Samouco, Portugal; 'Gha, Mor' is Oued Gharifa, Morocco.*

Population	**Sample size**	**Number of alleles (N_A)**	**Observed heterozygosity (H_O)**	**Expected heterozygosity (H_E)**	**F_{IS} (P)**
Boa, CV	11	4.75 ± 1.68	0.61 ± 0.23	0.68 ± 0.12	0.10 (0.05)
Mai, CV	25	5.80 ± 2.37	0.65 ± 0.16	0.66 ± 0.14	0.03 (0.31)
San , Az	25	8.00 ± 2.75	0.76 ± 0.16	0.77 ± 0.12	0.011 (0.33)
Fue, CIs	11	6.35 ± 2.23	0.72 ± 0.16	0.74 ± 0.14	-0.002 (0.57)
Por, Mad	2	2.31 ± 0.60	0.75 ± 0.26	0.66 ± 0.15	-0.31 (1.00)
Sam, Por	25	9.45 ± 3.85	0.75 ± 0.15	0.78 ± 0.12	0.03 (0.11)
Gha, Mor	25	4.75 ± 1.62	0.65 ± 0.19	0.65 ± 0.17	-0.02 (0.73)

Pairwise F_{ST} comparisons between archipelagos (mean F_{ST} between archipelagos) showed high genetic differentiation, and low between island differentiations within the same archipelago (Table 5, Boa Vista and Maio, $F_{ST} = 0.015$, P = 0.05).

Table 5. *Pairwise F_{ST} values (above diagonal) and P values for genetic differentiation (below diagonal) between Kentish Plover populations. Note: 'Boa' is Boa Vista; 'Mai' is Maio; 'San' is Santa Maria; 'Fue' is Fuerteventura; 'Por' is Porto Santo; 'Sam'* is Samouco; 'Gha' is Oued Gharifa.

Population	**Boa**	**Mai**	**San**	**Fue**	**Por**	**Sam**	**Gha**
Boa		0.0153	0.09	0.11	0.24	0.07	0.17
Mai	0.053		0.09	0.11	0.25	0.08	0.18
San	<0.00001	<0.00001		0.05	0.14	0.02	0.09
Fue	<0.00001	<0.00001	<0.00001		0.16	0.02	0.10
Por	0.01	<0.00001	0.003	0.02		0.09	0.18
Sam	<0.00001	<0.00001	<0.00001	<0.00001	0.002		0.07
Gha	<0.00001	<0.00001	<0.00001	<0.00001	0.002	<0.00001	

Results from STRUCTURE suggested that each archipelago is genetically differentiated from all other (Figure 1); without a location prior the most likely K value was four splitting all archipelagos populations except Madeira from the mainland population whereas when using the more sensitive method with location prior the most likely K value was five assigning also Madeira samples into a separate cluster. The archipelago populations were genetically distinct from mainland population, there was only a single cluster for the two mainland populations (Iberia and North Africa), and the samples from the two Cape Verdean Islands were grouped together (Figure 1b).

Genetic and morphological differentiation in relation to geographic distance

Genetic distance and the extent of neutral genetic variation estimated from microsatellites tended to correlate positively (Mantel test: $r = 0.365$, $P = 0.078$). However, once Bonferroni correction was taken into account for multiple testing, the relationships between geographic distance and morphological differentiation or neutral genetic distance were all far from significant (Table 6).

We also compared the extent of morphological differentiation over distance between males and females, although none of these differences was statistically significant (Mann-Whitney U test: $U = 4$, $N_1 = N_2 = 3$, $P = 0.83$) suggesting that morphological differentiation over geographic distance is comparable between male and female plovers. The low statistical power perhaps due to the small sample size.

Table 6. *Partial correlations between geographic, phenotypic (P_{ST}) and genetic (F_{ST}) distance matrices. Pairwise differences between populations in regards to genetic differentiation (F_{ST}) and geographic distance (log km, Mantel test: r = 0.365, P = 0.078).*

Variables	**Male**			**Female**		
	Body mass	**Wing**	**Tarsus**	**Body mass**	**Wing**	**Tarsus**
	r (P)	r (P)	r (P)	r (P)	r (P)	r (P)
P_{ST} vs F_{ST}	-0.03 (0.58)	0.05 (0.39)	-0.36 (0.86)	0.30 (0.19)	0.012 (0.48)	-0.27 (0.79)
P_{ST} vs geographic distance	0.07 (0.44)	0.20 (0.14)	-0.22 (0.88)	0.08 (0.39)	0.28 (0.02)	-0.27 (0.89)

Discussion

Our study found three major patterns. First, we show that body sizes differ between Kentish Plover populations that breed on different Macaronesian Islands. Kentish Plovers use fairly similar habitats across their vast geographic range that includes saltpans, sand dunes, and lake shores and the breeding habitats in several Macaronesian archipelagos are consistent with their habitat preference elsewhere. Therefore, local adaptation to different ecology does not seem to explain the body size variations between islands. We offer three potential explanations. Firstly, in spite of overall difference in ecology between the islands, there might be differences in microhabitats that can select for smaller or larger sizes. Foraging habitats may differ between sites, vegetation cover and thus the easy to catch insects and invertebrates in the mud, or the consistency of mud may make running and catching mobile prey more difficult in some locations than in others leading to differences in tarsus length. Secondly, the different wing length between archipelagos suggests that the overall mobility to move between islands may vary. A common sign of losing mobility and migratory behaviour is reduced flight musculature and wing length, and the differences we observed may reflect these tendencies. Thirdly, founder effects and genetic drift may create the different morphologies across islands. Spurgin *et al.* (2014) suggested that founder effects contributes significantly to the genetic and morphological differentiation between bird populations. The results of this study indicate that most pairwise tests of the morphological differentiation were not significant. A possible explanation for this might be that unequal sample sizes limited the power of the statistical analysis. Shaw & Mitchell-Olds (1993) reported that unequal sample sizes may raise the chance of type II error. Tamhane (2008) mentioned that P values are highly influenced by sample sizes.

Our second major result that each archipelago is genetically differentiated is in line with previous studies using native bird populations in Macaronesia (Pestano e*t al.* 2000, Dietzen *et al.* 2003, Kvist *et al.* 2005, Päckert *et al.* 2006, Illera *et al.* 2007). Using a superior clustering algorithm that can deal better with low sample sizes (Hubisz *et al.* 2009) we also found significant and meaningful genetic differences between Madeira Kentish Plovers and the mainland population despite having only sampled two unrelated birds in this archipelago.

We suspect that a major reason for the genetic difference we observed between archipelagos is change in life style. Once the ancestor population settled in an island, it became sedentary due to the all year round food availability. We also noted during extensive fieldwork on Cape Verde, that in contrast to mainland Kentish Plover that are highly polygamous the island plovers are more monogamous and highly site faithful and tend to return year after year to breed in the vicinity of their former territory. This limited dispersal over large numbers of generations may have produced the genetic difference we observed between different islands.

Finally, genetic differentiation is not linked to morphological differentiation. Genetic differentiation but not morphological differentiation follows isolation by distance pattern. We propose that island (or archipelago) specific selection pressures shape phenotypes. These are different from simple macroecological processes such as isolation by distance and need to be investigated further.

In conclusion, using a wide-spread shorebirds species, the Kentish Plover, as a model organism we show that Macaronesian archipelagos harbour genetically and morphologically unique populations. The differences could be the beginning of speciation after isolation by distance and gene flow seems to be reduced because of behaviour and/or distance. In one of these archipelagos (Canary Islands) the breeding population is rapidly declining, although the trends from other populations have not been reported. Since Macaronesia, similar to many oceanic archipelagos, are rapidly developing largely due to beach tourism, we are concerned that all of these populations may be declining. This requires actions to establish the proximate causes of population declines, and develop an action plan to safeguard the remaining breeding populations.

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Chapter 6

Conclusions and future directions

Mohammed Almalki

Main Conclusions

My PhD provided novel insights in three important fields of biology: breeding ecology, conservation genetic and population genetics, and these results have further implications of conserving and managing wetland bird species. Specifically,

- I investigated the sex-related variations in body size, breeding behaviour and parental care of the Crab Plover in Saudi Arabia. This endemic bird has a peculiar and rarely investigated breeding system. Using molecular sexing, I demonstrated that the most reliable morphometric trait to distinguish between male and female Crab Plovers is bill length. I also demonstrated that Crab Plover nesting burrows provide thermal stability in a harsh, hot environment. To our knowledge, this is the first study that has used molecular sex-typing to distinguish sexual differences in the daily rhythms of food provisioning and chick feeding in the Crab Plover. Overall, my work provided new insights into the breeding ecology of an enigmatic and unique burrowing shorebird, and suggest areas for future investigation to further elucidate the breeding behaviour of this species (Chapter 2).

- I reviewed the status of breeding Crab Plover populations along the Red Sea coast of Saudi Arabia. Comparing these new data to previous studies, we discovered two new colonies. Therefore, this update will contribute to filling the gap between the known number of breeding pairs and the global population that was estimated from censusing wintering birds. The breeding of crab plovers is restricted to the remote islands around the Arabian Peninsula. Therefore, we provided implications for conservation that summarise some potential threats that may face the Crab Plover during the breeding season (Chapter 3).

- I modelled suitable habitat and areas of high species richness using 22 wetland bird species along the Red Sea coast of Saudi Arabia. To date, this study has produced the first predicted potential habitat richness model for wetland bird species along the poorly conserved Red Sea coast of Saudi Arabia. I highlighted 17 potential richness areas; each area combines different numbers of endemic species. I argue that this model can contribute effectively in helping researchers and ecologists in identifying important areas to survey. I recommend establishing new protected wetland areas along the Red Sea coast in order to conserve the threatened and endemic wetland bird species (Chapter 4).

- Finally, using microsatellite markers, I investigated the genetic and morphometric differentiation in the Macaronesia region. The main objective of this paper was to provide training in microsatellite analyses that I am planning to use for genetic differentiation of waterbirds in Saudi Arabia. The main conclusion is that each archipelago population is genetically and morphologically differentiated. Therefore, this result improves our understanding and should help establish a strategy to conserve this species in the Macaronesia region. Additionally, further studies are required to identify the risks that may face Kentish Plover populations in this region and to set up an effective conservation strategy (Chapter 5). Since Saudi

Arabia has various islands in the Red Sea region, a study using an archipelago (Macaronesia) provided a useful baseline comparison.

Future directions

In course of this PhD, I obtained valuable skills in field biology and molecular ecology, and became familiar with scientific concepts in behavioural ecology and conservation biology. In future, I wish to use these skills and knowledge to follow-up the results in this dissertation.

Four research lines appear extremely promising to follow-up the research in this dissertation:

1. The Arabian Peninsula and the surrounding seas have numerous endemic species, although many of these endemic species have not been investigated in detail. I propose to investigate the mating systems and parental care strategies of three endemic waterbirds: Crab Plover, White-eyed Gull and Saunders’s Tern. The White-eyed Gull is an endemic species to the Red Sea and the Gulf of Aden (PERSGA/GEF 2003) and is considered Near Threatened (IUCN 2014). It is observed to breed in some islands along the Red Sea coast of Saudi Arabia (Shobrak & Aloufi 2007, Jennings 2010). The breeding and non-breeding areas are restricted to the Gulf of Aden and the Red Sea in Egypt, Sudan, Eritrea, Djibouti, Saudi Arabia, Yemen and Somalia (BirdLife International 2014). The local movements of the White-eyed Gull is poorly studied (Jennings 2010). Saunders’s Tern is also a poorly studied species; it is restricted to the north Indian Ocean and has a massive breeding range, extending from the Red Sea coast and Arabian Gulf to northwest India, Sri Lanka and the Maldives (BirdLife International 2013, del

Hoyo *et al.* 1996). It is recognised that it breeds in the Red Sea and the Arabian Gulf coast and the islands of Saudi Arabia (Bundy *et al.* 1989, Shobrak & Aloufi 2007, Jennings 2010). It is currently classified as a Least Concern by the IUCN Red List (IUCN 2014). However, the population is expected to decrease due to predation by rats and cats (in some areas), human disturbance and habitat loss due to development (del Hoyo *et al.* 1996).

Studying these three species is appropriate not only because of their importance in the ecosystems in which they occur and potentially declines in their populations, but also because they are little-known species, with baseline ecological data on their ecology, demography and behaviour largely missing. We know very little with regarding to the mating systems and parental care strategies of these species. Studying the mating system and parental care aspects of these species has become an urgent necessity, since these influence productivity and thus long-term population strategies. This research will ultimately help to understand and conserve these species.

Future studies must aim to a) gather baseline details on the behaviour and breeding ecology of the Crab Plover, White-eyed Gull and Saunders's Tern; b) describe the mating systems and parental care of these three species; c) identify factors that affect breeding success, distribution of care types, mate fidelity and nest-site fidelity, and nest attendance on these three species; d) investigate nest architecture of the crab plover. The latter seems especially important, because I believe that breeding burrows may be connected and there is a possibility that several adults may share breeding.

2. Investigate the post-breeding migratory behaviour of Crab Plover, White-eyed Gull, and Saunders's Tern

Little information exists on the movements of Crab Plover. De Marchi *et al.* (2006) indicated that the breeding areas of Crab Plover do not correspond entirely with those used in the non-breeding season. They leave their breeding sites rapidly when faced with extreme environmental conditions, thermal stress or limited food availability (De Sanctis *et al.* 2005). The wintering and breeding populations of Crab Plover were estimated in different countries. However, there is a lack information available about how the breeders migrate, that is, what routes they take and where the stop-over sites are.

Overall, understanding the movement of these species and the habitats they use is important for establishing the appropriate conservation planning.

3. Studying the genetic structure of the Crab Plover, White-eyed Gull and Kentish plover.

The genetic structure of a population can play a significant role in the description and comparison of the level of within-deme and between-deme genetic variations. It is useful to identify gene flow, natural selection, age structure and mating systems. The molecular differentiation in birds is less pronounced than in other vertebrates at both the population as well as species levels (Avise & Aquadro 1982). This might be because of the greater mobility of birds, higher levels of gene flow and larger effective population sizes (Barrowclough 1983). Only a few studies of genetic diversity have been conducted on some species of shorebirds, (Küpper *et al.* 2008, Miller *et al.* 2009); the latter observed differences in genetic structure between the interior and Atlantic piping plovers in North America. Küpper et al. (2012) mentioned that there are genetic differences between mainland and island populations of Kentish plover. Moreover, the remote island populations of Kentish Plover have lower levels of genetic diversity (Küpper *et al.* 2012). Several lines of independent research have indicated that the vast majority of extinctions have been in island species (Frankham *et al.* 2009) because they are unable to cope with diseases (Penn *et al.* 2002) and environmental changes (Bijlsma & Loeschcke 2005).

Following the same methods as those used in Chapter 5 of my thesis, I am currently analyzing the Kentish Plover microsatellite and morphological data from the Arabian Peninsula. This work can be followed up by microsatellite analyses of other shorebirds in Saudi Arabia including Crab Plover and White-eyed Gull. The former species would be particularly rewarding, since breeding colonies tend to be stable over long time period on a given island suggesting that neighbours (possibly offspring as well) create kin-related colonies over time. This could potentially enhance cooperation and may lead to shared parenting of young and/or cooperative breeding.

Concluding remarks

I believe that understanding bird ecology and behaviour in the Arabian Peninsula is important for 2 main reasons. First, there are very few bird studies in the region apart from raptors and falcons, although these desert-dwelling birds may show specific adaptations such as burrowing in the sand. Second, understanding evolutionary ecology of these species is important for predicting chances in future. Animals that live in the desert may be already at their edge of their tolerance in regards to heat physiology, and thus further changes in global climate may tip them over their physiological carrying capacity. Threats, primarily from habitat loss and pollution, likely to make an additional pressure on wild bird populations, with as yet unknown consequences.

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FOOD ABUNDANCE EXPLAINS THE BREEDING SEASON OF A TROPICAL SHOREBIRD, THE CRAB PLOVER *DROMAS ARDEOLA*.

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The timing of the breeding in birds is a life-history trait that generally depends on food availability, but other factors may play a role, particularly in tropical areas where food availability is less seasonal than in temperate or arctic areas. We studied the factors affecting the breeding season of the Crab Plover *Dromas ardeola*, a burrow nesting colonial shorebird endemic of the north-western Indian Ocean. A reduced risk of burrow flooding, high temperatures suitable for exploiting solar incubation, a reduced interference by Palaearctic shorebirds during foraging and a reduced risk of predation by Palaearctic raptors are all associated with the summer breeding season of the Crab Plovers, but none of these factors can explain the difference, up to one month, in the breeding schedule throughout their breeding range. On the contrary, we found not only a clear peak of food abundance during the breeding season but also a significant correlation between the start of the breeding season and the average annual chlorophyll-a concentration around the colonies, a proxy for marine productivity. We conclude that food abundance, together with the likely high intraspecific competition due to coloniality, is strongly supported as the critical factor determining the nesting phenology of this tropical species.

Key words: breeding season, burrow temperature, chlorophyll, competitors, Crab Plover, *Dromas ardeola*, food abundance, Indian Ocean, predators, rainfall.

INTRODUCTION

The timing of breeding has important fitness consequences for birds because reproductive success varies seasonally, and frequently declines with advancing dates (Verboven and Visser 1998, Williams 2012). A recent review (Verhulst and Nilsson 2008) pointed out that an effect of laying date explains the low fitness of late breeders, that there are costs for laying early, and that early breeders are individuals of higher quality, better able to cope with the costs of early laying (e.g. when food availability is still low). Late low reproductive effort is caused by a general decrease in habitat quality, in particular for species that lay a single clutch. Food abundance is the crucial factor most frequently suggested for such timing. This idea was championed by Davis Lack (1968), who suggested that the breeding phenology is ultimately determined by the food availability necessary in particular during the chick-rearing phase. While there is an overall accordance between breeding season and food abundance at least at the population level, Perrins (1970) pointed out that most females lay eggs too late for the offspring to fully exploit the seasonal peak in food abundance, and suggested that the proximate cause might be food shortage for egg production. Other researchers suggested that other factors might be important and that the laying date is a life-history trait that results from a trade-off between the interest of parents and the interest of offspring (Martin 1987, Drent 2006).

Climatic conditions, competition with other species and the risk of predations have been suggested to influence the laying date of some species in alternative to food abundance. In Arctic breeding species, like the Snow Geese *Chen caerulescens atlantica*, breeding phenology strictly depends on snow cover in spring (Dickey et al. 2008). Schreiber (1980) suggested that timing of the nesting season of the

Brown Pelicans *Pelecanus occidentalis* has evolved so as to avoid the hurricane season, which could put eggs and chicks at great risk. Species nesting on low islands in rivers, like some terns and pratincoles, are forced to breed at low water levels, during the dry season (Brosset 1979). The White Tern *Gygis alba* breeds when food availability is reduced but when interference competition by other species is lower (Catry et al. 2009). Clay-coloured Robins *Turdus grayi* and Bananaquits *Coereba flaveola* breed at a time when food availability is low but when nest predation is less prominent (Morton 1971, Wunderle 1982). The Moluccan Megapode *Megapodius wallacei,* a species that uses the heat generated by the sun to incubate its eggs, exhibits lunar synchrony in the timing of egg-laying, likely explained by the increased risk of predation during the dark nights (Baker and Dekker 2000).

Most researches on timing of reproduction involved well studied northern temperate species while tropical birds have been much less studied (Stutchbury and Morton 2001). In general, as a result of a less seasonal climate, many tropical areas have a much more extended breeding season, lasting between 6.6 and 9.8 months compared to 3.1 to 4.2 months of temperate areas (Ricklefs 1966, Stutchbury and Morton 2001). Few species, like the Sooty Tern *Sterna fuscata* and the Bridled Tern *Sterna anaethethus*, experience constant conditions and breed with subannual periodicity (Ashmole 1963, Diamond 1976, Jaquemet et al. 2007), even if they breed annually when marine productivity is more seasonal (Jaquemet et al. 2007). However, the seasonal breeding schedule is less clear than in temperate climates, even in a quite seasonal tropical region like the north-western Indian Ocean. There, the winter and spring breeding season of landbirds is clearly dependent on winter rainfall, even if it is a scarce rainfall (Jennings 2010), but the determinants of the breeding season of the sea dependent species are less clear (Jennings 2010). Some species like the Brown Booby *Sula leucogaster* and the Socotra Cormorant *Phalacrocorax nigrogularis* breed almost throughout the year, the Osprey *Pandion haliaetus* and Caspian Tern *Sterna caspia* breed during the winter, while many species of terns, two species of gulls and the Crab Plover *Dromas ardeola* breed during the summer (Semere et al. 2008, De Marchi et al. 2009, Jennings 2010). The summer breeding season of many species might be coincident with the period of fish abundance, but there is little research to date (Jennings 2010, Vosoughi et al. 2010, Shobrak and Aloufi 2013).

We studied the nesting phenology of the Crab Plover, whose food availability, coastal invertebrates (Rands 1996), can be more easily assessed than for seabirds. The Crab Plover is a shorebird that breeds endemically in north-western Indian Ocean, while it disperse more widely during the non-breeding season (Rands 1996). Crab Plovers breed colonially in burrows on islands devoid of terrestrial predators and lay a single egg (Rands 1996), which is huge for the female size, at about 23% of the female mass (De Marchi et al. 2012, Tayefeh. et al. 2013). The habit of breeding in burrows is unique among the shorebirds. The thermal properties of the burrows are almost optimal for solar incubation, which results in a low parental effort during incubation (De Marchi et al. 2008, De Marchi et al. In press). The single chick is provisioned by the both parents at the burrow until it fledges (Rands 1996) and even later on for some months on the wintering grounds (De Sanctis et al. 2005).

Their breeding season, (from April to September) was considered a puzzle by Hockey and Aspinall (1997). In their study area, in Abu Dhabi, shade temperature (but there was no shade in their breeding sand-banks) reached 48°C, was regularly over 40°C and was rarely below 28°C, and humidity was frequently above 90%.

Possibly as a consequence of thermal stress, the peak of foraging activity occurred early in the morning and late in the afternoon independently of the tide level (but see De Marchi et al. In press). We envisage five possible selective factors that can be responsible for the breeding phenology of this species. 1) A higher food availability as suggested by Hockey and Aspinall (1997). They hypothesized, but could not endorse, that reproduction was timed with maximal availability of invertebrate prey, mostly crabs, for feeding the nidicolous chicks in July-August. The importance of crab abundance might be increased by the high intraspecific competition for food due to the highly colonial breeding habits, with hundreds or even thousands pairs nesting on the same islet. A circumstantial evidence that the food-linked window for breeding is narrow was that birds were present at the colony for a short period only, arriving just before egg-laying and leaving just after chicks fledged (Hockey and Aspinall 1997). 2) A reduced risk of predation by Palaearctic raptors, in particular *Circus* harriers, that are common at the Crab Plover breeding sites during winter but much rarer during the breeding season of Crab Plovers (De Marchi et al. 2009, Aspinall 2010). 3) A reduced interference on the foraging grounds by the hordes of Palaearctic shorebirds that leave the mud-flats almost free for the Crab Plovers while breeding further north during the summer. The idea stems from the depression of food-intake rate, recorded in some other shorebirds, caused by large numbers of shorebirds through interference competition (Sutherland and Koene, 1982; Ens and Goss-Custard 1984). Interference could affect Crab Plovers because preys, as *Uca* crabs, hide in burrows at the approach of shorebirds, thus creating a halo devoid of prey around each birds (Van der Kam et al. 2004). 4) A reduced risk for the burrows to be flooded by rain, a well known cause of breeding failure in some burrowing species like penguins and shearwaters (Stokes and Boersma 1991, Thompson and Furness 1991). 5) The opportunity to exploit high soil temperatures during the early summer months, which reduced incubation costs through the adoption of partial solar incubation (De Marchi et al. 2006, De Marchi et al. In press), while avoiding higher temperatures that could be dangerous for the embryo later during the breeding season.

During several field campaigns in Eritrea, we collected data for checking the association between the breeding season and some environmental factors (food availability, presence of Palaearctic shorebirds and raptors, risk of flooding, and temperature of the burrows). Moreover, we investigated the correlation between the start of the breeding season and some environmental factors (latitude, marine productivity, air temperature and rainfall) that are correlated to the likely important environmental factors.

METHODS

Study area

Field data were collected in the Dahlak archipelago and on the nearby mainland of Eritrea. The area has a high coastal productivity (Sheppard et al. 1992, Butler et al. 2001) with great concentrations of migrating, wintering and nesting coastal and marine birds (Semere et al. 2008, De Marchi et al. 2009). The Dahlak archipelago alone hosts about 15 Crab Plover colonies, that with other colonies in the Howakil, Anfile and Assab Bays, make Eritrea a stronghold for this species (De Marchi et al. 2006, Semere et al. 2008). Data were variously collected in the period 2002-2013 on five islands with Crab Plover colonies (Dahret, Baradu, Kad Norah, Sarad and NN086), three islands without colonies (Durgham, Durghella, and Sheikh Said) and 2 coastal sites just north of Massawa (near Gurgussum and Hamasien hotels, Fig. 1).

Nesting phenology

Due to the difficulty of checking the content of many burrows without creating excessive disturbance to the colony, we used indirect ways to calculate the timing of breeding at Dahret island. In particular, we recorded the number of burrows and of hatched eggs. The colonies were searched for eggshells outside nests, and all newly found eggshells were crashed in order to avoid double counts. The number of hatched eggs could be calculated as number of eggshells found on the colony surface, since eggshells are ejected from the burrows by the parents soon after hatching (Tayefeh et al. 2013, personal observations). The timing of egg laying is back-calculated from the date of hatching, on the basis of an incubation period of about 33 days, observed both on Dahret island (De Marchi et al. 2008) and in the Persian Gulf (Tayefeh et al. 2013). The timing of the chick rearing phase is calculated using the 7 week long chick rearing phase recorded in a study in Iran (Tayefeh et al. 2013). We divided each phase (egg laying, incubation, hatching and chick rearing) in early (the first 25% of pairs), peak (the middle 50% of pairs) and late phase (the last 25% of pairs). Unfortunately, fishermen collected Crab Plovers eggs at Dahret island during the second half of May and beginning of June in all study years apart from 2005, a disturbance that might result in a significant bias in the calculation of the different phases of reproduction. Therefore, only the data of 2005 were used to calculate the breeding phenology at Dahret Island.

Data on timing of breeding by Crab Plovers were obtained for other regions from the literature, particularly about the start of nest digging, the breeding phase that had been recorded most easily. The period April-May was divided in 5-day periods, and each colony was assigned to a period (Table 1) depending on the start of the digging phase (e.g. : 1 for start on 1-5 April, 2 for 6-10 April and so on).

Prey abundance.

We selected three different habitats used for foraging by Crab Plovers and censused the density of burrows built by different species: 1) burrows of ghost crabs *Ocypode saratan* on sandy beaches; 2) all burrows, mainly of *Dotilla* crabs, on shallow intertidal sandy beaches; 3) all burrows, mainly of *Uca* crabs and callianassid mud-shrimps, on mud flats. All these dominant species are part of the diet of Crab Plovers in the study areas as a result of direct observation or by the analysis of food remains (data not shown). In the last two habitats the owner of the single burrows could not be identified with certainty, so the total number of burrows was recorded. On the contrary on sandy beaches the only vertical burrows are built by the ghost crabs. Therefore, we could calculate also the mass of ghost crabs from the size of their burrows, and estimate the density of their biomass. To this goal, we measured the relationships between burrow diameter and the carapax width at the anterior margin (diameter = 1.0372 width – 1.7811, $R^2 = 0.95$, n = 11), and the relationship between the mass and the carapax width (mass = 0.0003 $(\text{carapax width})^{3.1716}$, $R^2 = 0.99$, n = 40) for a sample of captured crabs. The diameter of 100-400 burrows was measured together with the length of the surveyed beach in order to compare the seasonal density (gr/meter of beach) of ghost crabs on 8 beaches (Dahret, Baradu, Sarad, Durgham, Durghella, Sheikh Said, Gurgussum and Hamasien (Fig.1) both in June-July and in December-January. On Dahret and Sheikh Said islands, we were able to collect more data, albeit more than in one year, and to obtain a profile of crab abundance throughout the seasons. The abundance of intertidal invertebrates in the *Dotilla* zone (Fishelson 1971) on the mud-flat of Gurgussum beach (15°39’29”N, 39°28’06”E) was measured monthly between January and September 2013 in 30 quadrats with the side of 1 m. The quadrats were placed approximately in the same area every month in five rows separated by 10 m. Each row was composed by the six quadrats separated by 5 m. The data of the 30 quadrats were averaged for each month. The seasonal abundance of intertidal invertebrates was measured on the mud-flat of Sheikh Said island (15°35’31”N, 39°28’45”E) monthly between April and September 2004. Twenty quadrats with the side of 1 m were positioned always in the same places thanks to metal sticks as signposts. The quadrats were in three rows at 5 m from the closest quadrats (one area could not be used for the presence of a mangrove tree). In each quadrat the burrows were divided into 3 size categories (less than 0.8 cm in diameter, between 0.8 cm and 2 cm in diameter, larger than 2 cm in diameter). The data of the 20 quadrats were averaged for each month.

As comparable data of seasonal food abundance were not known for other areas of the breeding range, we used chlorophyll-a concentration around colonies and measured correlation with the start of the breeding season. The use of chlorophyll-a concentration measured by remote sensing has already been used as a proximate of marine productivity in studies of the breeding phenology of a tropical seabird (Monticelli et al. 2007, 2014). For coastal species, the use of chlorophyll-a concentration is supported by its high correlation with the biomass for both grazers and filter feeder intertidal invertebrates (Bustamante et al. 1995). We obtained the average annual chlorophyll-a concentrations for the period January 2003-December 2013 from the 4 km resolution data collected by the Moderate Resolution Imaging Spectroradiometer (MODIS) aboard NASA’s Aqua satellite (http://disc.sci.gsfc.nasa.gov/giovanni). For each colony we selected the pixel containing the colony and the 24 surrounding pixels (a squared area of 20*20 km) corresponding to an average radius of approximately 11 km, the main foraging area recorded for the colony of Dahret as only 4 birds out of 17 partially used also area further away during the incubation phase (De Marchi et al. In press). We than excluded the pixels with no data, which correspond to landmasses, and averaged the chlorophyll-a concentration on the remaining pixels. Similar chlorophyll concentrations were obtained using the pixels containing the colony and the 8 closest pixels, corresponding to a radius of approximately 6 km. The data of colonies that are less than 100 km away were pooled in order to avoid spatial correlation.

Risk of predation

In order to measure the risk of predation, we recorded the number of Crab Plovers found partially eaten by raptors and the number of raptors dangerous for the Crab Plovers seen during more than 90 visits to Dahret island and occasionally on the nearby Baradu island. Accurate data on the presence of avian predators throughout the breeding season are not available on a geographical scale. The Palaearctic avian predators, the most common in the breeding range of Crab Plovers (Eriksen et al. 2003, Gregory 2005, De Marchi et al. 2009), are largely absent during the Crab Plover breeding season, when they are breeding in their Palaearctic range, but they can be dangerous at the start (April-May) and at the end of the Crab Plover breeding season . The timing of the risk posed by Palaearctic raptors likely varies depending on the latitude, as their migration is roughly in a north-south direction. If predators are important in determining the breeding season of Crab Plovers, we expect a positive correlation between the start of the breeding season and the latitude during spring in the nine areas (Table 1) as a result of the northward migration of dangerous raptors, or a negative correlation if the southward migration in autumn is more important.

Abundance of Palaearctic shorebirds

We carried out 20 censuses of shorebirds (all species excluding Crab Plovers) at Sheikh Said island, just offshore of Massawa in the period 2002 to 2005 in order to measure the seasonal trend of their presence in the study area. Censuses were carried out on an area of approximately 0.15 km^2 always at low tide using a 20X spotting scope placed on an elevated point, a ruined building (15°35’32”N, 39°28’44”E). In order to get a measure of the possible interference of Palaearctic shorebirds on foraging Crab Plovers, we measured by sight the proportion of Crab Plovers that had a shorebird within a distance of 5 m and correlated it with the abundance of shorebirds in the surveyed area. If interference with Palaearctic shorebirds is critical for the breeding phenology, we expect a correlation between the start of the breeding season and the latitude, as for Palaearctic raptors.

Risk of flooding.

If the risk of flooding could be considered a factor determining the different breeding seasons in different parts of the breeding range, we would expect to find a higher rainfall in April in the areas where Crab Plovers starts breeding in May and a lower rainfall where Crab Plovers starts breeding earlier, in April. Data on average April rainfall at the closest meteorological stations (see Table 1) were downloaded from the web site www.climatemps.com.

Risk of overheating

While a high nest temperature has been shown to be useful for partial solar incubation (De Marchi et al. 2008), the increasing temperature during the breeding season might reach values so high that embryo development might be at risk. Therefore, we reanalyzed the original data collected by De Marchi et al. (2006), who measured burrow temperatures for 4 years, 2003 to 2006, by placing two dataloggers (one at 100 cm and one at 200 cm from the burrow entrance) each year in an abandoned burrow and recording temperatures 6 times a day. These distances from the burrow entrances were chosen as eggs were located at 60-290 cm from the burrow entrance (De Marchi et al. 2008). In particular, we checked whether maximum burrow temperature could have ever been dangerous for embryo development during the incubation phase. In addition, we downloaded the maximum air temperature recorded in June from the web site www.climatemps.com in the nine breeding areas in order to check for correlations with the breeding season, as high temperature in June might force Crab Plovers to start nesting early, in April instead of May. The meteorological stations are the same as for the risk of flooding (Table 1).

Statistics

All statistical tests were two-tailed and were run using the software SPSS 18.0 (SPSS Inc., Chicago). All dispersion measures are Standard Deviations. Non parametric test were used when assumptions for the use of parametric test were not met (Zar 1999).

RESULTS

Nesting season.

The construction of nest borrows started at Dahret island with a similar timing in 6 study years, on the second week of May, with only two nests (30 and 60 cm long but without eggs) discovered as early as on 30 April 2007 (Fig. 2). Egg hatching followed by about 40 days the start of burrowing. The breeding phases for the central 50% of the breeders were: egg laying from 19 to 22 May, incubation from 19 May to 23 June, hatching from 21 June to 24 June and chick growth likely from 22 June to 11 August (Fig.3).

Food abundance.

On the mud-flat of Sheikh Said island there was no significant change in the density of medium (Pearson r = 0.013, n = 6, P = 0.981) and large burrows (Pearson r = 0.365, n = 6, P = 0.476) throughout the 2004 breeding season but there was a significant increase of small burrows (Pearson r = 0.948, n = 5, P = 0.014) of various species with high densities also in September, after the end of the breeding season (Fig.4).

The abundance of invertebrates on the mud-flat of Gurgussum beach in the *Dotilla* zone in 2012 was higher (Mann-Whitney test, $U_{4,7}$ = 2, P = 0.023) during the main breeding season, May-August, than during the non breeding season, September-April (Fig.4).

The abundance of ghost crabs followed a similar seasonal trend with a peak in spring-early summer in two different beaches (Fig. 4), on Dahret island, where Crab Plovers were present only during the breeding season (several hundred compared to 0-2), and on Sheikh Said island, where there were more Crab Plovers (t-test, $t_{18} = 3.059$, $P = 0.007$), during the non-breeding season, September-April (24.5 ± 7.6, $n = 11$ censuses), than during the breeding season, May-August (13.8 ± 8.0, $n = 9$ censuses). The seasonal decline was apparently delayed by about one month on Sheikh Said island (Fig. 4) compared to Dahret island. More generally, the abundance of ghost crabs was constantly lower (Wilcoxon exact test, $Z = -2.521$, $n = 8$, $P = 0.008$) in December-January than in June-July in 2011-2013 in eight well separated beaches (Dahret, Baradu, Gurgussum, Hamasien, Sheikh Said, Sarad, Durgham and Durghella, Fig.1). The ratio between the summer and the winter abundance was 4.1 ± 4.4 with an absolute range between 1 and 19 g/m of beach. Recruitment of ghost crabs took place mainly in June on Dahret island as there were more burrows (Mann-Whitney test, $U_{3,13} = 0$, $P = 0.004$) of the smallest size (5-10 mm) in June (1.50 ± 0.56 burrows/m, $n = 3$) compared to the rest of the year (0.23 ± 0.25, $n = 13$).

Abundance of Palearctic shorebirds

The abundance of wintering Palaearctic shorebirds was minimal in May-June and reached its maximum in autumn-winter on Sheikh Said island (Fig. 9).

There was a significant correlation (test $t_{15} = 3.910$, $P = 0.0014$, $R^2 = 0.505$) between the proportion of Crab Plovers that had a shorebird within 5 m and the logarithm of the number of shorebirds in the mud flat of Sheikh Said island (Fig. 6)

Risk of predation

The most frequently observed dangerous raptors were *Circus* harriers (Marsh Harrier *Circus aeruginosus*, Montagu's Harrier *Circus pygargus* and Pallid Harrier *Circus macrourus*) and the Lanner Falcon *Falco biarmicus* while Black Kites *Milvus migrans* were observed only once. During 90 surveys on Dahret island, many more *Circus* harriers (Mann-Whitney test, $U_{29,61} = -7.477$, $P < 0.001$) were observed on Dahret island during September-April (1.03 ± 0.73 *Circus* per visit, $n = 29$ visits) than during May-August (0.03 ± 0.18 *Circus* per visit, $n = 61$ visits). At least one *Circus* harrier was observed in 79.3% of the visits during September-April and only in 3.3% of the visits during May-August. *Circus* harriers are probably responsible for the killing of two of the last 3 chicks seen on the colony on 9 September 2006 and found dead, partially eaten, on 23 September 2006 (3 harriers were on Dahret on the second date).

Lanner Falcons were observed 4 times on Dahret island during the breeding season of Crab Plovers and they have been observed to prey once on an adult White-cheeked Tern *Sterna repressa* and once on an adult Crab Plover. Lanner Falcons were observed at all seasons on a larger neighbouring island (Baradu island, 5 km north east of Dahret island) throughout the study years, which suggests that the species was resident. Altogether, we found 6 adult Crab Plovers killed on the surface of the colony of Dahret island: five during the breeding season, between 2003 and 2009; one was found during the winter, but could have been killed during the breeding season as we were absent from the islands from before the end of the breeding season up to the visit when it was found.

Nest thermal conditions

The incubation of the 50% central breeding Crab Plovers took place when maximum burrow temperatures rarely exceeded 36 °C even at only 100 cm, a relative short distance, from the burrow entrance (Fig. 7).

Risk of flooding.

Nesting burrows of Crab Plovers were usually heavily damaged by the winter rains at Dahret island, but we never observed rainfall during 90 two or more day long visits during 11 breeding seasons (2003-2013). Fig. 8 shows that throughout the breeding range of Crab Plovers rainfall mostly decreases from April to May. It is higher in April in the northern Persian Gulf than in the south where breeding starts later. Rainfall is almost absent from the breeding area of Crab Plovers during the rest of the breeding season apart from the colony on Masirah island.

Geographical correlations

The start of the breeding season varies throughout the breeding range up to one month (Table 1). In the northern part of the Persian Gulf (Bubiyan island in Kuwait, Dara island and Mond islands in Iran), burrow digging starts in April, at least 3-4 weeks in advance of the other places. Burrow digging likely starts in April also on Shagaf island, off Masirah island, in Oman, as young were seen in May (Table 1).

There is no significant correlation between the start of the breeding season and either the maximum temperature in June (Spearman r = -0.169, n = 9, P = 0.664) or the rainfall in April (Spearman r = -0.231, n = 9, P = 0.549) or the latitude (Spearman r = -0.459, n = 9, P = 0.214), while there is a highly significant negative correlation with chlorophyll-a concentration in a radius of 11 km around the colonies (Spearman r = -0.853, n = 9, P = 0.003).

DISCUSSION

This is the first study that tries to correlate environmental factors with timing of breeding of the Crab Plover.

Our field data for Eritrea show that the summer breeding season offers a full set of advantageous environmental conditions: higher food abundance, lower risk of foraging interference by Palaearctic shorebirds, lower risk of predation by Palaearctic raptors, almost perfect thermal conditions for solar incubation, very low rainfall with minimal risk of flooding of the burrows. All these factors seem to largely overcome the risk of overheating due to the need to commute between the colony and the foraging areas when air temperature and humidity can be physically stressing (Hockey and Aspinall 1997). However, our study suggests that only food abundance is critical. This result stems from the following considerations based on field data from Eritrea and on correlation between with the start of the breeding season and some environmental factors on a geographical scale.

Solar incubation

Data shown in Fig. 7 are partly at odd with the hypothesis that the breeding season is timed in order to better exploit solar incubation. Indeed, maximum burrow temperatures for peak breeding Crab Plovers were well below the temperatures that may endanger avian egg development (Webb 1987), suggesting that Crab Plovers could have exploited even more favourable burrow temperatures for solar incubation if they had delayed incubation by one month (Fig. 7). A proof that the burrow temperatures were slightly suboptimal for solar incubation comes from the observation that Crab Plover eggs were incubated about 54% of the time and warmed on average about 1.7 °C, with incubation taking place also during the afternoon, the hottest hours for the burrows (De Marchi et al. In press). These observations show that eggs never faced dangerous temperatures, contrary to what happens to the eggs of various species of terns and plovers that breed on the ground in exposed places and whose parents are forced to wet their bellies in order to cool down the eggs during the hottest hours (Grant 1982, Amat and Masero 2007, personal observations). The lower burrow temperature experienced by early breeders may be a disadvantage as longer incubation spells are needed in order to reach the same average egg temperature, a pattern supported by a previous study that showed a higher incubation constancy in early breeders (De Marchi et al. 2008).

Rainfall

The hypothesis that the breeding season is timed so as to avoid flooding risk is not supported by the lack of any significant correlation between the rainfall in April and the start of the breeding season. In particular, the data in Table 1 show that rainfall is higher in northern Persian Gulf in April than in the south, where there is a delay of the breeding season. Altogether, rainfall is generally minimal along the breeding season and throughout the breeding range. Only the coast of Oman can be at risk as it can be battered by tropical cyclones that form in the Arabian sea. These cyclones are more frequent in May and June, a third of them occurring between 18 May and 14 June, while there are almost no cyclones in July, August and September (Membery 2012). Not much is unfortunately known on the breeding season on Shagaf island, off Masirah island, the only known colony of Crab Plovers in the Arabian sea, but the observations of young there in May (Rogers 1988) counter the hypothesis of a big delay in the breeding season, which could be useful in order to avoid the cyclone season. On the opposite, Crab Plovers of Shagaf island appear to start breeding earlier than in most of the breeding range (Table 1).

Predators and competitors

Dangerous resident raptors, like Lanner Falcons, are present on some of the Dahlak islands (De Marchi et al. 2009) but their year-round presence rules out their possible role as critical for constraining the breeding season of Crab Plovers. The breeding area of Crab Plovers is flooded by Sooty Falcons *Falco concolor* as summer breeding visitors, but these falcons prey on smaller species than Crab Plovers, up to the size of Bridled Terns (Jennings 2010). The lack of response of Crab Plovers to the presence of Sooty Falcons (personal observation) is an additional hint that they do not influence the breeding biology of the Crab Plovers. *Circus* harriers are likely the most dangerous Palaearctic raptors in the breeding range of Crab Plovers as they are frequently present during winter on Dahret island (see Results) and at other breeding colonies (Aspinall 2010). However, most of them disappear at the start of the breeding season of Crab Plovers at Dahret island, where we observed only two harriers in more than 60 visits during the breeding season. Their disappearance at the Dahlak islands during the breeding season of Crab Plovers is largely in accordance with observations for other parts of the Crab Plover breeding range (Eriksen et al. 2003, Gregory 2005, Ash and Atkins 2009). When *Circus* harriers are back at the beginning of September, they can prey on late chicks (we recorded two chicks being killed at Dahret island likely by *Circus* harriers at the beginning of September 2006). Chicks risk to be captured particularly during the fresher daylight hours, when they regularly stay at the burrow entrance or outside the burrows waiting for the parents to return from the foraging ground and exercising their flight muscles (personal observations). The presence of helpers at the nests that work as lookouts (Aspinall 2010, personal observations) might help to counter predation, but chicks are sometimes completely alone (personal observations). The risk increases at the end of the breeding season, because of the reduced number of occupied nests and consequently of adults visiting the colony. Palaearctic shorebirds as well are nearly absent from the southern Red Sea at the beginning of the Crab Plover breeding season, while they steadily increase in number already in July-August (Fig.5). In other areas within the Crab Plover breeding range, the number of Palaearctic shorebirds starts to rise significantly only from August (Safriel 1968, Eriksen et al. 2003).

Considering the risk for late chicks to be captured by *Circus* harriers and the schedule of presence of shorebirds, it looks like that the southward migration can be more important than the northward migration. However, if either the northward or the southward migration could be considered critical for the breeding phenology of the Crab Plovers, we should find a negative correlation between the latitude and the breeding season, considering that the migration throughout the latitudinal range of the breeding area may take weeks. For example, the average speed of spring migration of Marsh Harriers was measured at 161 km/day (Strandberg et al. 2008), which means that these raptors need almost two weeks to surpass the full length of the Red Sea. The correlation between the start of the breeding season and the latitude is negative suggesting that the influential migration could be the autumn southward migration. However, the correlation is not significant ($P = 0.358$).

Food abundance

We found that various preys exploited by breeding Crab Plovers had slightly different seasonal peaks, which however were all largely coincident with the breeding season of Crab Plovers (Fig. 4). On sandy beaches, the biomass of ghost crabs was everywhere lower in winter than in summer. The spring increase, well recorded at Dahret and Sheikh Said islands, was likely favoured by the stranding of large brown macroalgae, mainly *Sargassum* and *Turbinaria* (Ateweberhan et al. 2009) which provide large amounts of additional food as detritus to the otherwise poor supra- and sub-littoral zone (Sheppard et al. 1992). These macroalgae are almost absent in the northern Red Sea, while they become abundant in the southern part (Sheppard et al. 1992), a difference that may explain the higher density of ghost crabs observed in the south (Fishelson 1971). The sharp increase of the smallest holes (up to 10 mm) of ghost crabs in June, probably marking the peak of recruitment, accords with the April to August recruitment recorded for the same species in the central Red Sea (Al-Solamy and Hussein 2012). Food was more abundant during summer also in intertidal areas. In a mud-flat dominated by *Uca* crabs and callianassid mud-shrimps the density of small burrows (less than 0.8 mm) increased from April to July-September (Fig. 4). Similarly, the density of burrows of invertebrates in an intertidal area dominated by the small *Dotilla* crabs increased sharply during spring, and remained high throughout the Crab Plover breeding season. Altogether, the food abundance increased during summer to 3-4 times the level in winter on all the three surveyed habitats (Fig. 4). Zwarts (1990) similarly reported an increase in availability of crabs during spring in another tropical area of Africa at roughly the same latitude, the Banc d’Arguin in Mauritania, an availability that allowed premigration hyperphagia in Whimbrels *Numenius phaeopus*.

We compensated the lack of field data on food availability throughout the breeding range of Crab Plovers by relying on an indirect measures, the concentration of chlorophyll-a in the sea surrounding the colonies. We found a strong negative correlation between the average chlorophyll-a concentration during 11 years and the start of the breeding season in nine well separated areas. The three areas where Crab Plovers bred earlier (the extreme north-west of Persian Gulf, the Mond islands and Shagaf island) are surrounded by seas with high or very high average annual chlorophyll-a concentration. The first two areas are close to the estuaries of the two major rivers discharging into the Persian Gulf, the Shatt Al-Arab and the Mond Rivers (Abaychi et al. 1988, Nezlin et al. 2007, Al-Yamani 2008). The third area where Crab Plovers likely start breeding early, Shagaf Island, off Masirah Island in the Arabian Sea of Oman, lays in a hotspot of chlorophyll-a concentration, particularly in summer, due to wind driven upwelling of deep, cold, nutrient rich water (Sheppard 1992).

In summary, it looks likely that the summer breeding season has been selected in order to exploit the availability of food, as hypothesized for this species by Hockey and Aspinall (1997). The observation that the peak phase of burrow starting, and of egg hatching, last only four days for the 50% peak breeders, suggests that the best time span for nesting season is short. Our data on food availability do not allow to distinguish whether the stage of egg formation or that of chick feeding is more critical for the timing of the breeding season. At first sight, judging from the density of burrows in the mudflats, late nesters do not seem to be at a disadvantage in comparison to early and to peak nesters (Fig. 4). The quite long peaks of food abundance might even offer a chance to Crab Plovers to renest if eggs are lost to human predators, a possibility suspected in two recent studies (De Marchi et al. 2006, Tayefeh et al. 2013). However, late nesters and renesters could suffer from several disadvantages: 1) they may face interference from a growing number of returning Palaearctic shorebirds (Fig. 5) so that their foraging efficiency may be decreased (Sutherland and Koene, 1982; Ens and Goss-Custard 1984); 2) they may face an increased risk of predation by *Circus* harriers (see Results); 3) they might have to forage on areas close to the colonies that have already been exploited by a large number of conspecifics due to coloniality. We underline that our data on food abundance in intertidal areas are for two areas, Sheikh Said island and Gurgussum beach, that are too far from any colony to be regularly used by breeding Crab Plovers (De Marchi et al. 2006, De Marchi et al. In press), so these areas are likely not overexploited during the summer. On the contrary, the foraging areas close to the colonies might undergo a decreasing food abundance during the breeding season, forming the so called Ashmole's halo (Gaston et al. 2007) as suggested for the Crab Plovers by Hockey and Aspinall (1997). This phenomenon can force late breeders to spend more energy in longer commuting flights between the foraging areas and the colony. This interpretation is in accordance with our observation of an earlier decrease in abundance of ghost crabs on Dahret island, which holds a colony of Crab Plovers, compared to Sheikh Said island. The formation of an Ashmole's halo is also in accordance with the unusually low body mass of the Crab Plover fledglings compared to the adults (only 55% on average of the adult mass) measured at a colony in the northern Persian Gulf (Tayefeh et al. 2013). The most likely explanation of the low mass of the fledglings is that they have to leave the colony as soon as possible in order to reduce the energy spent in flight energy by their parents (Tayefeh et al. 2013) that can only bring a single food item in their beak back to the colony.

The expected advantage of early breeding leaves open the question of why not all pairs start breeding at the same, optimal time. The likely explanation, generally assumed for other avian species, is that females vary individually and differ in their ability to find enough food to produce their eggs when food availability starts improving in spring but it is not yet at its highest (Verhulst and Nilsson 2008).

Conclusions

Lower rainfall, lower concentration of raptors and shorebirds and suitable nest temperature certainly favour Crab Plovers during their summer breeding season, but cannot specifically explain the variability of their breeding phenology. The presence of Palaearctic raptors and shorebirds at the end of the breeding season certainly favours an early as possible start of the breeding but only food abundance, under the constraints imposed by colonial life, resulted critical to explain the breeding season of Crab Plovers. As a result, the following scenario can be proposed, largely in support of the hypotheses of Hockey and Aspinall (1997). Crab Plovers cannot breed during the winter when food is less abundant and colder water temperature, higher rainfall and locally cold winds (Sheppard 1992) would reduce the availability of crabs that would tend to hide inside their burrows (personal observations). When foraging conditions improve in spring, females that either wintered in the area or arrived from the wintering grounds start producing their eggs as soon as possible, because late nesters would face a food depletion due to intraspecific competition during chick-rearing. However, females manage to lay their single but huge egg in April only in regions where marine productivity is very high, while in the less productive areas they need to wait until May. This conclusion supports the idea that food availability is the main determinant of the bird breeding season of many birds not only in temperate areas, but also in tropical areas (Poulin et al. 1992, Jaquemet et al. 2007).

Future studies should try to substantiate the food depletion that occurs around colonies along the breeding season, to test the importance of food abundance on a larger sample of colonies (including for example data from Sudan and Yemen), and to distinguish whether food abundance is more critical during the egg-laying or during the chick rearing phase.

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Table 1. The start of the burrowing activity and environmental variables, in different parts of the Crab Plover breeding range

ISLAND ARCHIPELAGO COUNTRY	COORD IN.	START OF DIGGING, PERIOD STARTING FROM 1 APRIL. REFERENCE	CHLOROPH. (mg/m3)	RAINFALL APRIL (mm) (meteor. station)	MAX. AIR TEMP. (°C) IN JUNE (meteor. stat.)
Marbat Al Wajh Bank North Red Sea Saudi Arabia	25.88N, , 36.603E	Middle of May? 9 (Almalki et al. 2014)	0.664 (Jeddah)	5 (Jeddah)	41
Dahret and Sarad Dahlak Islands South Red Sea Eritrea	15.904N 39,578E and 15.82N 39.907E	Second week of May 8 (This paper)	2.518	4 (Massawa)	40 Massawa)
Saacada Diin Gulf of Aden Somalia	11.433N 43.466E	Middle of May 9 (Archer, Godman 1937)	1.446	29 (Djibouti)	38 (Djibouti)
Bubiyan and Dara Northern Persian Gulf Kuwait and Iran 2003,	29.935N 48.685E and 30.101N	Middle of April? 4 (Al-Nashrallah, Gregory Tayefeh, pers. observ.)	3.817	15 (Kuwait City)	44 (Kuwait City) 49.109E
Nakhilu and Omol-Karam Mond Islands Northern Persian Gulf Iran	27.821N 51.473E and 27.834N 51.564E	About 20 April 5 (Tayefeh et al. 2013)	2.672	9 (Boushehr)	37 (Boushehr)
Abu el Abyad Southern Persian Gulf Abu Dhabi	24.211N 53.807E	From 10 May 9 (Aspinall 2010)	2.665	9 (Doha)	41 (Doha)
Shaghaf Arabian Sea Oman	20.453N 58.746E	April? young in May 4 (Rogers 1988)	6.674	10 (Masirah)	35 (Masirah)
Mandhar and Humr Farasan islands Southern Red Sea Saudi Arabia	16.962N 41.802E and 16.781N 42.011E	Middle of May 9 (Almalki et al. 2014)	2.303	18 (Jizan)	38 (Jizan)
Umm al Quronatain Al Batain Central Red Sea Saudi Arabia	19.266N 40.977E	Middle of May 9 (Almalki et al. 2014)	1.063	18 (Jizan)	38 (Jizan)

Fig. 1. The study sites around the coastal town of Massawa and in the Dahlak archipelago, central Eritrea.

Fig. 2. Number of nests at the beginning of the breeding season (Late April-early May) on Dahret island in 7 different years.

Fig 3. Cumulative proportion of started nests and of hatched eggs measured at Dahret island in 2005.

Fig. 4. Seasonal abundance of invertebrates in central Eritrean coastal habitats. Top = density of burrows of various species (mainly *Uca* crabs and *Callianassa* mud-shrimps) at Sheikh Said island in 2004. Center = density of burrows of various species (mainly *Dotilla* crabs) at Gurgussum in 2013. Bottom = density of ghost crabs *Ocypode saratan* on Dahret island and Sheikh Said island in various years. The lines are 3rd order polynomial interpolations.

Fig. 5. Abundance of Palaearctic shorebirds at low tide on a mudflat of approximately 0.15 km^2 around Sheikh Said island in the period 2002-2005.

Fig. 6. Correlation between the proportion of Crab Plovers that had a shorebird within 5 m and the abundance of shorebirds on Sheikh Said island.

Fig. 7. Temperatures (maximum, average, and minimum with SD) measured by 8 dataloggers inside the 4 nest burrows at 100-200 cm, averaged for each 10-11 day span, 2003 to 2006. Shading marks the incubation period (19 May-23 June) for the central 50% of the nests .

Fig. 8. Average monthly rainfall during the breeding season throughout the Crab Plover breeding range downloaded from www.climatemps.org for Jeddah (Northern Red Sea), Jizan and Massawa (Southern Red Sea), Djibouti (Gulf of Aden), Masirah (Arabian Sea), Dubai (Southern Persian Gulf), Kuwait City and Bushehr (Northern Persian Gulf).

Fig. 9. The 2003-2013 surface chlorophyll-a concentration in the breeding range of Crab Plovers and location of 10 Crab Plover colonies or group of colonies, whose breeding phenology is known (Downloaded at http://disc.sci.gsfc.nasa.gov/giovanni).

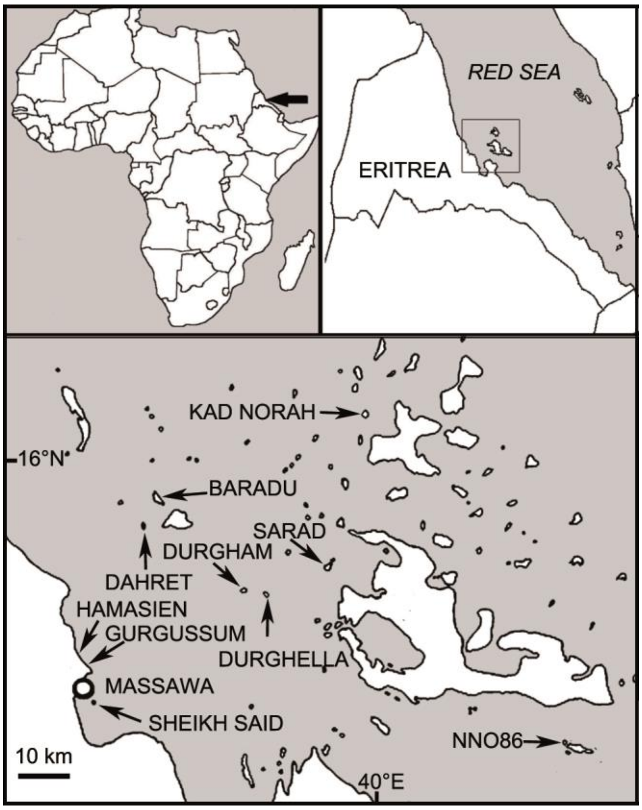
RED SEA
ERITREA
KAD NORAH
16°N
BARADU
SARAD
DURGHAM
DAHRET
HAMASIEN
GURGUSSUM
DURGHELLA
MASSAWA
SHEIKH SAID
NNO86
10 km
40°E

Fig. 1.

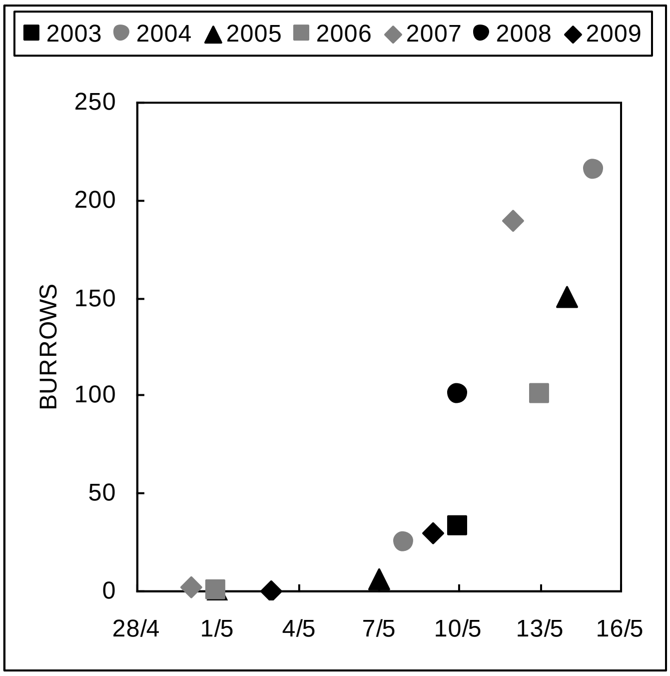
■ 2003 ● 2004 ▲ 2005 ■ 2006 ◆ 2007 ● 2008 ◆ 2009
BURROWS
250
200
150
100
50
0
28/4
1/5
4/5
7/5
10/5
13/5
16/5

Fig. 2.

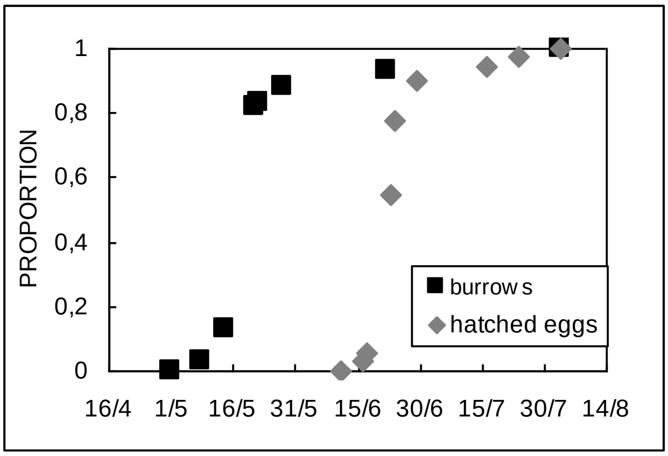
PROPORTION
1
0,8
0,6
0,4
0,2
0
16/4
1/5
16/5
31/5
15/6
30/6
15/7
30/7
14/8
burrows
hatched eggs

Fig 3.

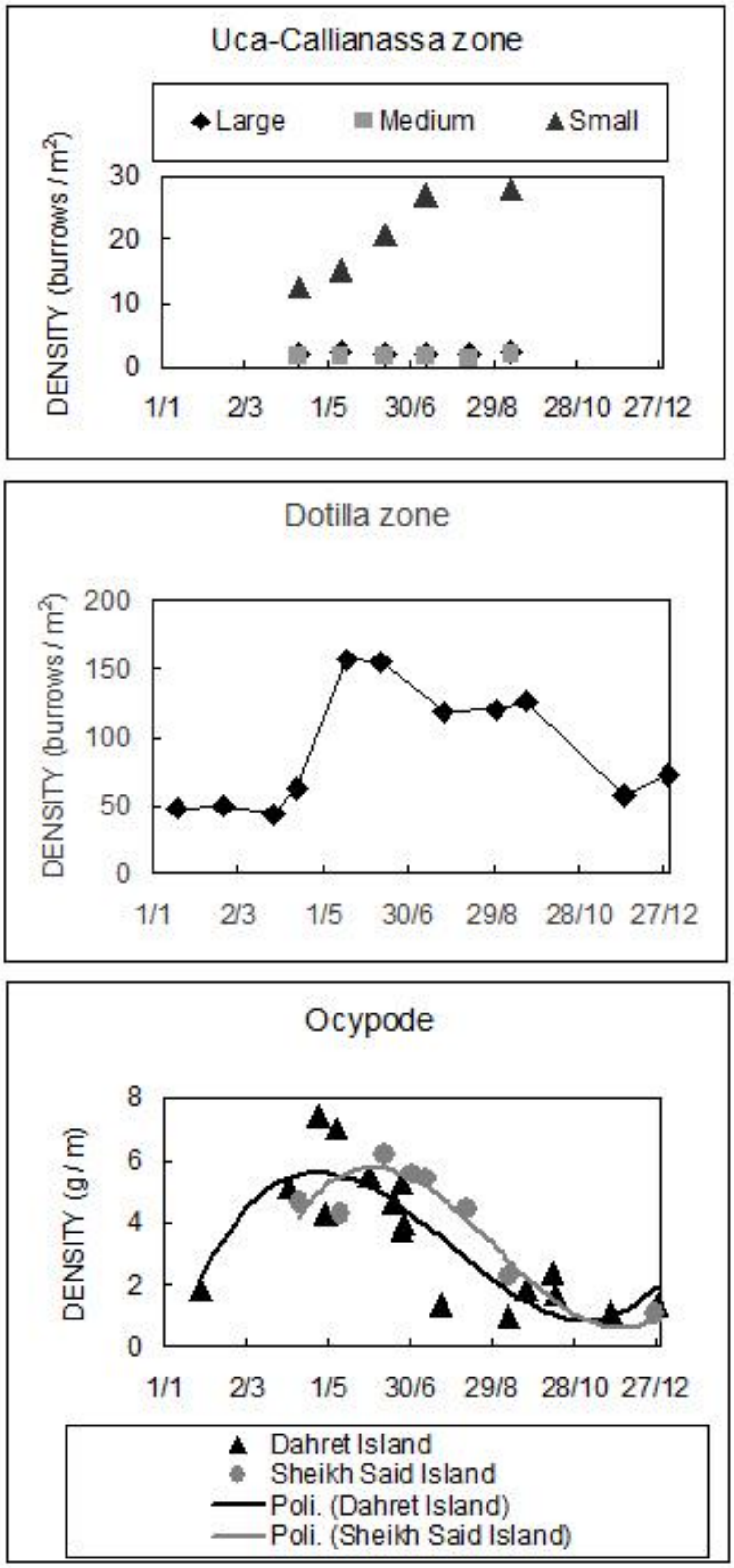
Uca-Callianassa zone
Large
Medium
Small
DENSITY (burrows / m²)
30
20
10
0
1/1
2/3
1/5
30/6
29/8
28/10
27/12
Dotilla zone
DENSITY (burrows / m²)
200
150
100
50
0
1/1
2/3
1/5
30/6
29/8
28/10
27/12
Ocypode
DENSITY (g / m)
8
6
4
2
0
1/1
2/3
1/5
30/6
29/8
28/10
27/12
Dahret Island
Sheikh Said Island
Poli. (Dahret Island)
Poli. (Sheikh Said Island)

Fig. 4.

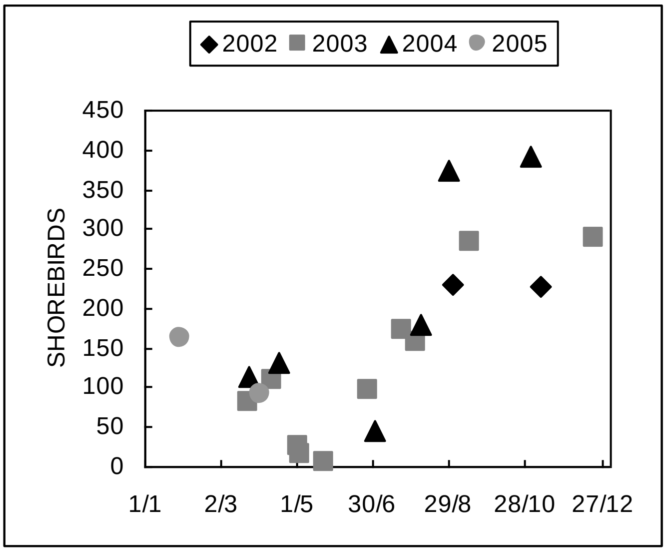
◆ 2002 ■ 2003 ▲ 2004 ● 2005
SHOREBIRDS
450
400
350
300
250
200
150
100
50
0
1/1
2/3
1/5
30/6
29/8
28/10
27/12

Fig. 5.

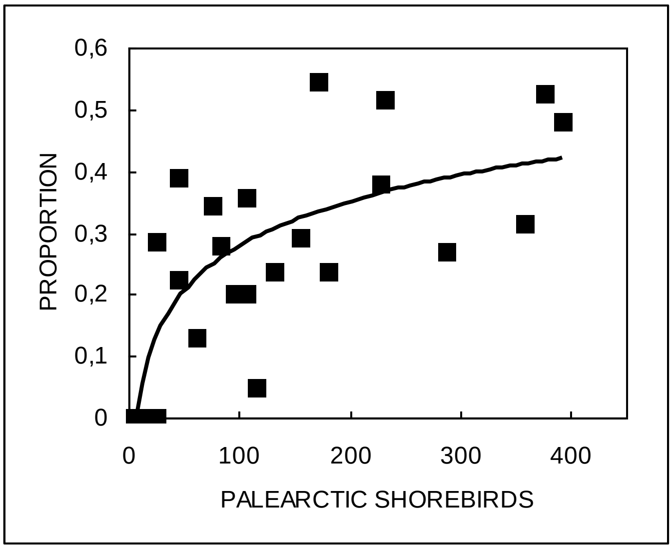
PROPORTION
0,6
0,5
0,4
0,3
0,2
0,1
0
0
100
200
300
400
PALEARCTIC SHOREBIRDS

Fig. 6.

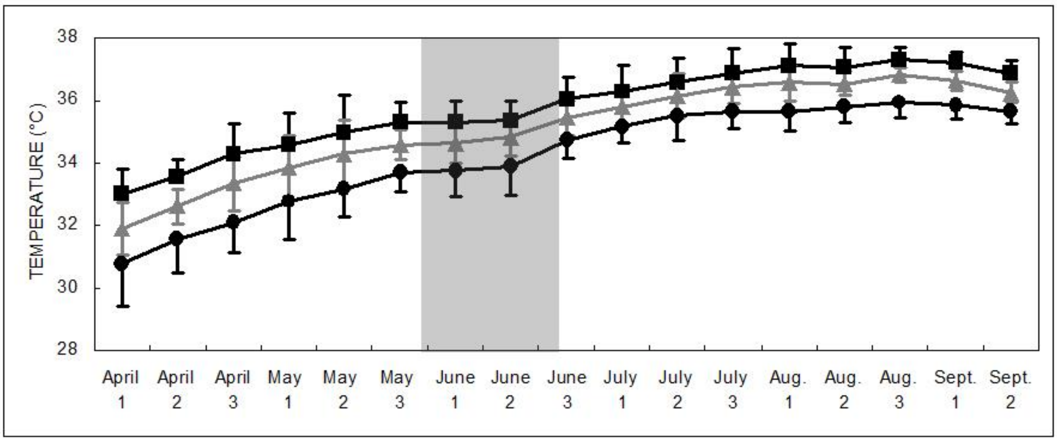
TEMPERATURE (°C)
38
36
34
32
30
28
April 1
April 2
April 3
May 1
May 2
May 3
June 1
June 2
June 3
July 1
July 2
July 3
Aug. 1
Aug. 2
Aug. 3
Sept. 1
Sept. 2

Fig. 7.

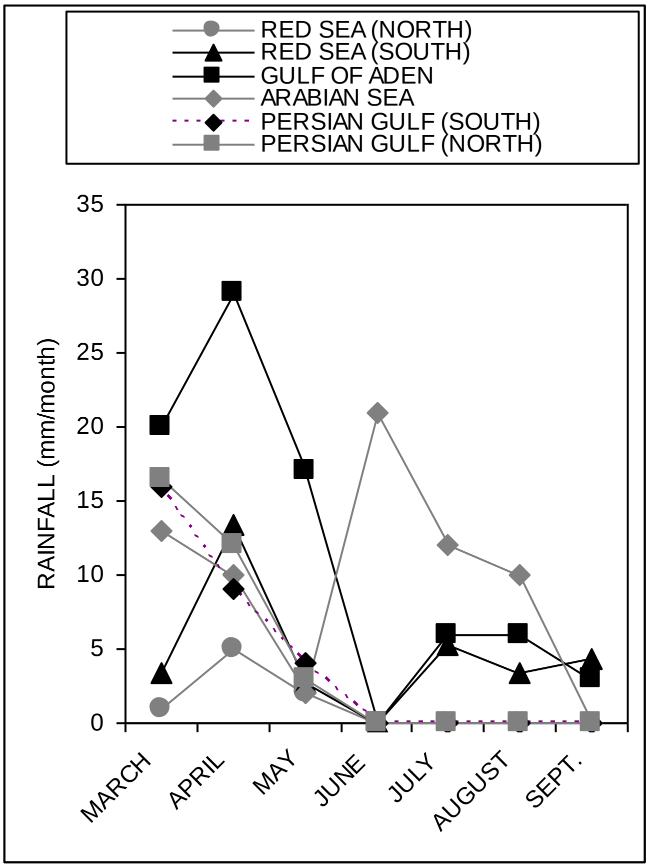
RED SEA (NORTH)
RED SEA (SOUTH)
GULF OF ADEN
ARABIAN SEA
PERSIAN GULF (SOUTH)
PERSIAN GULF (NORTH)
RAINFALL (mm/month)
35
30
25
20
15
10
5
0
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPT.

Fig. 8.

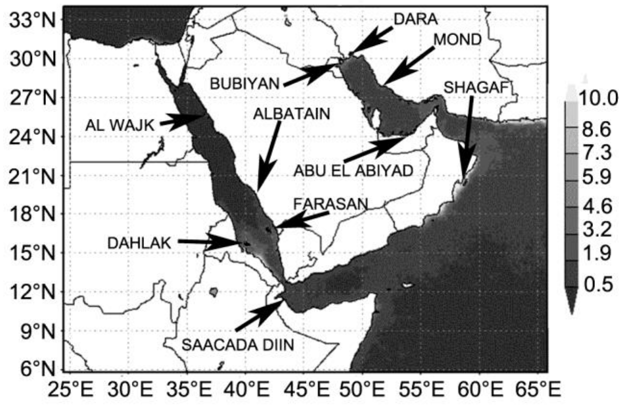
33°N
30°N
27°N
24°N
21°N
18°N
15°N
12°N
9°N
6°N
25°E
30°E
35°E
40°E
45°E
50°E
55°E
60°E
65°E
DARA
MOND
BUBIYAN
SHAGAF
AL WAJK
ALBATAIN
ABU EL ABIYAD
FARASAN
DAHLAK
SAACADA DIIN
10.0
8.6
7.3
5.9
4.6
3.2
1.9
0.5

Fig. 9.

Appendix 2

Breeding ecology and conservation of Saunders's tern *Sterna saundersi*, and Kentish plover *Charadrius alexandrinus* in Farasan Islands in Saudi Arabia

Fieldwork report 2013

Mohammed Almalki

28th of June 2013

Summary

Waterbirds are an important indicator of ecosystem health in many natural systems in the world. I investigated the Saunders’s terns and Kentish plovers in the Farasan Islands from the 5th of March to the 4th of June 2013. I used the funnel traps on the nests to capture both species, and I measured and marked the captured birds with individually coloured rings. I recorded the incubation rate of the ringed Saunders’s Tern for an uncompleted 24 hours at 10 nests using automatic cameras. In addition, iButton dataloggers were planted inside and outside preyed upon nests to measure the temperatures inside and outside the nests. I found that the temperature inside the nests during the hottest time in the day was higher than the temperature outside the nests of Saunders’s tern.

The Saunders’s tern faces serious threats from predation and human disturbance in Farasan Islands. Nest predation of the Saunders's tern in Farasan Islands occurred at a high rate of 64.9%, whereas only 14.8% of clutches produced chicks. In addition, the two main predators of the Saunders’s tern eggs were the Mongoose and the Egyptian vulture.

Kentish plover blood samples were calculated from six sites along the Red Sea coast of Saudi Arabia.

Introduction

Saunders’s tern is a seabird that belongs to the *Sternidae* family. It has a massive breeding range, extending from the Red Sea coast and Arabian Gulf to northwest India, Sri Lanka, and the Maldives (BirdLife International 2013). This species inhabits an array of coastal regions, including shallow tropical and subtropical inshore waters, estuaries, tidal lagoons, and harbours (Del Hoyo et al. 1996, Snow & Perrins 1998). It nests on the ground up to 2 km inland in exposed sand, shingle, or dried mud, and it nests in individual pairs or small colonies of 5-30 pairs (Del Hoyo et al. 1996, Snow & Perrins 1998). The Saunders's tern's breeding season occurs between February and late April (Newton 2006). The female of this species lays two pale eggs (Del Hoyo et al. 1996). This tern is currently classified as a Least Concern by the IUCN Red List (www.iucn.org, accessed in June 2013). However, the population trend is declining (Wetlands International 2006) due to predation by rats and cats in some areas, human disturbance, and habitat loss due to development (del Hoyo et al. 1996).

The Kentish Plover *Charadrius alexandrinus* is a wader that belongs to the *Charadriidae* family. It is has a massive geographic distribution through Europe, Asia and Northern Africa (del Hoyo *et al.* 1996; Wetlands International 2006; Delany *et al.* 2009). The populations of Kentish Plover are suffering from a decline in their range. There are several factors leading to the decrease of Kentish plover populations, for instance, degradation and loss of coastal habitats, environmental pollution, land reclamation and human activities (del Hoyo *et al.* 1996; Barter 2006; Kelin & Qiang 2006). The breeding system of Kentish plovers is distinguishing (Székely *et al.* 2006). Several patterns of breeding systems occur in within a single population: polygyny, polyandry and monogamy (Lessells 1984; Székely *et al.* 2006). The eggs are incubated by both males and females (Fraga & Amat 1996). Furthermore, after the egg hatches, one parent—usually the male—provides the care for the chicks whereas the female may desert the offspring (Amat *et al.* 2008).

Objectives

The objectives of this study were to:

1. collect data on the basic demography and behaviour of the Saunders’s tern;
2. quantify the incubation rate of Saunders’s Tern;
3. determine the predation rate and the main predators of the Saunders’ tern eggs;
4. collect data on mate and site fidelity of the Kentish plovers in Farasan Islands;
5. collect some blood samples from the Kentish plovers and Saunders’s tern in many sites along the Red Sea coast of the Saudi Arabia.

Finding colonies, ringing adults and collecting blood samples from the Saunders’s tern and the Kentish plover are required to achieve these goals.

Methods

Study Area

Fieldwork was carried out between the 5th of March 2013 and 4th of June. I visited many sites along the Red Sea coast such as Al Sarum south of Jeddah city, Al Qunfudah city, Jazan city and Farasan Islands, and several sites along the Arabian Gulf of Saudi Arabia such as Sabkhat Al Fasl and Ras Abu-Ali Island in Al Jubail city. The aim of these visits was to find colonies of the Saunders's tern *Sterna saundersi* (Table 1). After finding these colonies, the Farasan Islands were chosen as a suitable study area for collecting detailed data, because it contains more Saunders’s tern nests than the other sites.

I visited several sites along the Red Sea coast of Saudi Arabia between the 16th of April and the 26th of May 2013 to collect data on the Kentish plovers. These sites included Al Sarum south of Jeddah city, Al Qunfudah city, Jazan city, Farasan Islands, Umlug city and Alwajh city. In addition, I visited several islands in Umlug and Al wajh archipelago using boats to catch and collect some blood samples from the Kentish plovers *Charadrius alexandrines* (Fig. 1).

Fieldwork Methods

The Saunders's tern build nests sparsely up to 1 km away from the coast in exposed sites, whereas, the Kentish plover build nests sparsely up to 1 km away from the coast in either exposed or unexposed under bush sites. Two methods were used to discover nests: 1) by driving a car at slow speed and flushing the incubating birds; or 2) by following parents when they came back from the shoreline to their nests. For each nest, I recorded the time, the date, the clutch size, and the measurements of the eggs as well as the geographic coordinates using a GPS device. To identify a nesting site, I used a straw, placed 10 m from the nest. The majority of nests were visited every 2-5 days. Therefore, the fates of the nests were recorded using following of these criteria: (1) “hatched” when one or more eggs hatched; (2) “predated” when the eggs were eaten; (3) “abandoned” when no adults were observed at the nest, or there were no Saunders’s Tern footprints around the nest; or (4) “unknown” when the fate of the nest was not followed or the eggs disappeared and neither predation nor hatching was confirmed (Alrashidi *et al.* 2011).

Parents were captured by funnel traps on the nest or funnel traps fitted to the chicks (Székely et al. 2008). The chicks were covered with a suitable sieve that accommodated all the young, and the funnel trap was put around the sieve. All captured adults of the Saunders's Tern were ringed with one metal ring provided by the Saudi Wildlife Authority (SWA), and two coloured rings because their legs are very short, whereas, chicks were ringed with one metal ring and one coloured ring. On the other hand, all of the Kentish Plover that were only captured for their blood samples were ringed with only metal rings. However, all of the captured adults or families of the Kentish plover in Farasan Islands that I was attempting to follow using a spotting scope were ringed with metal rings and 1-3 coloured rings. I also recorded the number of chicks and the number of attending parents. All the families with coloured rings were visited every 2-5 days.

For both species, I measured the body mass with a spring balance, the right tarsi and the length of bill only Saunders’s tern with sliding calipers, and the length of the right wing with a ruler. In addition, I took pictures of each nest I found. Blood samples were taken from the brachial vein of the Saunders's Tern and the Kentish plover adults, and the tarsal vein of their chicks for DNA analyses.

Recording Incubation Behaviour of Saunders’s tern

The Reconyx (SC950 HyperFire Security IR) cameras were used to record the nest attendance behaviour at 10 nests (I could not record more nests because the predation risk was high, and because this bird nests in exposed areas which increases their sensitivity to any thing around their nests). The camera was positioned about 1 m from the nest. The Reconyx camera was set up to record an image every five seconds for an uncompleted 24 hours. Because the Saunders's terns nests in exposed areas, they are easily disturbed by anything around their

nests; therefore, it is difficult to set up the camera around their nests. So, to solve this problem, I painted the cameras with a sandy colour. In addition, because the camera was noticeable for the Egyptian vultures, I placed the camera inside a woody box (Fig. 2).

Ambient temperatures of the area around the nests were recorded by iButton dataloggers every minute. Furthermore, iButtons were placed inside ten predated nests to measure the nests temperature.

Results

Saunders's tern

Breeding dynamics

In total 49 nests were found: 40 in the Farasan Islands and nine in Al Sarum south of Jeddah city (Appendix 1). The nest is a hollow in exposed sand; some of these nests are surrounded by marine shells, and the material inside the nest contains small marine shells. Some species place the nest on the flat sandy area while others nest above small mounds surrounded by some coral (Fig. 3). Egg laying season of the Saunders's tern began in March 2013. The eggs had started to hatch by the end March 2013. We did not follow any eggs from laying to hatching because the predation risk was very high.

Each female lays one or two eggs. The eggs are usually a pale cream with brown and grey markings. Considering the Saunders's tern nest in exposed areas, the parents built the nests close to car tracks. I made new car tracks around one nest and I noticed that the parents took around 15 min to find their nest (during this period one bird went to the shoreline and brought their mate back to find the nest location). Usually both parents came to their nest and flew around when I tried to place the funnel trap or set the camera on the nest.

Based on the date that the eggs hatched, the breeding season may start as early as March and the breeding peak between March and April. Furthermore, based on the nesting date records, I think the breeding season may continue into late June.

On the Farasan Islands, the distance between the water and nests was between 50 and about 500 m. Furthermore, the nest is usually placed between 20 and about 100 m apart from the other nests. I noticed that there was fighting between neighbours when the distance between their nests was around 20 m. The older neighbours, which have two eggs, tried to exposed their neighbour's nest by standing and/or flying above their neighbour's nest.

In Al Sarum, I found a small colony that included around 15 nests located around Al Sarum Lake (Fig. 4). I could not get to this island because the area between the island and the mainland is a risky area. The water was not deep at about half a metre and its ground was very muddy. It will pull anything down. On this island, I estimated the distance between the nests and the distance between the water and nests to be around 5 m. On the mainland, the distance between the nests was between 30 and about 100 m apart. In addition, the distance between the nests and water were between 5 and 100 m.

Clutch Size and Hatching

A total of 40 breeding pairs of Saunders's tern was recorded on the Farasan Islands; 24 had two eggs (60%) and 16 had only one egg (40%). An overall, average clutch size of 1.6 (± SD 0.5) eggs per clutch was evaluated.

A total of 18 breeding pairs were recorded in Al Sarum; 17 had one egg (94.4%) and only one had two eggs (5.6%). An overall, average clutch size of 1.1 (± SD 0.33) eggs per clutch was evaluated.

Capture and Measurements

I captured 33 Saunders's tern on the Farasan Islands (28 adults and 5 chicks), and seven Saunders's tern adults in Al Sarum south of Jeddah city. Thirty-nine blood samples were collected (32 from the Farasan Islands and seven from Al Sarum). The average weight, wing length, tarsus length and bill length of captured adults in Farasan Islands 45.58 grams ± SD 4.97, 167.95 mm ± SD 2.72, 17.51 mm ± SD 1.53 and 28.62 mm ± SD 1.3 respectively. The average weight and wing length of captured adults in Al Sarum were 47 grams ± SD 3.87 and 164.3 mm ± SD 11.3 respectively. All data were recorded (Appendix 2).

Parental Behaviour

I setup the cameras in front of 15 nests: 10 in the Farasan Islands and five in Al Sarum, but cameras could not take photos of nest attendance for a completed 24 hours for several reasons. The first reason is that the camera sometimes snapped pictures intermittently not every five seconds, as it was set up; in some cases, the camera failed to take a picture for several hours. The second reason is that several nests were exposed to the predation by mongooses or Egyptian vultures. The third reason is that some nests were abandoned because of the cameras.

Nevertheless, the cameras photos show us some incubation behaviour such as, both parents sharing the incubation duties approximately equal numbers of both parents attended to the nest to incubate the eggs or chicks. In addition, they attend to the nest consecutively. One partner would bring some food to their mate who was incubating the offspring. I recorded adults bringing only one kind of food (small fish) to their chicks or partners (Fig. 5) (Fig. 6). When the chicks hatched, they usually stayed around the nest and their parents incubated them around the nest area. In three families, both parents were captured with their chicks. I did not notice any attempt at nesting again by any ringed bird whose nest was predated in the study area.

Nest Temperature

I measured the temperatures inside and outside ten nests of Saunders's tern. I found that there were some differences between the temperatures inside and outside the nests. The temperatures inside the nests during the hottest time in the day are higher than the temperatures outside the nests. The maximum temperature inside the nests recorded was 61.02 °C whereas the temperature outside the nest was 58 °C. Furthermore, the minimum temperature inside the nests recorded was 24.06 °C whereas the temperature outside the nest was 25.13 °C. Figure 7 plots of how the ambient temperature and the temperature inside nest of Saunders's tern change throughout the day.

Predation

In regards to the Farasan Islands, the fate was known for 59 eggs laid in 37 nests studied, five failed to hatch (Appendix 1). The predation on the Farasan Islands population appears to be high and occurs at a high rate of up to 64.9 % (n=24 nests) whereas the hatching rate was 20% (n=8 nests). Mongoose and Egyptian vultures are the main predators for the Saunders tern clutches and chicks in Farasan Islands. The cameras nest identified a mongoose and an Egyptian vulture (Fig. 8). Their footprints were all around the predated nests. Thirteen nests were predated by Mongooses whereas eight were predated by Egyptian vultures. At the end of the peak of the Saunders's tern breeding season, the numbers of Sooty gulls began to increase. Therefore, I think the Sooty gulls *Larus hemprichii* represent a potential predator of the Saunders's tern eggs and chicks. Although I have no evidence that they predated any chick or egg, I show some Sooty gulls flying around the Saunders tern nests, and standing on the shoreline in groups.

In Al Sarum, only two nests were predated. One predated by a cat as evidenced by their footprints nearby the predated nest. The other nest was predated by an unknown predator that opened the egg and threw it along with its liquids out of the nest (Fig. 9). No footprints were found close to the nest.

Kentish plover

Capture and Parental Behaviour

I captured 55 Kentish Plover on the Farasan Islands, 12 in Jazan, 31 in Al Sarum, 14 in Umluj and 8 in Alwajh city (Appendix 3). On the Farasan Islands, I followed six colour-ringed families (Appendix 4). I noticed both parents with the chicks except in one family where the male was not observed (I saw a Kentish plover male dead on the road having been run over by a car not far from the location of this family, so maybe this male belongs to this family).

For the site and mate fidelity, I caught 11 coloured-rings birds in their nests whereas I caught one of these birds on the shoreline with its family. All of these birds were ringed in the last years (Appendix 3).

Bird Distribution

While visiting some islands in Umluj city and Al Wajh archipelago, I observed some bird species during their breeding seasons (Table 2). For examples, I recorded two colonies of Brown booby Sula leucogaster on Bareem Island in Al wajh archipelago. In addition, I recorded one colony of Crab plover on Al Shack Marbat Island. Furthermore, I recorded a number of bird species such as White-eyed gull Larus leucophthalmus, Sooty gull and Osprey Pandion haliaetus.

Conclusion

The 2013 fieldwork has given some important results. First, the numbers of Saunders’s tern that breeds in the mainland is low. Second, the Saunders's tern population on Farasan Islands suffers from high predation pressure. Third, Mongoose and Egyptian vultures are the main predators for the Saunders tern eggs and chicks in Farasan Islands. Fourth, the temperatures inside the nests of Saunders’s terns during the hottest time in the day are bigger than the temperatures outside the nests. Fifth, in regard to Kentish plover I found that both male and female of Kentish plovers in Farasan Islands care for the chicks. Finally, I recommend that the population of Mongoose on Farasan Islands should be controlled to enhance nest success of ground nesting birds.

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Table 1. *The number of individuals and nests of Saunders's tern on the visited sites.*

Site	Date of visit	Individual bird numbers	Nest numbers
Al Sarum South of Jeddah city	06-03-2013	33	24
Al Qunfudah city	08-03 & 16-04 2013	407	No nests observed
Jazan city	26-05-201	4	2
Farasan Islands	21-03 & 24-04 2013	123	40
Umlug city	13-05-2013	No birds observed	No nests observed
Al Wajh city	18-05-2013	No birds observed	No nests observed
Sabkhat Al-Fasl Lagoons in Al Jubail city	11-05-2013	No birds observed	No nests observed
Ras Abu Ali Island in Al Jubail	13-05-2013	No birds observed	No nests observed

Table 2. *The bird species that observed on the visited Islands in Umluj city and Al Wajh archipelago.*

city	Site	Date of visit	Bird species	Individual bird numbers	Comments
Umluj	Umm Sahar Island	13-05-2013	White-eyed gull	147	
			Sooty gull	85	
	Maliha Island	13-05-2013	White-eyed gull	18	
			Sooty gull	5	
	Ataweel Island	13-05-2013	White-eyed gull	41	
			Sooty gull	19	
			Caspian Tern	3	
	Umm Al Malik Island	13-05-2013	Sooty gull	2	
Al Wajh	Barreem Island	18-05-2013	Sooty falcon	4	
			Osprey	2	
			Grey heron	3	
			Brown booby	39	Two colonies
			Ruddy Turnstone	1	
			Crab Plover	5	
			Kentish Plover	2	
			Sooty gull	11	
			Caspian Tern	12	
	Al Shack Marbat Island	18-05-2013	Osprey	4	
			Crab plover	119	Colony (79 burrows)
			Sooty gull	27	
			Little tern	34	
			Caspian tern	7	
			Greater crested tern	8	
			Sooty tern	30	

			White-cheeked Tern	36	

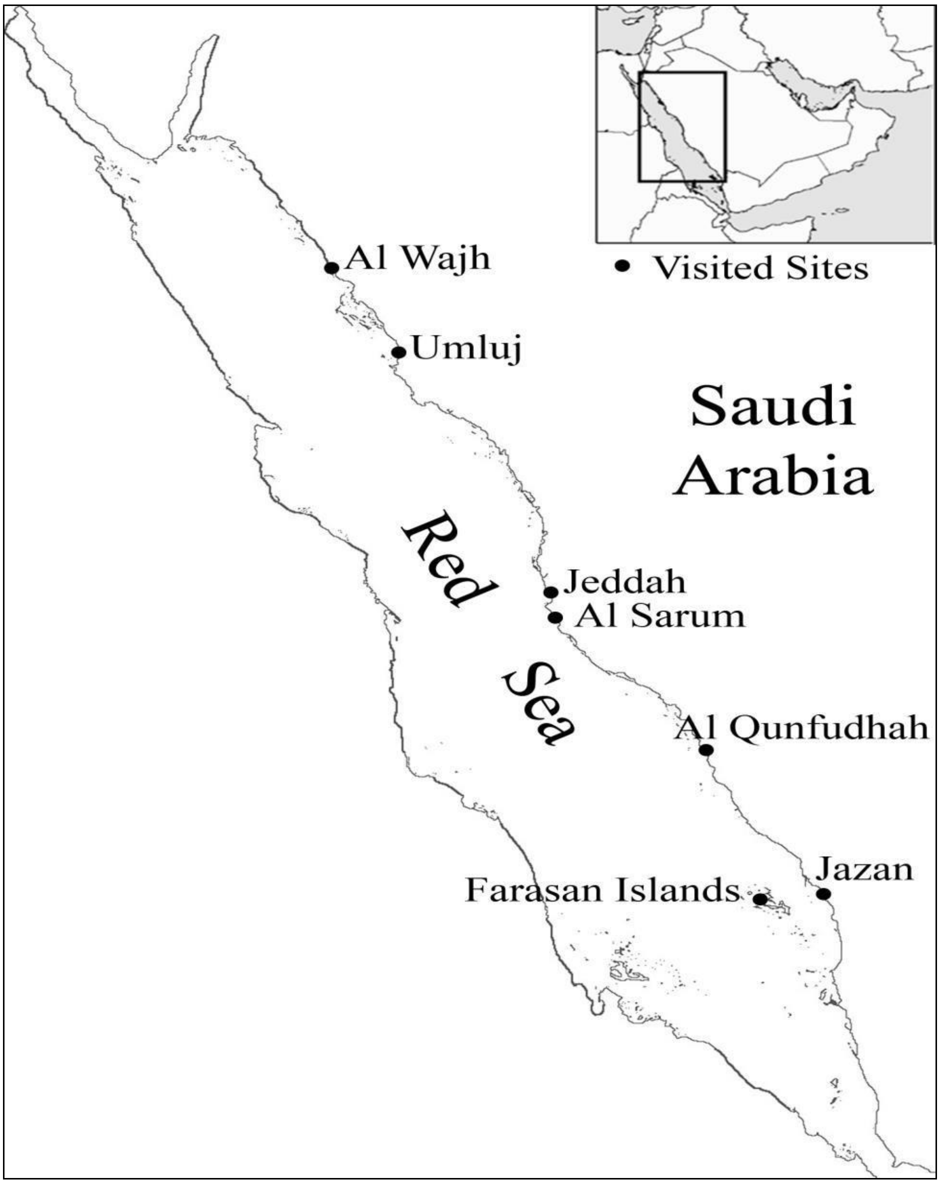

Figure 1: *Map showing the visited area on the Red Sea coast of Saudi Arabia.*

Figure 2: *Painted camera with wooden box.*

Figure 3: *The nest site selection and the nest material of the Saunders's tern.*

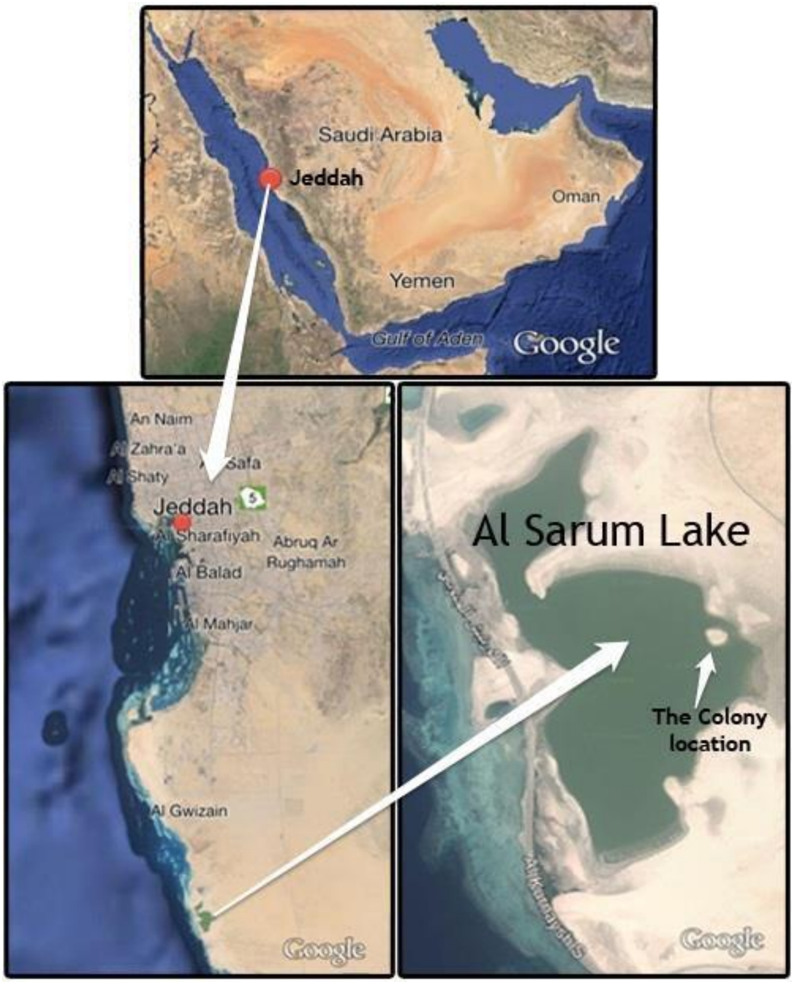

Figure 4: *Map showing Al Sarum Lake and the location of the Saunders's tern colony.*

Figure 5: *Saunders's terns bring food (small fish) to his partner.*

Figure 6: *A small fish collected from the nest of Saunders's tern.*

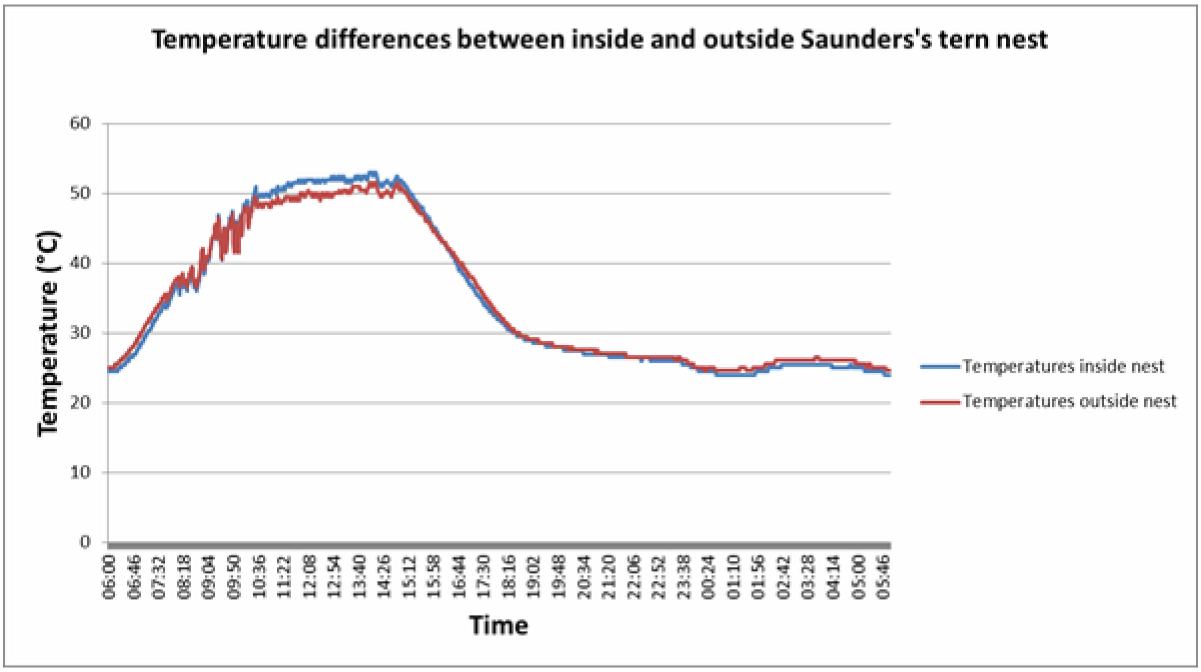

Figure 7: *Showing how the ambient temperature and the temperature inside nest of Saunders's tern change throughout the day.*

Figure 8: *Saunders's tern nests predated by Egyptian vulture and Mongoose.*

Figure 9*: Nest predated by an unknown predator that opened the egg and threw it along with its liquids out of the nest.*

Appendix 1. *Nest records of Saunders's tern nests in Farasan Islands and Al Sarum.*

PRED: Predated HAT: Hatched

Year	Site	Nest ID Latitude	Longitude	Found date	Laying date	End date Fate	Fat	No. chicks	Clutch size	L1	B1	L2	B2	Comments
2013	Farasan Islands	99796	36894	322		401	PRED	0	2	32	23	31.5	23	Mongoose
2013	Farasan Islands	95949	37930	321		402	PRED	0	2	30.5	22	30	22.5	Mongoose
2013	Farasan Islands	95856	38333	321		402	PRED	0	1	33	24			Egyptian vulture
2013	Farasan Islands	95486	38475	323		329	HAT	2	2	30.5	23	29.5	23	
2013	Farasan Islands	95491	38919	321		425	HAT	2	2	31.25	23	30	23	
2013	Farasan Islands	95490	38972	320		425	PRED	0	2	30	23	31	23.1	Mongoose
2013	Farasan Islands	95461	38371	323		425	HAT	2	2	31.5	24	31.5	24	
2013	Farasan Islands	97780	35848	323		402	HAT	1	1	31	24			
2013	Farasan Islands	99917	37132	323		404	PRED	0	2	29	23	29.5	23	Mongoose
2013	Farasan Islands	97971	36166	323		425	UNKOWN	0	1	30	23			
2013	Farasan Islands	98337	35817	324	325	424	PRED	0	2	32	23.25	30	23	
2013	Farasan Islands	99208	36135	324		401	PRED	0	2	33	23	32	22.5	Mongoose
2013	Farasan Islands	99487	36183	324		329	PRED	0	2	31.5	23	32	23	Mongoose
2013	Farasan Islands	99549	36482	324		330	PRED	0	2	31	23	29	23	Mongoose
2013	Farasan Islands	99110	36083	325		328	PRED	0	1	32.5	22.25			Mongoose
2013	Farasan Islands	99112	36109	325		330	PRED	0	2	30.75	23.1	30.25	23	Egyptian vulture
2013	Farasan Islands	95776	37300	326		330	PRED	0	2	30	23	29.5	22.25	Mongoose
2013	Farasan Islands	99009	35832	326		401	PRED	0	1	32	23.5			Egyptian vulture
2013	Farasan Islands	97585	35975	326		402	HAT	0	2	30	23	30.5	23.25	
2013	Farasan Islands	95387	38435	327		328	HAT	2	2	32	24	29.75	23	
2013	Farasan Islands	95559	38262	327		425	ABANDONED	0	1	31	23			
2013	Farasan Islands	95626	38804	328		425	PRED	0	2	30.5	23.25	31	24	Mongoose
2013	Farasan Islands	95689	38161	330		425	ABAND	0	2	33	23.5	34	23	
2013	Farasan Islands	98900	41262	331		401	ABANDONED	0	2	30	23	29.75	23.25	Nest Camera

2013	Farasan Islands	99976	37195	331		404	PRED	0	2	30.5	22.5	30	22.5	Mongoose
2013	Farasan Islands	99238	36193	401		401	PRED	0	2	32	24.5	30.5	24	Egyptian vulture
2013	Farasan Islands	99788	36826	401	401	403	PRED	0	1	32	23.5			Egyptian vulture
2013	Farasan Islands	98335	35888	402		424	UNKOWN	0	1	30.75	24			
2013	Farasan Islands	98295	43749	403		424	PRED	0	2	32	23.5	29.75	23.25	Mongoose
2013	Farasan Islands	99807	36936	403		404	PRED	0	1	31	23.5			
2013	Farasan Islands	98397	44078	403		404	PRED	0	1	33.5	24			Egyptian vulture
2013	Farasan Islands	98494	35796	405		428	HAT	2	2	31.75	24	30.75	23	
2013	Farasan Islands	97664	36240	424		424	PRED	1	0					Egyptian vulture
2013	Farasan Islands	96599	36353	424		503	HAT	1	1	31.25	23.5			
2013	Farasan Islands	97381	35785	424		425	PRED	0	1	31.5	23			Egyptian vulture
2013	Farasan Islands	97492	36141	425		502	PRED	0	2	29	23	29.5	23.25	Mongoose
2013	Farasan Islands	97491	36203	425		501	PRED	0	1	35	23.25			Egyptian vulture
2013	Farasan Islands	97852	36174	428		501	PRED	0	1	32	22			
2013	Farasan Islands	98329	44202	527			UNKOWN	0	1	32.25	22			
2013	Farasan Islands	98257	44075	602			UNKOWN	0	2	30.5	22.5	30.75	23	
2013	Al Sarum	519010	2336191	416			UNKOWN	0	1	29.75	23.25			
2013	Al Sarum	518363	2336383	416			UNKOWN	0	2	34.25	23.25	32.1	23	
2013	Al Sarum	518764	2337169	416			UNKOWN	0	1	32.5	23.5			
2013	Al Sarum	518261	2340214	416			UNKOWN	0	1	33	23			
2013	Al Sarum	517927	2340279	417			UNKOWN	0	1	32.25	23			
2013	Al Sarum	518254	2341362	417			PRED	0	1	31.75	23.25			
2013	Al Sarum	518670	2340683	417			PRED	0	1	31	23.75			
2013	Al Sarum	517884	2340340	418			UNKOWN	0	1	34	23.5			
2013	Al Sarum	518310	2341406	417			UNKOWN	0	1	32.5	23			

Appendix 2. *Morphometric data of Saunders's tern on Farasan Islands and Al Sarum.*

Date: given as MDD (M = month, D = day), Sex: M = male, F = female, J= juvenile

Colour code: Metal=metal ring, W=white ring, O=orang ring, R=Red ring, D=black ring, Y=yellow ring, G=green ring, B= Blue

Rings	Year	Site	Nest ID	Bill	Date	Time	Weight	Wing length	Right Tarsus	Sex	Code	Blood samples
Coo3951	2013	Farasan Islands	1	30	321	08:45	38.5	170.1	17		Metal	2
Coo3952	2013	Farasan Islands	1	29	323	09:32	44	167	17		Metal	1
Coo3953	2013	Farasan Islands	4	28.5	324	09:30	43	167	12		Metal	2
Coo3954	2013	Farasan Islands	4		325	06:45	5	11	7	J	Metal	0
Coo3955	2013	Farasan Islands	11	29.5	325	10:50	46	167	17.5		Metal	2
Coo3956	2013	Farasan Islands	17	28	326	09:55	45	168	18		Metal	2
Coo3957	2013	Farasan Islands	13	28.25	326	02:30	48	175	22		Metal	2
Coo3958	2013	Farasan Islands	12	29	326	03:38	46	174	19		Metal	2
Coo3959	2013	Farasan Islands	3	30.25	327	10:15	44	169	17		Metal	2
Coo3960	2013	Farasan Islands	20	28	328	01:50	46	168	18		Metal	2
Coo3961	2013	Farasan Islands	21	27	330	02:15	47.5	167	18.25		M/YG	2
Boo4249	2013	Farasan Islands	24	27	331	11:00	48.5	167	18.25		MY/R	2
B004248	2013	Farasan Islands	24	29.25	331	12:00	45	162.5	18		M/G	2
B004247	2013	Farasan Islands	25	27.25	401	01:50	41.5	165	17.25		M/Y	2
B004250	2013	Farasan Islands	16	29.25	401	03:44	45	170	18		M/R	2
B004201	2013	Farasan Islands	SHORELINE	30.5	402	02:00	48	170	18		M/G	1
B004202	2013	Farasan Islands	SHORELINE	31	402	02:00	54	168.5	18		M/R	2
B004203	2013	Farasan Islands	SHORELINE		042	02:00	9			J	M	1
B004204	2013	Farasan Islands	SHORELINE		402	02:00	12			J	M	1
B004205	2013	Farasan Islands	29	26	403	05:30	47	165	17		M/Y	2
B004206	2013	Farasan Islands	31	30	404	09:30	44	170	17		M/R	2
B004207	2013	Farasan Islands	7	29	406	11:00	59.50	169	17.5		M/G	2
B004208	2013	Farasan Islands	7	28.25	406	11:00	58	167.5	18		M/R	2
C007233	2013	Farasan Islands	33	30	424	12:00	42.5	168.5	18		M/YG	1
C007234	2013	Farasan Islands	33		424	12:15	9			J	M	1

C003962	2013	Farasan Islands	34	28	424	03:00	43	169	17		M/YO	1
C003963	2013	Farasan Islands	35	28	424	04:30	43.50	166	18		M/YR	2
B004001	2013	Farasan Islands	36	25.25	425	12:15	38	162	16.5		M/YB	1
B004002	2013	Farasan Islands	32	29	427	10:15	45	168	18.25		M/YW	1
B004003	2013	Farasan Islands	32		427	10:30	6	11	12	J	M	1
B004004	2013	Farasan Islands	32		428	11:50	6	9	12	J	M	1
B004068	2013	Farasan Islands	39	28.75	527	04:36	39.25	167.5	16.25		M/W	2
B004086	2013	Farasan Islands	40	28.75	402	05:30	41	167	16		M/O	2
B004209	2013	Al Sarum	1		416	08:15	51	163			M/OY	2
B004210	2013	Al Sarum	1		416	10:20	44	136			M/By	2
B004211	2013	Al Sarum	2		416	12:05	53	165.2		F	M/GY	2
B004221	2013	Al Sarum	8		418	10:50	43.5	162.5			M/WY	2
B004222	2013	Al Sarum	6		418	12:03	43.5	163.5			M/RY	2
B004223	2013	Al Sarum	6		418	12:30	48.5	162.5			M/YY	2
B004235	2013	Al Sarum	4		419	08:30	45.5	171.5			M/OW	2

Appendix 3. *Morphometric data of Kentish plover on Farasan Islands, Jazan, Al Sarum, Umluj and Al Wajh.*
Date: given as MDD (M = month, D = day), Sex: M = male, F = female, J= juvenile

Colour code: Metal=metal ring, W=white ring, O=orang ring, R=Red ring, D=black ring, Y=yellow ring, G=green ring, B= Blue

Rings	Year	Site	Nest ID	Bill	Date	Time	Weight	Wing length	Right Tarsus	Sex	Code	Blood samples
B004005	2013	Farasan Islands	SHORELINE	F	428	1440	34	106	28	Metal	1	
B004006	2013	Farasan Islands	SHORELINE	J	428	1450	8	9	22	Metal	1	
B004007	2013	Farasan Islands	SHORELINE	J	428	1459	8.5	12	22	Metal	1	
B004008	2013	Farasan Islands	1	F	428	1740	38.5	103	28.25	Metal	1	
B004009	2013	Farasan Islands	2	M	429	1015	37	105	28.25	Metal	1	
B003713	2013	Farasan Islands	3	M	429	1600	36	107	28.25	Metal	1	Ringed previously
B004010	2013	Farasan Islands	4	M	429	1755	33	102	29.25	Metal	1	
B004011	2013	Farasan Islands	5	F	430	0910	37	102	27.25	Metal	1	
B004012	2013	Farasan Islands	5	M	430	0920	39	109	29.25	Metal	1	
B004013	2013	Farasan Islands	SHORELINE	M	430	0950	33	106	28.25	Metal	1	
B004014	2013	Farasan Islands	6	M	430	1030	34	105	28.75	Metal	1	
B004015	2013	Farasan Islands	4	F	430	1100	39	105	29	Metal	1	
B004016	2013	Farasan Islands	7	M	430	1155	36	109	29	Metal	1	
B004017	2013	Farasan Islands	7	F	430	1210	38	110	28.25	Metal	1	
B004018	2013	Farasan Islands	8	F	430	1650	36	103	28.75	Metal	1	
B004019	2013	Farasan Islands	9	J	430	1700	6			Metal	1	
B004020	2013	Farasan Islands	9	J	430	1710	7			Metal	1	
B004021	2013	Farasan Islands	9	M	430	1749	36	105.5	29	Metal	1	
B004022	2013	Farasan Islands	9	F	430	1820	37	105	29	Metal	1	
B004023	2013	Farasan Islands	3	F	501	1830	36	99	27	Metal	2	
B007023	2013	Farasan Islands	6	F	501	1010	38	98	27	MO/GR	1	Ringed previously
B004024	2013	Farasan Islands	10	M	501	1315	37.5	110	31	Metal	1	
B004025	2013	Farasan Islands	11	M	501	1345	55.5	102.5	28.25	Metal	1	
B004026	2013	Farasan Islands	10	F	501	1415	42	102	28	Metal	1	
B004027	2013	Farasan Islands	11	M	501	1505	32.5	105	29	MW/GR	1	

B004028	2013	Farasan Islands	5	J	502	0958			6	Metal	1	
B004029	2013	Farasan Islands	5	J	502	1007			5.75	Metal	1	
B004030	2013	Farasan Islands	8	M	502	1019		105	36.5	Metal	2	
B004075	2013	Farasan Islands	16	M	530	1111	36	105	27.25	MW/OW	2	
B004076	2013	Farasan Islands	16	F	530	1124	36.5	104	26.25	MW/BW	2	
B004077	2013	Farasan Islands	17	M	530	1250	32.5	104	27.5	MW/OY	2	
B004078	2013	Farasan Islands	17	F	530	1305	37	105.5	27.5	MW/YW	2	
B004079	2013	Farasan Islands	18	F	530	1417	36.5	106	27.5	MW/YY	0	
B004080	2013	Farasan Islands	18	M	530	1430	36.25	106	30	MW/RY	0	
B007100	2013	Farasan Islands	13	F	527	12:00	38.5	106	29	MO/GY	3	Ringed previously
B007099	2013	Farasan Islands	13	M	527	1300	35	106		MO/GB	2	Ringed previously
B008610	2013	Farasan Islands	14	F	528	1428	33	100		..Y/MR	0	Ringed previously
B008612	2013	Farasan Islands	14	M	528	1527	37.5	109		MO/.. ..	0	Ringed previously
B004073	2013	Farasan Islands	11	F	529	0830	36.75	101.5		MW/GB	0	
B007080	2013	Farasan Islands	15	F	529	1015	48	109		MO/GLO	0	Ringed previously
B007079	2013	Farasan Islands	15	M	529	1100	36	107		MO/GLY	0	Ringed previously
B004085	2013	Farasan Islands	20	F	530	1200	40.25	109	28.5	OY/MR	2	Ringed previously I put only metal ring
B004069	2013	Farasan Islands	SHORELINE	J	528	1745	12	11.5	24.25	MW/RY	0	
B004070	2013	Farasan Islands	SHORELINE	J	528	1820	7.75		22.25	MO/RY	0	
B004071	2013	Farasan Islands	SHORELINE	J	528	1830	7		21.75	MO/RO	0	
B004072	2013	Farasan Islands	SHORELINE	J	528	1844	7.25		22	MO/RW	0	
B003760	2013	Farasan Islands	SHORELINE	M	529	1700	34.75	106	29	MG/-R		Ringed previously
B004074	2013	Farasan Islands	SHORELINE	J	529	1800	4.50	10	20.50	MW/OY		
B004081	2013	Farasan Islands	SHORELINE	J	529	1720	6	12	20.50	MW/RG		
B004010	2013	Farasan Islands	SHORELINE	J	529	1730	4.50	10	20.50	MW/RO		
B004082	2013	Farasan Islands	SHORELINE	J	601	1705	9.50	13	22	MY/GW		
B003802		Farasan Islands	20	M	530	1414	34.5	109	28	OB/MR	2	Ringed previously
B004056	2013	Jazan	1	F	526	1130	43	107	27.75	metal	2	

B004057	2013	Jazan	1	M	526	1139	35	106.5	27.25	metal	2	
B004058	2013	Jazan	2	M	526	1225	33	102	26.25	metal	3	
B004059	2013	Jazan	2	F	526	1400	30	99	27.25	metal	3	
B004060	2013	Jazan	3	M	526	0700	34.5	104	28	metal	3	
B004061	2013	Jazan	6	F	526	0600	38	109	28.25	metal	3	
B004062	2013	Jazan	4	F	526	0715	36.5	107.5	28.75	metal	2	
B004063	2013	Jazan	4	M	526	0815	36.25	105	29	metal		
B004064	2013	Jazan	7	M	526	0840	38.5	108.5	30.25	metal	2	
B004065	2013	Jazan	5	F	526	0956	42	108	28	metal	2	
B004066	2013	Jazan	5	M	526	1015	39	109.5	28.75	metal	2	
B004067	2013	Jazan	7	F	526	1100	45.5	109.5	28	metal	2	
B004212	2013	Al Sarum	1	F	416	1415				Metal	2	
B004213	2013	Al Sarum	2	F	416	1515	36.5			Metal	2	
B004214	2013	Al Sarum	3	M	417	1045	35			Metal	2	
B004215	2013	Al Sarum	2	M	417	1115	34			Metal	2	
B004216	2013	Al Sarum	4	F	417	1155	34			Metal	2	
B004217	2013	Al Sarum	4	M	417	1208	33			Metal	2	
B004218	2013	Al Sarum	5	F	417	1420	50			Metal	2	
B004219	2013	Al Sarum	6	F	417	1458	37			Metal	2	
B004220	2013	Al Sarum	7	F	417	1540	36			Metal	2	
B004024	2013	Al Sarum	8	M	418	1320	37.5			Metal	2	
B004025	2013	Al Sarum	9	M	418	1445	38			Metal	2	
B004026	2013	Al Sarum	9	J	418	1445	6			Metal	1	
B004027	2013	Al Sarum	9	J	418	1445	6.5			Metal	1	
B004028	2013	Al Sarum	9	J	418	1500	6			Metal	1	
B004029	2013	Al Sarum	9	F	418	1500	41			Metal	2	
B004030	2013	Al Sarum	SITE 10	J	418	1700	6.25			Metal	1	
B004031	2013	Al Sarum	SITE 10	J	418	1700	6			Metal	1	
B004032	2013	Al Sarum	SITE 10	J	418	1710	5			Metal	1	
B004033	2013	Al Sarum	SITE 10	M	418	1720	34			Metal	2	

B004034	2013	Al Sarum	SITE 10	F	418	1730	37			Metal	2	
B004036	2013	Al Sarum	SITE1	J	419	1120	9			Metal	1	
B004037	2013	Al Sarum	1	J	420	1128	5			Metal	1	
B004038	2013	Al Sarum	1	J	420	1226	13.5			Metal	1	
B004039	2013	Al Sarum	BIG LAKE	J	420	1540	20.5			Metal	1	
B004040	2013	Al Sarum		J	420	1515				Metal	1	
B004041	2013	Al Sarum		J	421	1138				Metal	1	
B004042	2013	Al Sarum		J	421	1200				Metal	1	
B004043	2013	Al Sarum		J	421	1420				Metal	1	
B004044	2013	Al Sarum		J	421	1445				Metal	1	
B004045	2013	Al Sarum		J	421	1540				Metal	1	
B004046	2013	Al Sarum		M	421	1650				Metal	1	
B004038	2013	Umluj		J	514	1655	21	29	26.5	Metal	2	
B004039	2013	Umluj		J	514	1715	8		20		1	
B004040	2013	Umluj		J	514	1729	7.5		21.25		1	
B004041	2013	Umluj		J	514	1800	6		20.25		1	
B004042	2013	Umluj		J	515	0942	31.5	43	26		2	
B004031	2013	Umluj		J	513	1402	22.5	41	27		2	
B004032	2013	Umluj		J	513	1419	19	16	26.25		1	
B004033	2013	Umluj		J	513	1500	8	16	26		1	
B004034	2013	Umluj		M	513	1530	39	110	29.25		2	
B004035	2013	Umluj		J	513	1600	12.5	18	23		2	
B004036	2013	Umluj		F	513	1630	39	111	28		2	
B004037	2013	Umluj		F	513	1729	39.5	118	29		2	
B004044	2013	Umluj		F	515	1345	36.5	112.5	29.25		2	
B004045	2013	Umluj		J	515	1455	6		18.25		1	
B004046	2013	Al wajh	1	F	516	1031	42	107.5	27		2	
B004047	2013	Al wajh	1	M	516	1043	40	109	28.25		3	
B004048	2013	Al wajh	2	F	516	1415	40.5	113	29		3	
B004049	2013	Al wajh	2	M	516	1445	35.75	113	29		2	

B004050	2013	Al wajh		M	517	1000	43	108	26.75		2	
B004051	2013	Al wajh		F	517	1350	46.5	106	27		2	
B004052	2013	Al wajh		J	517	1615	14	21	25.25		2	
B004053	2013	Al wajh		F	517	1900	40.25	110.5	28.5		2	

Appendix 4. *Variables recorded for brood encounters of Kentish plover in Farasan Islands.*

Note:

***Brood ID**: negative signs indicate that the brood hatched from a nest we did not find*

***Parent** : number and sex of parents (4 – both parents, 3 – only male, 2 – only female)*

Year	Site	Brood ID	Date	Time	Parent	Chicks	Latitude	Longitude	Habitat	Comments
2013	A	-1	528	1500	4	2	96683	35901	shoreline	
2013	A	-1	528	1435	4	1	96209	37187	100 far from the shoreline	
2013	A	-1	528	1820	4	3	95860	37197	Shoreline	
2013	F	-1	529	1700	4	1	99517	39290	shoreline	
2013	B	4	530	1721	4	3	96824	35832	Shoreline	
2013	A	5	601	1705	2	1	95399	37754	shoreline	The nest was contains 3 eggs