

**THE UNIVERSITY OF HULL**

**Breeding Ecology, Migration and Population Genetics of  
Lesser Crested Terns *Thalasseus bengalensis emigrata***

being a Thesis submitted for the Degree of  
Doctor in Philosophy

in the University of Hull

by

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**Glossary**  
**List of abbreviations used in this thesis**

EGA: The Environment General Authority

IBAs: Important Bird Areas

IECS: Institute of Estuarine and Coastal Studies

ISPRA: The Italian Institute for Environmental Protection and Research

LSPA: The Libyan Spatial Planning Agency

MAP: The Mediterranean Action Plan

MBRC: The Marine Biology Research Centre

NGC: The National General Congress

NOC: The National Oil Corporation-Libya

NSA: The National Safety Authority

RAC/SPA: The Regional Activity Centre for Specially Protected Areas

SST: Sea Surface Temperature

UNEP: The United Nations Environment Program

UNDP: United Nations Development Program

WCT: Waterbird Census Team

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## Abstract

The ecological requirements of the Mediterranean breeding population of Lesser Crested Tern *Thalasseus bengalensis emigrata* were studied from 2009 to 2012 at its three breeding sites on the Libyan coast Libya: Gara, Elba islands and Jeliana islet. Four main research aspects were investigated: Breeding ecology, young diet structure and differences between sites, migration and recruitment, and population genetics. Threats and conservation measures were also discussed. This Mediterranean endangered population starts breeding at one site (Jeliana) three weeks earlier than in the other breeding sites, possible causes were discussed, including difference in migration routes and food diversity and availability among sites. Incubation period and nesting density were variable among sites; large colonies had more packed nests than in small colonies. A fourth breeding site was discovered in 2010 at Fteha Island. Nest counts at Jeliana colony tripled in 2012, following site restoration work that increased both the islet surface breeding area and height, to control nest inundation and competition on nesting space. Regurgitated food during young ringing was collected to study diet structure and diversity. Fish were the most common prey type with a small proportion of Cephalopods at Gara. Dominant fish families were Clupiedae, Exocoetidae, Hemiramphidae, Carangidae, Sparidae and Blennidae. Prey diversity was highest at Jeliana. There were more differences in prey structure among sites than within sites and seasons. Total length (TL) of fish was smaller at Elba compared to both Gara and Jeliana. Annual variability of prey mass were significant among sampling years, possible causes were discussed. Prey fish length increased with the progress of provisioning period, reflecting increased dietary demand by the growing young. Adults are potentially selecting actively for larger sizes. The relationship between the increase of Sea Surface Temperature and the primary productivity and fish spawning season coinciding with the Lesser Crested Tern breeding season have been discussed. 544 young terns were ringed during the study years, in addition to 808 that were previously ringed between 2006-2008 seasons. Ring sighting and recoveries constitute 2.07% of the total ringed terns; this allowed the gathering of more data on post-natal movements (staging and wintering ranges), breeding site philopatry and recruitment levels, in addition to a preliminary estimation the duration of migration

journey. The population genetics of the study subspecies have been studied using two mitochondrial DNA molecular markers (ND2 and Cyt b). DNA samples were collected from Libya, the Red Sea and the Persian Gulf breeding populations. Irrespective of the relatively small sample size and the limited number of genetic markers used, there was significant genetic variability among the three populations. Several private haplotypes have been firstly identified for each population; few others were shared among all subspecies populations. Haplotype diversity was highest at the Persian Gulf subspecies *T. b. torresii*. The present three subspecies classification is found to be valid, which in turn making the Mediterranean breeding population a special conservation unit, given its relatively small population size and the limited breeding range. A model presents the breeding temporal and spatial aspects, related habitat requirements and present threats have been designed, based on data collected in this study. Habitat requirements and factors known to affect the population at wintering and staging sites were included in the model. The lack of legal protection, the potential oil pollution and site disturbance by visitors, poachers and habitat degradation were the top threats facing the Mediterranean subspecies. Mitigation measures and a proposed Action Plan was presented and discussed. The present research has answered several questions on the status, ecology, feeding, genetics and management of the Mediterranean breeding Lesser Crested Tern colonies in Libya. It is considered a significant step towards the conservation of this localised un-protected population.

## Chapter 1 Introduction





## Chapter.1 Introduction

### 1.1 Introduction

The avifauna of Libya has been estimated recently to comprise about 350 species (Clements and Clements, 2007), although it is considered to be one of the least known African countries in terms of avian studies. The lack of data on avian diversity in Libya and its distribution is considered to have been affected by the political situation of the country, particularly during the period 1969-2011, with limited access for non-national scientists in addition to the absence of qualified Libyan ornithologists and birdwatchers for several decades. The main professional work collating information on the Libyan avifauna, *The Birds of Libya* (Bundy, 1976), gathers together information published during the Italian occupation period (1911-1945) and data published after Independence (1951) until the early 1970s including that author's own observations. Several general books on the birds of Libya were published in the early 1980s and mid-2000s (Al-Awami, 2007), however little was added to the data in Bundy (1976).

Since 2005, there have been more bird studies, with the annual wintering waterbird census across most of the coastal regions of the country (Smart et al., 2006). Data collected during 2005-2010 have been compiled into the *Atlas of Wintering Waterbirds of Libya* (EGA-RAC/SPA-WCT, 2012). One of the breeding seabirds in Libya during the summer months is the Mediterranean population of Lesser Crested Tern *Thalasseus bengalensis emigrata* (Newmann, 1934). This Thesis aims to add to the knowledge of the ecology, feeding, migration patterns and genetic variability of this localised population breeding off the Libyan coast.

Breeding ecology data represent essential baseline information for preparing conservation plans aimed at protecting the subspecies and its limited breeding areas. Breeding dates, habitat requirements, productivity and threats to both adults and their young are needed in order to produce population size trends and to indicate future steps to conserve and develop the Mediterranean population. Chapter 2 of this Thesis aims to investigate breeding habitat requirements, breeding dates, colony formation and breeding synchrony, egg-volume differences, nesting density and inter-nest distance, incubation period, reproductive success and causes of nest failure in the Mediterranean population

of Lesser Crested Tern *Thalasseus bengalensis emigrata* breeding off the Libyan coast during three breeding seasons 2009-2010 and 2012. The study of the breeding ecology of this localised population is one of the recommendations listed for the species in the Action Plan on Birds listed in Annex II of the Protocol concerning Specially Protected Areas (SPAs) and Biological Diversity in the Mediterranean (UNEP-MAP-RAC/SPA, 2003), and to fill gaps in knowledge of their little known breeding sites, discovered during past expeditions to Libya in the late 1930s (Moltoni, 1938) and early 1990s (Meininger et al., 1994).

Food delivered by adult breeding seabirds to their young is one of the limiting factors that control the breeding initiation date and population productivity (McLeay et al., 2008, Anderson et al., 2007, Barrett et al., 2007, Burger and Gochfeld, 1991, Safina and Burger, 1985). Any change in prey population may have adverse consequences on the breeding seabird population (Crawford, 2009, McLeay et al., 2008, Weimerskirch, 2007, Furness and Tasker, 1999, Furness and Tasker, 2000). Studying the diet structure of the young birds is an essential aspect in understanding their growth requirements. Food regurgitated by the young near nest sites can provide vital information on diet structure, in addition to adult preference for specific types of prey species and to prey availability at foraging areas used by the adults (Golet et al., 2000, Weimerskirch, 2007, Aygen and Emslie, 2006, Barrett et al., 2007, Duffy and Jackson, 1986, Hamer et al., 1991). Chapter 3 investigates and discusses prey species composition in the Mediterranean Lesser Crested Tern young diet and potential prey species differences among breeding sites, in terms of frequency and mass, with an emphasis on changing prey size during the later provisioning period in order to fulfil the increasing requirements of the growing young.

Being a population migrating between wintering sites in West Africa and breeding sites in Libya, while utilising staging sites to refuel by food, some of the young Lesser Crested Tern hatched in Libya have been ringed with metal and colour rings since 2007 (Hamza et al., 2007) in order to track post-natal movements and identify staging and wintering areas, through ring sightings and recoveries. Chapter 4 presents the results of the ringing study to date, as well as providing data on the importance of some staging and wintering sites. The

protection of such sites complements the protection of breeding habitats, for the conservation of this important Mediterranean population.

The conservation status of the species globally is Least Concern, based on the International Union of Nature Conservation's Red List of Endangered species (BirdLife-International, 2013). The red list defines a subpopulation as "geographically or otherwise distinct groups, between which there is little demographic or genetic exchange". The geographic separation among the Mediterranean subspecies *T. b. emigrata* and the other two subspecies breeding in both the Red Sea *T. b. bengalensis* and the Persian Gulf *T. b. torresii* can result in long-term isolation and genetic variation. Proving such differentiation can help to enhance the conservation status of the Mediterranean breeding subpopulation in the Red List, being the smallest among other populations of the species, with an immediate need for protection of its breeding and foraging areas. The genetic structure study (Chapter 5), have been conducted using the amplification of two mitochondrial genetic markers (the The NADH Dehydrogenase subunit 2 and the Cytochrome b). The current subspecies division of this species adopted in this Thesis was tested using a phylogenetic approach, and recommendations for future genetic research are proposed.

## **1.2 Ornithological literature review**

Efforts to study and document bird species in Libya started later than for neighbouring Tunisia and Egypt mainly due to historical, political and logistical reasons (Al-Awami, 2007). However, the oldest published information on the Libyan avifauna date from the late 19<sup>th</sup> century when Fraser (1844) listed 28 species (Toschi, 1969), which were reported during the travels of Dixon and Ross between Tripoli and Fezzan (NW and SW Libya respectively), the small number of species recorded may be attributed to limited number of areas visited. Several Italian ornithologists published data on Libyan avifauna during the Italian occupation of the country (1911-1945) including the discovery of what was then called Bird Island, where Lesser Crested Tern were found to breed in the late 1930s (Moltoni, 1938). A book was published later to include this period and later works were also published after Independence in 1951 (Toschi, 1969).

Bundy (1976) compiled a review of ornithological literature of Libya, listing 317 bird species, among them 28 seabird species. Only 2 tern species were reported as breeding in Libya, Lesser Crested Tern and Little Tern *Sterna albifrons*. Bundy's (1976) publication is still considered as one of the main sources of information on the Libyan avifauna, however it was an annotated checklist of species, included some limited data on breeding months without any further details on feeding ecology.

Meininger et al.(1994) conducted a survey to document waterbirds along the Libyan coast in July 1993 and visited the Gara island colony, and discovered the second Libyan breeding site of the species at Elba Island. Hadoud and Zgouzi (1995) reported the breeding of Common Tern *Sterna hirundo* to the west of Farwa Island (NW Libya).

In Libya there are eight sites listed as Important Bird Areas (IBAs) among them sites important for seabirds such as Gara, Elba and Farwa Islands (Robertson and Essghaier, 2001). An update of this list is needed, after the publication of Wintering Waterbirds Atlas, which showed several other sites that meet the criteria of IBAs (EGA-RAC/SPA-WCT, 2012). Between 1999 and 2001, the breeding of Caspian Tern *Hydroprogne caspia*, Common Tern *Sterna hirundo* and Little Tern *Sterna albifrons* on the western part of Farwa Island were reported (Etayeb and Essghaier, 2007). It was the first and only report of Caspian Tern breeding in Libya so far. Information on 21 different seabird species has also been presented (Brehme, 2003) along the coastal plain. New observations from 2004 and 2005 on the status and distribution of some Libyan birds were published by Gaskell (2005), who also mentioned coupling behaviour of roosting Lesser Crested Terns in the Benghazi lagoon.

### **1.2.1 Breeding seabirds of Libya**

Only a small number of seabird species are known to breed in Libya, either due to lack of breeding habitat or simply due to a lack of observers. Seabirds that are known to breed at present in addition to the Lesser Crested Tern, at both Gara and Elba Islands (Meininger et al., 1994, Hamza et al., 2007) are: the Mediterranean population of European Shag *Phalacrocorax aristotelis*

*desmarestii*, the Mediterranean population of Yellow-legged Gull *Larus cachinananus michahellis* at Gara, Elba and Barda'a Islands (Meininger et al., 1994), Caspian Tern *Hydroprogne caspia*, Common Tern *Sterna hirundo* and Little Tern *Sterna albifrons* at Farwa island (Hadoud and Zgouzi, 1995, Etayeb and Essghaier, 2007). Available data at present seem insufficient to provide a definitive list of breeding seabird species of the country, as more surveys and studies are needed to report and follow trends at each colony.

### 1.2.2 Non-breeding (wintering) Seabirds of Libya

Seabirds and other migratory species pass through Libyan waters during both autumn and spring passage. Some species overwinter in large aggregations, mainly Gulls and Cormorants (Smart et al., 2006). The number of seabird species reported to winter in Libyan coastal areas during the winter surveys of 2005-2010 has ranged from 15-22 species (EGA-RAC/SPA-WCT, 2012). Some species (including some waterbird species) are found to winter in good numbers, over 1,000 individuals of Shovelers *Anas clypeata*, Great Cormorants *Phalacrocorax carbo*, Greater Flamingos *Phoenicopterus roseus*, Dunlins *Calidris alpina*, Kentish Plovers *Charadrius alexandrinus*, Little Stints *Calidris minuta*, and Black-headed *Chroicocephalus ridibundus*, Slender-billed *Chroicocephalus genei*, Lesser Black-backed *Larus fuscus* and the Yellow-legged *L. Michahellis* / Caspian Gulls *L. cachinnans*. Significant numbers of Audouin's Gulls *L. audouinii* were also recorded and believed to belong to the Greek breeding population. Few individuals of Lesser Crested Terns are also known to winter in Libya (see Chapter 4).

### 1.2.3 Lesser Crested Terns in Libya

The Lesser Crested Tern *Sterna bengalensis* belongs to the Order Charadiformes, Family Sternidae, comprising 10 genera (with species belonging to the genus *Sterna*), 44 species and 123 taxa (del Hoyo et al., 1996, Cabot and Nisbet, 2013). The Lesser Crested Tern was recently regrouped with other "crested" terns (having elongated crest feathers) in the genus *Thalasseus* and thus became *Thalasseus bengalensis* (Bridge et al., 2005). Nevertheless the earlier *Sterna* name of the genus is still fairly widely used in the literature, but the more recent genus (*Thalasseus*) is used in this thesis.

The species is generally recognised as having three subspecies, differing mainly in size and minor plumage details:

- ***T. b. emigrata*** (Neumann, 1934) - Breeds in Libya, some isolated breeding occurred in France (Isenmann, 1972), Italy and Spain (Brichetti and Foschi, 1987), hybrid breeding with Sandwich Tern *T. sandvicensis* (Cramp, 1985). Most overwinter off the West African coast, with small numbers remaining in Mediterranean region.
- ***T. b. bengalensis*** (Lesson, 1831) Red Sea S along the coasts of Sudan, Eritrea, Djibouti & Somalia. Non-breeding: Along the coasts of Kenya, Tanzania, Mozambique, Natal (South Africa) and Madagascar, Comoros, Aldabra Atoll, Seychelles & Sri Lanka.
- ***T. b. torresii*** (Gould, 1843) - Persian Gulf, Indian Ocean coast of Pakistan, Maldives & Laccadive Islands East to the Malay Peninsula, Sumatra, Java & Sulawesi to New Guinea, Aru Islands (East Indonesia) and Bedout & Adele Island (Western Australia) to Capricorn & Bunker group of islands (Southern of Great Barrier Reef). Non-breeding: Northern Indian Ocean & Southwest Pacific Ocean.

The species is not globally endangered, as its world population is estimated at 225,000 pairs of which 50,000-60,000 pairs are found in the Middle East and 10,000 in North Africa (Gochfeld and Burger, 1996). However, the Mediterranean breeding population *T. b. emigrata* does not exceed 2,400 pairs based on recent field census (Hamza et al., 2007). A further 2,500 pairs are based on some islands in the Egyptian Red Sea (Goodman and Meininger, 1989). The Mediterranean breeding population is confined to Libyan waters and depends on only a few as yet unprotected sites off the Libyan coast. It has been listed as Endangered at the Mediterranean level, based on limited breeding range and human disturbance and marine pollution (Azafzaf et al., 2006). The action plan lists another 24 breeding seabird species in the Mediterranean region as Endangered as well (UNEP-MAP-RAC/SPA, 2003), the Action plan have identified several priorities for research on each of its listed species. The lack of protection and limited ecology and biology information are the main drivers to conduct the present study to fill the gaps in knowledge about this special population in the Mediterranean.

The Mediterranean population *T. b. emigrata* is characterised by a longer bill compared to the Red Sea and Indian ocean populations (Zotier et al., 1999). The body is distinctly larger with slightly paler upper parts, in adults, compared to the nominate subspecies *bengalensis* from eastern and southern India, Sri Lanka and westward to East Africa and the Red Sea (Cramp, 1985). Neumann (1934) described the Mediterranean subspecies from individuals caught in Morocco during their autumn passage after leaving their Libyan breeding sites (Figure 1.1).

The eggs of Lesser Crested Tern are laid on the bare soil of islands and islets with sparse vegetation, or on coral islands. In cases of large tidal range, nests are established further away from the beach area (Cramp, 1985). Some colonies are established at the centre or periphery of an island, close to a wetland (salt marsh) that is connected to the sea (e.g. at Elba Island, Libya). This site selection may provide a safe escape route for the young to the sea when faced with terrestrial predators or colony disturbance (*Personal field observation*).

The first mention of colonial Lesser Crested Terns breeding in Libya was in the early 1930s when Italian hydro-biologist Giorgio Bini and his team made four visits during two successive years to an Island in the Gulf of Sirte along the coast of Libya. They found eggs in July and tern chicks in August, originally identified as Caspian Tern *Hydroprogne caspia*. However, two years later the Italian ornithologist Edgardo Moltoni visited this island (Gara island), on August 21<sup>st</sup> 1937 and found a large colony of Lesser Crested Terns (Moltoni, 1938) Estimating the population size at 2,000 birds (adults and nestlings)

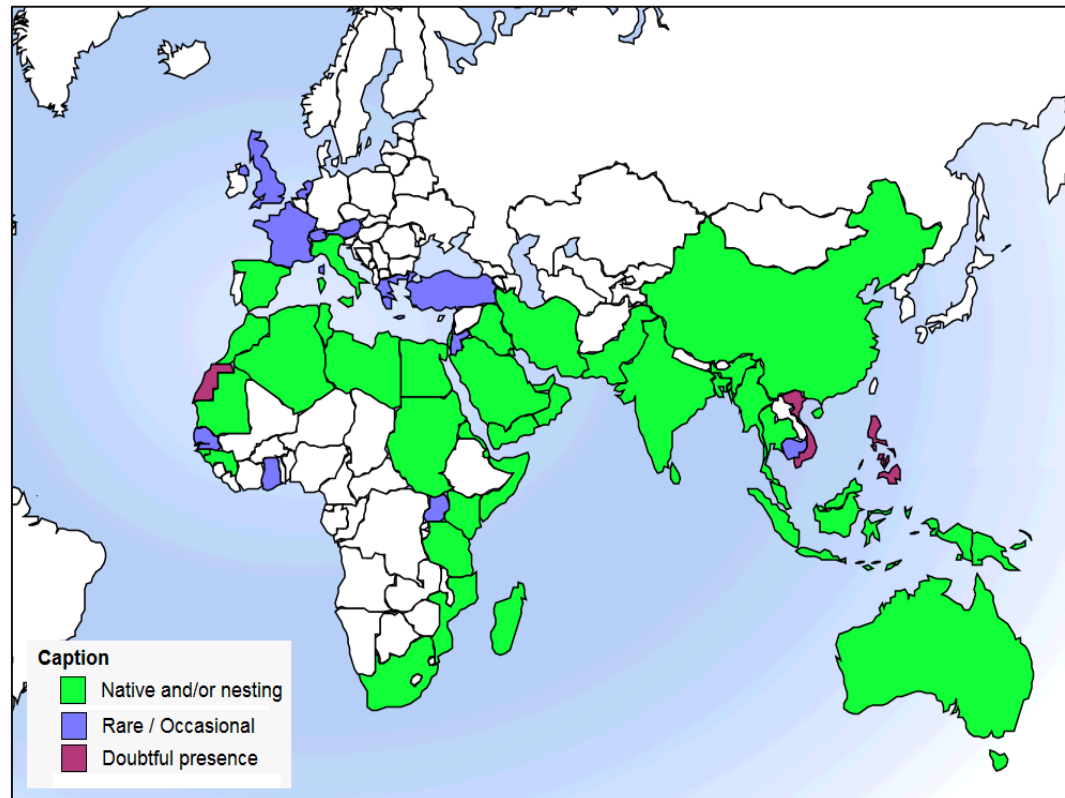


Figure 1.1 World distribution (by country checklist) of Lesser Crested Tern *Thalasseus bengalensis* ©1996-2013 Oiseaux.net

Meininger *et al.* (1994) estimated a total of 1,700 pairs on Gara Island and 40 pairs on Elba Island during 1993 season. Elba became the second reported colony for the species in Libya (Figure 1.2). From mid-June 2004 to March 2005 Gaskell (2005) visited some sites along the Libyan coast. He mentioned for the first time a breeding colony of over 50 pairs on an inshore island in one of Benghazi's lagoons. Azafzaf *et al.* (2006) visited the two known breeding sites (Gara and Elba) in early August 2006, for the first time in 13 years, and found that both islands are still hosting breeding Lesser Crested Tern, in an almost stable condition to that reported by Meininger *et al.* (1994).

The species was also recorded in the Benghazi lagoon, but with no confirmed breeding. In 2007 a ringing programme was initiated using metal rings provided by Birdlife Malta and Italian colour colour-rings (Chapter 4). Breeding was confirmed on a small inshore islet within Sebket Jeliana (the southern side of Benghazi Lake); no ringing was conducted at Jeliana in 2007 due to the increase of water level leading to colony inundation. During the 2008 season, 288 young terns were ringed at Jeliana, resulting in a subsequent sighting of a juvenile tern



at Ceuta (Spanish Morocco) and a recovery from Turtle Island off Sierra Leone, in October 2007 and January 2008 respectively (Hamza et al., 2008).

A few individuals are known to remain in Libya during the winter period. Hering (2009) observed three individuals sitting on a small island near the ruins of Sabratah during December 2009. Other winter records suggest regular occurrence of isolated individuals in the winter months (EGA-RAC/SPA-WCT, 2012)

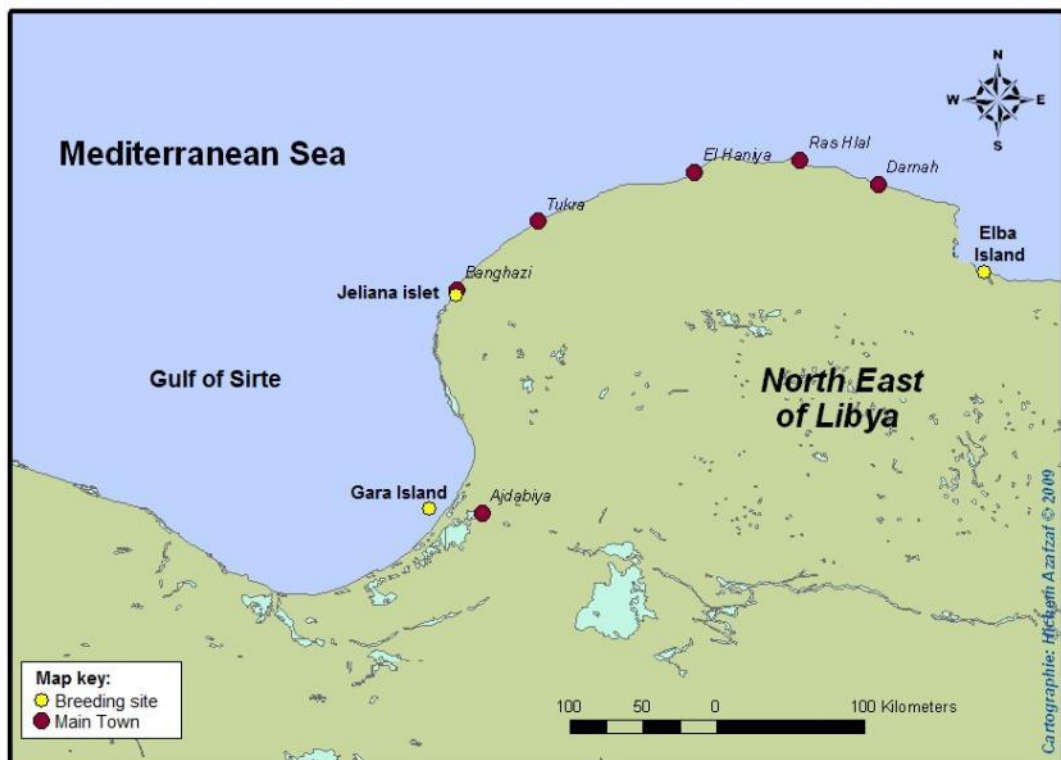


Figure 1.2 Map of the breeding sites of Lesser Crested Terns in Libya.

#### 1.2.4 Lesser Crested Terns in North and West Africa

The species has been reported from North and Northwest African countries (Figure 1.1), during migration from and to the breeding sites in Libya. In Egypt the species other nominate *T. b. bengalensis* is a summer breeder in the Northern region of the Red Sea. Table 1.1) summarises published information of the species in Egypt and Northwest African countries. The table shows the chronological sequence of observing the migratory groups of Lesser Crested Tern, during their movements between wintering and breeding sites, and vice

versa. It also shows that in most West African countries, data on wintering population size varies from few individuals to several hundred, which may reflect both the relative distribution of the species among these countries and the monitoring differences between them.

### **1.2.5 Conservation status**

The species is considered to be not globally threatened and of “Least Concern”, as the global population is classified as stable, thus the species does not approach the thresholds for Vulnerable status under the population trend criterion (<10,000 mature individuals with a continuing decline estimated to be >10% in ten years or three generations, or with a specified population structure (BirdLife-International, 2013). The species is included in the following conventions:

- Appendix II of the Bonn Convention on Migratory Species (African and South-West Asian populations).
- Appendix II of the Bern Convention on the Conservation of European Wildlife and Natural Habitats.
- Appendix II - (African pops.) Convention on the Conservation of Migratory Species of Wild Animals (1979).
- European Union Regulations on technical measures for the conservation of fishery resources in the Mediterranean (1626/94 (EC) 1994).
- Annex II of the Barcelona Convention for the Protection of the Mediterranean Sea against Pollution, under the Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean, for which a Mediterranean Action Plan for the Conservation of Marine and Protected Birds has been adopted (UNEP-MAP-RAC/SPA, 2003).
- The African-Eurasian Water-bird Agreement- AEWA Action Plan (Column A Category 1/c).

Table 1.1 Published data on Lesser Crested Terns in North and West Africa.

Country	Status	Reference
Egypt	Common local breeder in Red Sea region, rarely passing along the Mediterranean coast. One individual of the subspecies breeds in Libya controlled in Gulf of Suez (connectivity with Libyan population uncertain).	Goodman and Meininger (1989)
Tunisia	Spring (May-June) and autumn (September-October) passage to and from Libyan breeding sites. Single breeding record in 1983, in a Sandwich Tern colony.	Isenmann et al. (2005)
Algeria	Spring (May-June) and autumn (September-November) passage migrant, in small flocks.	Jacob (1983)
Morocco	Early spring passage (late March/early April) with peak in May/ early June. Some birds summer along Mediterranean and Atlantic coasts. Autumn passage occurs late August to early September. Some birds overwinter in the Moulouya estuary/Mediterranean Morocco.	Thévenot et al. (2003)
Mauritania	Prenuptial passage late March/early April to late May/June (peak May) and postnuptial passage in late August to early November (peak October), some individuals overwintering at the Banc d' Arguin National park.	Isenmann et al. (2010)
Senegal	Spring passage. Some birds overwinter and/or summer (non-breeders or sub-adults), About 30-50 non-migrating birds seen daily off the N'Gor area in October 2003; During 3-16th October 2005 a total of 825 recorded at Calao.	Holmstrom and Mangsbo (2003)
The Gambia	Frequent to common along the coast, e.g. Tanji Bird Reserve, lowest numbers during May-September. Never reported in large numbers.	Barlow et al. (1997)
Guinea-Bissau	Main wintering area with 400 birds, estimate of up to 1,000 birds present in winter.	Altenburg et al. (1992)
Sierra Leone	Hosts >5% of West Africa's Flyway population. In 2005 midwinter census, 534 birds counted in three wetlands. Estimates of 52% of total wintering population occurring in the country.	Van der Winden et al. (2007)

### **1.2.6 The Mediterranean Seabirds Action Plan**

The Action Plan for the Conservation of Bird Species listed in Annex II of the Protocol concerning Specially Protected Areas (SPAs) and Biological Diversity in the Mediterranean follows a series of four Action Plans adopted by the Parties to the Convention for the protection of the marine environment and the coastal region of the Mediterranean. The Action plan identifies and lays out priorities and activities that need to be undertaken to attain their specific objectives. It also urges and encourages coordination and cooperation amongst Mediterranean states to work towards the achievement of conservation of a species or a group of species within this region. The main purpose of the Mediterranean Action Plan for the conservation of marine and coastal birds is to *maintain and/or restore the population levels of bird species to a favourable conservation status and to ensure their long-term conservation.*

Measuring the favourable conservation status of a species depends largely on the factors that affected the quality of the population size of that species (Sutherland et al., 2006). Based on European Bird's directive, The conservation status of a species is deemed favourable when the species 'is maintaining itself on a long-term basis as a viable component of its natural habitats' and 'there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long term basis (Trouwborst, 2011)

The development of this Action Plan follows various initiatives taken by other organisations, such as Bird Life International and its partners in the Mediterranean countries, WWF, IUCN, Medmaravis, Tour du valat, on the conservation of biological diversity, particularly with respect to birds, and their breeding and wintering habitats.

In 1995 the Parties to the Barcelona Convention adopted a new Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean. Annex II of this new protocol lists endangered or threatened species found in the Mediterranean, including 15 bird species. In 2009 the contracting parties to Barcelona Convention adopted the addition of further 10 species of seabirds to the Action Plan, following a recommendation from the first Symposium on Mediterranean Seabirds, in Villanova, Spain in 2005.

Five species of terns are included in the Action Plan: The Sandwich Tern *Thalasseus sandvicensis*, Lesser Crested Tern *Thalasseus bengalensis*, Little Tern *Sterna albifrons*, Caspian Tern *Hydroprogne caspia*, Gull-billed Tern *Sterna nilotica*. The last two species were added to the Action Plan in 2009.

The Action Plan aimed to maintain and increase the population size of the Mediterranean breeding population of Lesser Crested Terns, via the following action:

- Confer strictly protected status on the species.
- Prohibit all types of disturbance to breeding colonies, including the taking of eggs and young.
- Monitor and warden colonies, which may be under threat of disturbance.
- Create specially protected areas (SPAs) where the species' breeding colonies exist and prohibit access to known sites except for scientific purposes.
- Investigate whether local fisheries impact on breeding success.
- Prevent oil spills and chemical pollution of the sea.
- Establish population size and trends.

The present thesis is conducted in the framework of implementing some of the Mediterranean Action Plan objectives related to the breeding population of Lesser Crested Tern *T. bengalensis emigrata* in Libya.

### **1.2.7. The evolutionary context of the present research**

The following four chapters of this study are introduced with respective introductions that include the literature review on each of the topics discussed, i.e. Breeding Ecology, Feeding Ecology, Migration and recruitment and Population Genetics. However some basic ecological concepts needs to be clearly identified prior to the study of each of the above mentioned chapters.

A breeding colony is defined as a single location supporting breeding birds located close enough in distance to interact socially (Gochfeld, 1980). Colonial breeding is wide spread phenomenon in seabirds, and it is the principal form of social organization for 98% of marine bird species (Wittenberger and Hunt, 1985), several research efforts has been directed toward understanding the

evolution of avian coloniality (see review by Brown and Brown, 2001), some even found that colonial breeding has evolved even before the establishment of birdlife in the marine environment (Cecile Rolland et al., 1998). Some authors found that colonial breeding have evolved in order to exploit a dispersed unpredicted food resources, which in turn lead to increased vulnerability to predation, rather than being a strategy to combat predation (Clode, 1993).

Several benefits are believed to support the evolutionary evolvement of colonial breeding other than the predation control, such as enhanced ability to locate food (Guilford et al., 2008, Crawford, 2003, Forbes and Mock, 1996, Shealer, 2002), and information regarding the selectivity of breeding habitat suitability (Jones, 2001, Naves et al., 2006, Rosenzweig, 1991) outweigh costs such as competition for nest sites or foraging resources predictability (Lewis (Burger, 1988, Oro et al., 2009) increased prevalence of disease and parasites and predator attraction (Brown and Brown, 2001, Varela et al., 2007, Votier et al., 2009).

Over 98% of seabirds are colonial, they acquired special characteristics that allowed them to use colonial breeding as a reproductive strategy (Schreiber and Burger, 2002), of these characteristics the following:

- Colony size is related to feeding range.
- Colonial breeding evolved several times through the evolutionary history of seabirds.
- They can found at all latitudes.
- Colonial seabirds have wide spectrum of size and structure.
- Both nocturnal and diurnal seabirds are breeding in colonies.
- Colonial breeding increases social attraction of conspecifics.
- Coloniality exerts a constraint on new recruitment, due to competition on limited space.
- Colonial seabirds usually philopatric, and forming new colonies is used under special circumstances, mainly caused by depleted food resources or habitat degradation.
- Most colonial Seabirds are monogamous.
- Seabirds rarely rear more than one brood a year.

- A within-colony segregation exists between experienced better breeders and poor inexperienced breeders.
- Possibility of individual movement among colonies.
- Formation of dense groups is only noticed during breeding season.

However, regardless of all of the above benefits and feature that colonial breeding offers to seabirds, these advantages might require several years to be evident, and in some cases the environmental change in breeding or foraging environments can make colonial breeding no longer that advantageous for some seabird species, making them to choose to change their breeding behaviour, or be resilient colonial breeders as the only breeding strategy, or extinct when the disadvantages exceeds the tolerance of the species population (Schreiber and Burger, 2002),

Productivity in seabirds reflects conditions in the marine ecosystem (Saunders et al., 2013), any change in the prey population structure or density can lead to severe consequences on the survival of that seabird population, being interlinked directly through food chain (Crawford, 2009, Dänhardt and Becker, 2011, Dänhardt et al., 2011, Pedersen et al., 2012), this change may be caused by natural changes in prey population or induced by other climatic (Wanless, 2006) or other anthropogenic factors, such as overfishing or pollution (McLeay et al., 2008) . At the chapter 3 on feeding ecology, a detailed review on feeding requirements and trade-offs taken by seabirds in order to fulfil their own bodies and their offspring energetic requirements during the breeding season (Aygen and Emslie, 2006, Barrett et al., 2007, Horn et al., 2010, Dänhardt and Becker, 2011).

The migratory nature of lesser crested terns, from wintering grounds in West Africa to the Mediterranean breeding sites in Libya makes them a model of south-south migration, and an opportunity of collaboration between ornithologists in the range states. The ringing programme of the species started in Libya during 2006 with metal rings only donated by Birdlife Malta (Azafzaf et al., 2006), and then continued until the start of this study (Hamza et al., 2008, Hamza et al., 2007) the ringing from 2009-2012 aimed at gathering more information on migration pattern and dispersal of the newly fledged nestlings, in addition to monitoring recruitment at each of the breeding colonies in Libya.

### 1.3 Aims, objectives, hypotheses and structure of the Thesis

#### 1.3.1 Aims and objectives

1. To understand the breeding, feeding ecology and migration movements of the Mediterranean population of the Lesser Crested Tern *Thalasseus bengalensis emigrata* breeding in Libya,
2. To examine the genetic structure in comparison with other subspecies breeding in the Red Sea and Persian Gulf areas
3. To provide baseline data for management and conservation of the subspecies and its breeding, feeding and wintering habitats.

The hypotheses of the related research topics are outlined at the beginning of the Chapters of the Thesis.

#### 1.3.2 Structure of the Thesis

**Chapter 1** presents an introduction to the history of avian studies in Libya, together with the Lesser Crested Tern classification, distribution and conservation status, and lists the overall aims of the Thesis. **Chapter 2** examines the breeding biology of Lesser Crested Terns in Libya, including observations made at a breeding site in the Red Sea during summer 2009. It documents the data collected on basic breeding biology parameters for the subspecies, adding a new breeding site in 2010-2011 season to the three known breeding sites in Libya, and comparing it with similar studies for the species worldwide distribution range. **Chapter 3** examines the feeding ecology of Lesser Crested Tern young, particularly by studying regurgitated food items obtained from the colonies and during ringing operations. It also explains the differences in diet species frequency and mass of the breeding colonies in Libya, and discusses the link between oceanographic conditions and the arrival at, and breeding initiation in the colonies. **Chapter 4** discusses the results of ringing operations conducted for the species since 2007, in terms of sightings and recovery information obtained from breeding, staging and wintering areas of this Mediterranean breeding population. It also indicates the potential cause(s) of differences in breeding initiation at the Jeliana colony before the other breeding sites. **Chapter 5**



introduces the results of the phylogenetic study of Lesser Crested Tern using two genetic markers and samples from the Libyan, Red Sea and Persian Gulf populations. **Chapter 6** presents a general discussion on the results of Chapter 2, 3, 4 and 5 and concludes with proposals for conservation measures to be put in place for the protection and development of the Mediterranean population of Lesser Crested Tern *Thalasseus bengalensis emigrata*. The latter is in the light of threats identified during fieldwork for this Thesis and also the recommendations of the Mediterranean Action Plan on Seabirds.

**Chapter 2 The breeding biology of  
Lesser Crested Tern  
*Thalasseus bengalensis* in Libya**



## Chapter.2 The breeding biology of Lesser Crested Tern *Thalasseus bengalensis* in Libya

### 2.1 Introduction

The study of breeding biology provides the basis for monitoring and management of populations through assessing their breeding success and factors that control it, in addition to population size estimation and population trends. The breeding biology parameters also provide baseline data for the preparation and implementation of conservation management plans for that population. These form one of the aims of the regional Mediterranean Action Plan on marine birds, adopted under the protocol on Marine and Coastal Biodiversity in the Mediterranean (UNEP-MAP-RAC/SPA, 2003). This Action Plan lists 25 endangered seabird species with breeding ranges confined to the Mediterranean Sea region. This chapter discusses the breeding biology of Lesser Crested Tern at its Mediterranean breeding range along the coast of Libya.

Terns and other seabird species invest effort in their breeding by generally laying single egg. This strategy is linked to the limited availability of food resources, and as an adaptation to overcome predation risk by laying a replacement clutch (Frost and Shaughnessy, 1976). Although the Lesser Crested Tern generally lays a single egg, a small proportion of the population has clutches of two eggs (Cramp, 1985). The latter clutch proportion can range between 2% for the Persian Gulf population (Behrouzzi-Rad and Teyefeh, 2008) and 4% for the Australian breeding population (Nicholson, 2002). In the Mediterranean breeding population *T. b. emigrata*, this ratio was estimated as 12.7% at the Gara Island colony during the 1993 breeding season (Meininger et al., 1994).

Predation and food availability are the main limiting factors for the survival of seabird colonies (McLeay et al., 2010, Furness and Tasker, 1999, Anderson et al., 2005, Burger, 1988). The Mediterranean colonies were thought to have suffered predation of eggs and chicks by Yellow-legged Gulls

*Larus michahellis*, because that species occurs at the tern colonies of both the Gara and Elba Islands (Meininger et al., 1994), during this study no sign of gull predation have been reported, although the sympatric presence of these Gulls presents a probability of such predation to occur. In addition, non-avian predators (dogs) were proved to predate eggs and chicks during the present study (See Chapter 3), thus affecting the survival of the whole colony, particularly at Jeliana, which is located within an urban habitat and poorly protected.

The incubation period of Lesser Crested Tern ranges from 21 to 26 days with both parents alternating incubation. A pale black-spotted nestling then hatches led from the nest after two to four days and fed by the parents at the perimeter of the colony (Cramp, 1985). After seven days the chicks gather in a crèche that is guarded by the adult terns. The chicks fledge at 30 to 35 days, becoming fully independent at 5 months of age with first breeding at around 2 years (del Hoyo et al., 1996). Breeding success of a seabird depends on several factors including food availability, nest predation or failure, availability of suitable habitat, displacement by other colonial seabird species, disease and human disturbance (Anderson et al., 2007, Canova and Fasola, 1993, Ghasemi et al., 2011, Hamer et al., 1991, Parsons et al., 2008, Ramos, 2001, Suddaby and Ratcliffe, 1997). For the Lesser Crested Tern, breeding success is high for the early clutches, but reduces with the date of laying in the sedentary Australian population because of vulnerability to predation (Nicholson, 2002). In Libya most research conducted in recent decades concentrated on reporting population size and nest counts. Ringing of nestlings then started for identifying migration routes and wintering areas (Meininger et al., 1994, Hamza et al., 2008, Hamza et al., 2007, Gaskell, 2005, Bricchetti and Foschi, 1987, Baker, 1984) breeding and fledging success were not documented in the earlier studies due to the nature of that research being limited to the recording of population size and ringing of nestlings.

The overall aim of this chapter is to understand and update the breeding ecology information on the Mediterranean population, which will form the baseline data for conservation and management plans for this population. The Mediterranean Action Plan on seabirds calls for such field studies to fill

knowledge gaps, and will help to fulfil the Libyan government's commitment towards the protection of this Mediterranean breeding seabird population.

## **2.2 Aims, Objectives and hypothesis**

1. To update information on breeding, incubation, hatching and fledging dates of Lesser Crested Terns at their breeding sites in Libya.
2. To estimate the hatching, fledging success and breeding productivity; to investigate the relationship between colony productivity and diet diversity.
3. To investigate the potential relationship between breeding synchrony and colony size.
4. To study the relationship between colony size and inter-nest spacing.
5. To determine the causes of nest failure and colony disturbance.

The hypotheses tested in this chapter are:

1. Breeding initiation is expected to start at the most western site (Gara Island) followed by other sites to the east.
2. Synchrony is expected to be higher at larger colonies as in other tern species.
3. Eggs at the centre of the colony are expected to be larger in size compared to those laid on the periphery (by late less experienced birds).
4. Nesting density and inter-nest distance are negatively correlated with colony size (the larger the colony the more compact the nest spacing).
5. Breeding success is dependent on diet diversity of the young birds.

## **2.3 Description of study areas and methods**

The following is a description of each known breeding site in Libya (Figure 1.2 & 2.2), in addition to one breeding site visited in the Egyptian Red Sea (Table 2.1).

Table 2.1 Description of breeding sites in Libya and one breeding site in the Red Sea, Egypt

Colony name	Location	Description	Climate
<b>Gara Island</b>	Within the eastern side of the Gulf of Sirte at 30°48'N 19°54'E, ca.12Km off the coast near Ajdabiyah (Figure 2.3)	4.5 h, Sandstone Island with cliffs and gentle slopes surrounding low hills with scattered rocks to the north, east and western sides. The southern side have a short sandy beach. Low halophytic plants and some shrubs, with flat sandstone plain.	Air temperature: 13.5°C in January to 27°C in August. Rainfall: scarce (76mm in December to nil in June).
<b>Jeliana islet</b>	Located at Benghazi city centre, in the western lake of Jeliana at 32°05'N 20°03'E. A very small low lying islet (Figure 2.5)	Small (area c.35m <sup>2</sup> ), rocky base with mud and litter accumulation. Located at the northern section of Sebkheth Jeliana (30 ha. permanently flooded salt-marsh). In winter used by Cormorants and other water birds as a roosting site.	Air temperature: 13°C in January to 27°C in August. Rainfall: 67mm in December to nil in June.
<b>Elba Island</b>	Small, low-lying Island situated in the vicinity of the Gulf of Bumbah, 32° 14'N 23° 17'E, 2 km from the mouth of Ain Al Ghazalah bay (Figure 2.6 & 2.7)	Covering about 20 ha. Halophytic plants are the dominant vegetation, with a few shrubs and rocky beaches to the north and east, while the southern and western beaches are covered with dead sea weed banks.	Air temperature: 12°C in January to 23.5°C in July. Highest rainfall in December (54mm).
<b>Fteha Island</b>	A flat Island in the Gulf of Bumbah (Figure 2.8), at 32° 23' N, 23° 9' E).	Rectangular in shape, with a salt marsh wetland covering most of the west and southwest of the Island. Surface area of ca. 6 ha, elevated an average of <1m above sea level.	Air temperature and rainfall: similar to Elba Island
<b>Ashrafi archipelago, the Red Sea, Egypt</b>	A group of three coral Islands, located at the mouth of the Suez Gulf 27°46'N 33°41'E. Some other smaller islets located to the southeast of the main Islands. Average elevation 0.5-2m above sea level (Figure 2.9 & 2.10).	Formed with a coral substrate and sandy/rocky surfaces. The central Ashrafi Island has a depression which forms a wetland in the rainy season. The Island was arid at the time of the visit in August 2009, except for very few halophytic plants and a few shrubs on the southern tip of the Island. The colony found at Abukirsh Island south of central Ashrafi Island	Air temperature: 12°C in January to 37°C in July. Rainfall is rare, only 5.26 mm annually.

The habitat requirements of seabirds include access to suitable areas for breeding and foraging. These requirements are inter-dependent as without an adequate food supply parents are unable to raise their young to independence. Habitat requirements for breeding vary between species, but commonly include an area free from disturbance by terrestrial predators, storms and tide; presence of conspecific individuals, including potential mates and suitable access to foraging grounds. (Hulsman et al., 1997).

The above site description indicate the breeding habitat type required by the Lesser Crested Tern, that is mainly breeding on Islands, with sparse vegetation (Gara, Elba, Fteha and the Ashrafi Islands), these Islands are all offshore, with differences in location relative to the coast, as Gara is the furthest from the coast with more than 10 nautical miles, while both Elba and Fteha are located within smaller distance from the coast. In the case of Ashrafi Islands, it is located further offshore in the southern part of the Suez channel. The only exception was the islet of Jeliana, as an inshore site, close to the coast.

The breeding habitat requirements of this species are found to be diverse, the main feature in all breeding sites is to provide protection from predators and a breeding site utilised by the Lesser Crested Tern to form a single-species colonies at Gara, however, other breeding sites hosted additional breeding species around the Lesser Crested Tern colony border, such as the little tern *Sterna albifrons* and black winged stilt *Himantopus himantopus* in Jeliana islet, the little tern at Elba and the Yellow legged Gull *Larus michahellis* at Elba and Gara, and the white-cheeked tern *Sterna repressa* at the Red Sea colony of Ashrafi Island (Goodman and Meininger, 1989, Meininger et al., 1994, Personal Observations).

Short and sparse vegetation was found at and around the colony (except at Jeliana islet), however the site used in 2012 was also characterized with sparse halophytic vegetation. This plant cover provides shelter and a refuge to young terns from both heat stress and predators (*Personal observations*).

The nest is a scrape in sand or gravelly soil, in close proximity to other nests (Cramp, 1984). In Libya, the breeding colonies of this species are made of sandstone Islands (Gara Island); islets of mud and organic wastes (Jeliana) or

an Island composed of gravel and sand accumulations (Elba Island). The general shape of the colony varies: in Gara Island (the biggest colony) nests are made in bare sandstone soil with some soft sand around dry plants, on the North West edge of the Island. On Elba Island, the colony is established on soft sand gathered around halophytic plant communities at the centre of the Island.

In the Red Sea, the colony shape was quite different, where the colony is a strip measuring 2-3 metres in width and extends to 5-20m; inter-nest distances were tight as incubating birds were packed, probably as an anti-predator response to the high opportunistic predation pressure by the Red Sea endemic species of White-eyed Gull *Larus leucopthalmus* (Figure 2.1)



Figure 2.1 Habitat type and colony shape of Lesser Crested Tern at Ashrafi Islands, Red Sea. (a) Nest and colony pattern; (b) High-alerted Terns and predation pressure from White-eyed Gull.



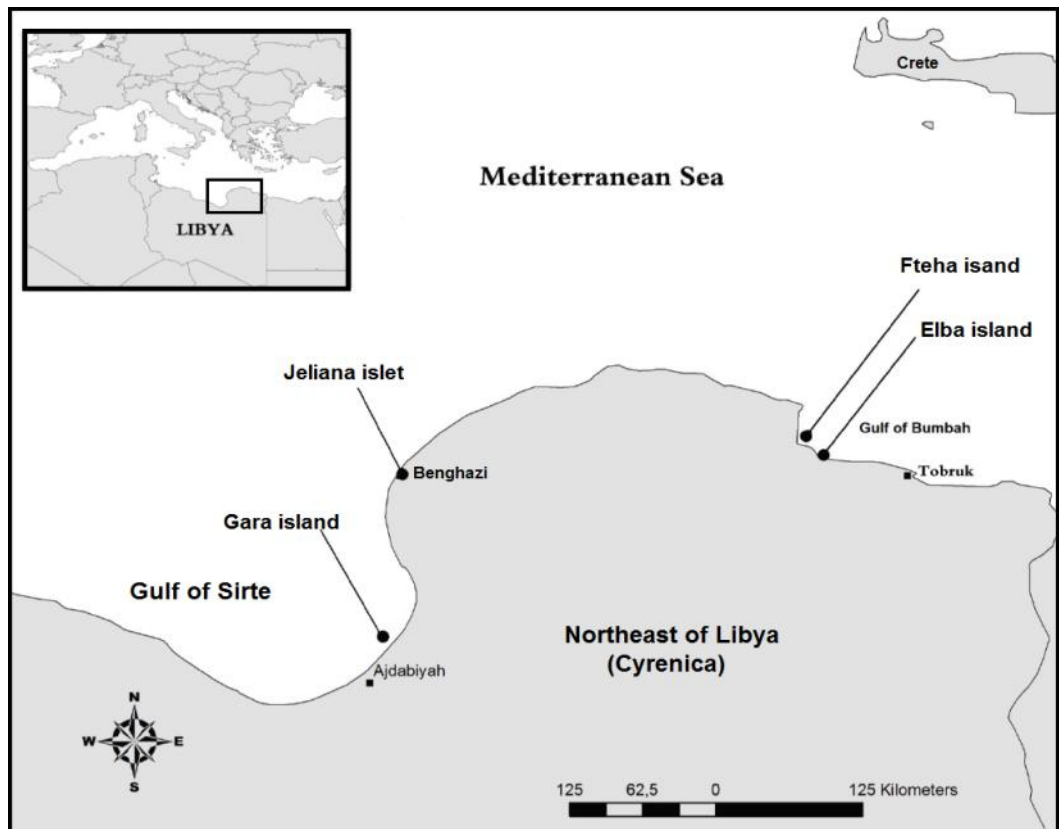


Figure 2.2 Breeding colonies of Mediterranean Lesser Crested Tern



Figure 2.3 General view of Gara Island.



Figure 2.4 The Lesser Crested Tern colony at Gara Island in 2009.



Figure 2.5 Jeliana islet in 2012, nestlings gathered in a crèche.



Figure 2.6 Satellite image of Elba Island (© Google Earth, January 2013).



Figure 2.7 Side of the Lesser Crested Tern colony at Elba Island in 2009.



Figure 2.8 Satellite image of Fteha Island (© Google Earth, January 2013).

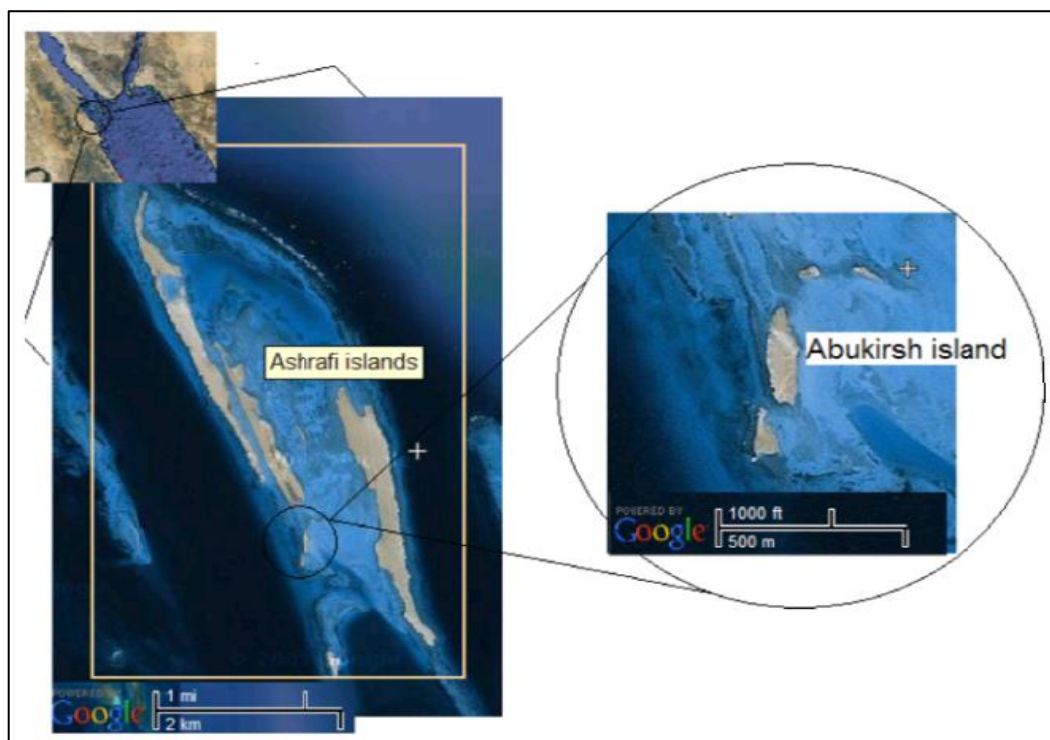


Figure 2.9 Location of Ashrafi archipelago, The Gulf of Suez, The Red Sea. Note the position of Abukirsh Island (© Google Earth, January 2010).



Figure 2.10 Lesser Crested Tern colony at Ashrafi archipelago, the Red Sea, Egypt in 2009.

## **2.4 Breeding Ecology parameters**

### **2.4.1 Breeding dates**

Field visits were made from June to July between 2009 and 2012 (excluding 2011 because of the events in Libya). One trip was made to the Ashrafi archipelago in the northern Red Sea, in August 2009. Comparable breeding ecology data from the Gulf region have been obtained from published literature on Saudi Arabian (Symens and Evans, 1993) and Iranian populations of the species (Ghasemi et al., 2011).

Sites were visited before the expected breeding initiation date, to document the presence of newly arrived adult birds that form the colony. Table (2.2) shows the visit frequencies for each site during the study period. Visits to Gara and Fteha Islands were not possible in 2012 due to the security situation in the area.

It is important to mention that the research methodology used was non-systematic, due to the nature of the study sites and limitations to access them equally and timely enough to conduct systematic research. The study sites are

all not legally protected at the time of fieldwork, which prevented investing in experimental field tools, as it was removed by locals when used, because of vandalism and to minimize attention to the breeding colonies, the research team preferred not to use such equipment.

Table 2.2 Dates of field visits to the breeding sites during the study seasons.

Breeding site	Field visits			
	2009	2010	2012	Total
Gara Island	5	5	non	10
Elba Island	6	4	5	15
Jeliana islet	10	1	4	15
Fteha Island	0	1	non	1
Ashrafi archipelago	1	non	non	1

#### 2.4.2 Colony formation and breeding population size

Based on personal observations during the present study, the Lesser Crested Tern colonies are formed by a founding group of breeding adults that arrive ahead of other colony members, and lay the first group of clutches usually in a compact pattern. These initial groups range from a few nests at Elba to about two hundreds at Gara. More birds then establish nests around those of the founding group. It is expected that as in other tern species the initial breeding pairs are more experienced individuals that select the breeding area within the site then followed by other less experienced pairs.

Several factors shape the population size and recruitment levels of a seabird population; such as immigration and emigration, survival rates (nestlings and adults), food availability, habitat quality, colony disturbance, predation levels and nest failures being the main factors that affect the breeding recruitment and consequently population size for a colonial seabird species (Burger and Gochfeld, 1991, Schreiber and Burger, 2002).

Population size at each breeding site has been estimated by doubling the total nest counts at the end of the egg laying period (assuming that each pair would

have one active nest). Data on the population size of previous seasons was also obtained from the literature. Non breeding population ranged from few individuals at Elba to hundreds at Gara.

### **2.4.3 Breeding synchrony**

Breeding synchrony has been described as “the tendency of individuals to carry out some stages of the reproductive cycle simultaneously with other members of the colony” (Findlay and Cooke, 1982). It is determined by recording the egg laying dates of the female birds and calculating the variance around the mean. When this variance is small, the population is considered to be “synchronous” and *vice versa* (Stutchbury and Morton, 1995). Breeding synchrony can be evolved due to local process (Physiological and behavioral), together with environmental cues, that can synchronize breeding between neighbours and through a whole colony (Jovani and Grimm, 2008).

The study of breeding synchrony in the present study is restricted to the stage of breeding initiation and egg laying period at each of the study colonies. It was estimated using the standard deviation value of clutch initiation date (Gochfeld, 1980) from the date of first clutch laid to the end of the egg laying period. When the synchrony value (SD) is low, it indicates high synchrony and vice versa (Burger and Gochfeld, 1991). A between-colony synchrony index has been compared using One-way ANOVA.

### **2.4.4 Egg volume, nesting density and inter-nest distances**

Egg dimensions (length and breadth, in mm) of the whole colony (at Elba and Fteha Islands) or a sample of randomly selected clutches (Gara , Jeliana and Ashrafi) were measured using digital calliper to the nearest 0.1mm, then weighed using a 100g digital scale, on the first or second day after being laid. Clutch size was estimated during the last week of incubation (Except at the Red Sea site). Nest and egg counting were made by direct counts at small colonies (Elba and Fteha Islands) otherwise they were counted from photograph transects that covered the whole colony (Jeliana, Gara and Ashrafi) to avoid both heat stress on nests and nest abandonment due to long visit disturbance. The volume of each fresh egg was calculated according to the following

equation (Meininger et al., 1994):

$$\text{Egg Volume} = 0.509 \times \text{Egg length (mm)} \times (\text{Egg breadth (mm)})^2 / 1000$$

Early arrivals lay their eggs at the colony centre due to experience, while late arrivals lay at the colony edges (Burger and Gochfeld, 1991, Hulsman, 1988). At the Gara colony, to assess the impact of laying order on Egg volume (experienced vs inexperienced adults), a sample of clutches from the colony centre and similar clutches from the colony edges were measured and compared in size and weight.

Nesting density was measured using quadrats of 100 cm<sup>2</sup>, Stratified sampling method were used to deploy quadrates throughout the colony area. The quadrates were placed along transect lines that passed through the colony area at several distance intervals. In small colonies (Elba and Fteha Islands), the quadrats were positioned to result in a stratified random sampling of the colony area and some adjacent vegetated areas and bare soil (Walsh et al., 1995)

At each colony, some nests were chosen for measuring inter-nest distance between each of a focal nest and all neighbouring nests in all directions. Measurements were taken between nest rims following method used in Collins and Taylor (2008).

#### **2.4.5 The incubation period**

Determining the incubation period is one of the fundamental breeding biology parameters that can be used in designing future conservation plans. Determining incubation duration at each colony, allows forecasting both hatching and fledging dates for that colony population. The incubation period in Lesser Crested Tern spans three weeks and incubation is carried out by both parents to maintain a suitable temperature for embryonic development. Personal field observations of Libyan and Egyptian breeding populations indicate that in hot climates, parent terns spend most of the time shading their eggs by their bodies, oriented to create a cool current rather than actual incubation, to prevent the lethal effect that heat stress has on eggs. This behaviour has also been observed in the Persian Gulf region Lesser Crested Tern colonies (Connor, 1980). Incubation (days) was estimated for all nests with



known laying and hatching dates. Laying dates were determined by the observation of newly laid clutches, which at small colonies (Elba) were numbered using a pencil on the broad edge of the egg, and at larger colonies a sample of 60 nests was monitored (Gara and Jeliana) to document accurately the duration of incubation period. Incubation days are calculated by the subtraction of egg hatching date from egg laying date (Meininger et al., 1994, Burger and Gochfeld, 1991).

#### **2.4.6 Reproductive success**

To assess reproductive success, nests were monitored up to four days after hatching. The nestlings would then join a crèche of older chicks and move away from the nest sites. It was possible only at Elba and Jeliana to count (or estimate) the crèche size at each visit and follow up the fate of the chicks, either surviving or lost. When a chick was reported as lost, it would have died due to lack of feeding by the parent terns (starvation) or when no trace of the chick was found, it is assumed either to have been predated (presumably by Yellow-legged Gulls or feral dogs at Jeliana) or collected by local poachers (at Elba).

#### **2.4.7 Hatching and breeding productivity**

Hatching success is defined as the proportion of eggs laid that hatched (i.e. % eggs hatched / eggs laid) and breeding productivity as the proportion of nestlings that fledged (i.e. % chicks fledged / nestlings). Visits to sites at dates of expected hatching were made to accurately record the first day of hatching. The expected hatching date was estimated by calculating the incubation period of known dated nests (Meininger et al., 1994, Burger and Gochfeld, 1991). Breeding productivity has been estimated by dividing the ground count of all chicks present at the colony just prior to fledging by the number of breeding pairs (Burger, 1979, Burger and Gochfeld, 1991).

#### **2.4.8 Nest failures**

In the study nest failure includes all eggs (clutches) that failed to hatch or nestlings that failed to survive after hatching. Un-hatched eggs and dead young were counted throughout the season until the fledging phase. Failed nests/dead nestlings were observed at all breeding sites in Libya (but not at the Ashrafi site

in Egypt as it were visited only during the early incubation period). A nest was defined as failed if no parent bird was seen incubating for three consecutive visits (Burger and Gochfeld, 1991). Signs of abandonment also include the accumulation of guano on the egg (personal observations). An egg is considered infertile if the parent bird was incubating it for more than 26 days (Cramp, 1985). Infertile/addled eggs were defined as eggs which were either not fertilized or failed to hatch because of some deformity during egg production (Braby et al., 2001 ); All infertile, un-hatched eggs and dead nestlings were pooled as failed nests /site/season.

#### **2.4.9 Statistical analyses**

Egg volume and weight have been compared between Libyan breeding sites and the site visited in Egypt using One-way ANOVA. Data were tested for normality, and found to be normally distributed. Where variance was not homogeneous, ANOVA was then used after the logarithmic transformation of egg volume value, to overcome the violation of unequal variances in ANOVA. Comparison of egg volume between two seasons for the same site (Elba) was conducted using t-test on log transformed data. Breeding synchrony (Standard deviation of mean egg laying date) was calculated by changing each cell containing a date (day/month/year) into a number value, then average values and standard deviations were calculated in Excel (Burger and Gochfeld, 1991).

### **2.5 Results**

#### **2.5.1 Breeding dates**

Identifying breeding initiation dates (clutch initiation date) is necessary in order to calculate the length of the incubation period, hatching and fledging dates. In the present study, adult terns first arrived in early May at Jeliana, Benghazi (May 10<sup>th</sup>). Then after a whole month, during the second and last third of June they initiated breeding at Gara and Elba colonies, respectively. The geographic location of Jeliana islet, midway between Gara to the southwest and Elba to the east, indicates that adult terns do not initiate breeding at the most proximal site to the west (Gara) as expected, the adult terns arriving from a westerly direction. This could be explained by possible differences in migration dates and/or routes

used by subpopulations of Lesser Crested Tern which apparently travel in separate groups to reach the different breeding sites.

### **2.5.2 Colony formation and breeding population size**

In the 2009 season the colony at Gara Island was initiated in mid-June with two sub-colonies of 390 and 115 nests (counted three days after the actual breeding initiation date). With the progress of the egg laying period, both sub-colonies then grew and merged in to one large colony of 1,935 nests containing 2,125 eggs. In the following 2010 season, the number of nests reached 1958 with 2,200 eggs (Table 2.3). The colony site within the Island had moved in an anticlockwise direction from the previous year's location (reported also in other Lesser Crested Tern colonies (PERSGA/GEF, 2003)). No breeding data were collected for the 2011 and 2012 seasons due to the security situation near the port of Zouitina, which used to cross by sea to Gara Island.

At the at Elba Island colony during the 2009 season, three sub-colonies of 16, 27 and 5 nests had eggs laid between June 26<sup>th</sup> and July 2<sup>nd</sup>. The sub-colonies were positioned at an average distance of 2.3m from each other in a triangular layout. In the 2010 season, on a different site within the same Island (to the northeast of the 2009 colony location), terns built 16 nests and laid a total of 19 eggs between June 27<sup>th</sup> and July 1<sup>st</sup>; while another 25 single-egg nests were laid on Fteha Island . In the 2012 season the Elba colony consisted of 28 nests with egg laying between June 24<sup>th</sup> and 30<sup>th</sup> (Table 2.3).

A new breeding site was discovered in 2010 at Fteha Island; it is located eleven nautical miles to the northeast of Elba (see the above description of breeding sites). This site became a new active colony due to the separation of the breeding population at Elba into two groups; one group remained at Elba and a second group of 25 pairs initiated breeding at Fteha Island (this study). This was a potential response to the disturbance near the Elba colony, caused by local visitors (see Chapter 6 Final Discussion).

It was not possible to visit the colony at Fteha Island during the 2012 season, because of adverse sea weather conditions. The Etesian (annual) winds locally known as Meltem, are northerly summer winds blowing in the whole of the

Aegean Sea and eastern Mediterranean from late May to early October. These result from a high pressure system over the Balkans/Hungary area and a relatively low pressure system over Turkey, which affects most of southern Mediterranean Libyan waters, preventing sailing for several days or weeks (Bulugma and El-Gzeri, 1997). Breeding at Fteha would be expected, due to the reduced colony size at Elba, as happened in 2010 when the population of Elba split and part of it bred at Fteha Island.

At Jeliana in 2009 the colony was composed of 68 nests, but only 4 nests survived the flooding of the whole islet that took place when the outlet channel to the sea was blocked due to municipal works. In the 2010 season the colony contained 72 nests prior to the expected hatching in June, Feral dogs had access to the islet and with a decreasing water level due to municipal development to deepen the lake, and this led to a complete loss of the colony for that season. Table 2.3 shows the number of nests, mean breeding dates and duration of egg laying periods.

Table 2.3 Egg laying dates of Lesser Crested Terns in Libya.

Site	Year	Nests	Mean laying	SD	First egg	Last egg
Jeliana islet	2009	62	24 May	±2.74	20 May	28 May
	2010	70	19 May	±2.73	15 May	23 May
	2012	402	24 May	±3.03	20 May	29 May
Gara Island	2009	1935	22 June	±4.47	15 June	29 June
	2010	1958	22 June	±3.89	16 June	28 June
Elba Island	2009	48	29 June	±2.45	26 June	2 July
	2010	16	29 June	±1.58	27 June	1 July
	2012	28	27 June	±2.16	24 June	30 June
Fteha Island	2010	25	28 June	±2.45	25 June	2 July

In 2012, some financial support was obtained from the French Ministry of Environment, to rehabilitate the Jeliana islet and increase its surface area and elevation, to avoid flooding and provide better breeding capacity for the terns. The surface area of the islet was increased to 114m<sup>2</sup>, the works being conducted by volunteers from Tripoli and Benghazi and coordinated by the author in March. In June 2012, the colony size increased by 300%, and

effectively used the whole rehabilitated islet, as 402 nests were laid there. In addition a further 390 nests were built on a new Island resulting from the deepening works of 2010. The good wet season in spring 2012, helped to flood the lake after it had dried out in 2011 and further shape the new Island (Figure 2.10).

Monitoring of colony chronology in the present study showed that parental care of the young started from the second week of June (at Jeliana) through to the end of August (at the other three sites). Fledging started from the first week of July through to mid-August (Jeliana) and in late August to early September (the other sites).

Population size showed different trends at each of the three colonies. At Gara (Figure 2.11) the average population size (mean  $\pm$ SD) was 1743 ( $\pm$ 374) breeding pairs, with a slight annual increase of 20% between 2006 and 2009. During 2010 there was a slight decrease in population size, possibly due to changes in recruitment of new breeding birds, as disturbance, inundation and predation were not observed at Gara during the monitoring seasons.

At the colony of Jeliana the average population size was 158 ( $\pm$ 108) breeding pairs (Figure 2.12). The high standard deviation in population size at Jeliana is caused by the recent 300% growth in population size following restoration of breeding habitat in 2012, by extending the surface area of the islet. At the colony of Elba, the average population size was 26.6 ( $\pm$ 9.34) breeding pairs, with inter-annual fluctuations from 1993 to 2012 (Figure 2.13).



Figure 2.11 Google Earth images of Jeliana Lake before (above) during 2010 and after restoration works (below) in 2012

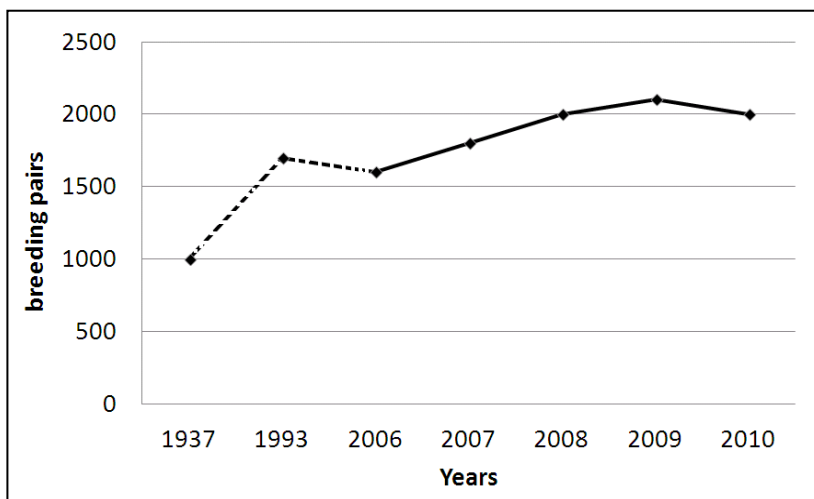


Figure 2.12 Breeding population size of Lesser Crested Tern *Thalasseus bengalensis* at the Gara colony (1937-2010).

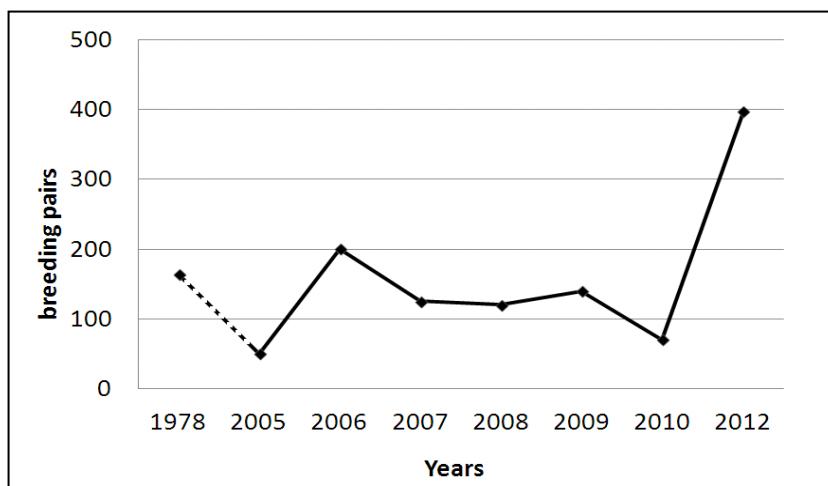


Figure 2.13 Breeding population size of Lesser Crested Tern *Thalasseus bengalensis* at the Jeliana colony (1978-2012).

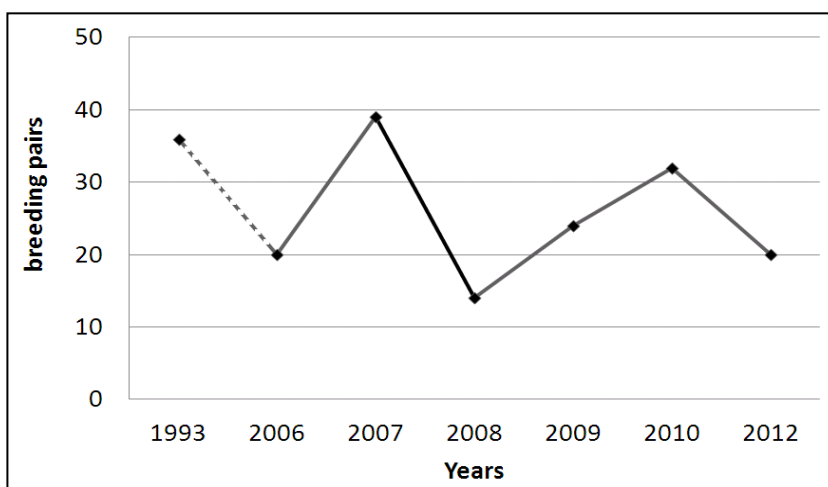


Figure 2.14 Breeding population size of Lesser Crested Tern *Thalasseus bengalensis* at the Elba colony (1993-2012).

### 2.5.3 Breeding synchrony

The study of breeding synchrony of these colonies was restricted to the observation of the egg laying period, starting from the first clutch laid until the end of the egg laying period. Table 2.4) shows within-colony synchrony (as SD of mean egg-laying date) for each colony. Synchrony ranged from 1.58 at Elba during 2010 to 4.47 at Gara during 2009. Both colonies of Jeliana and Elba had significantly higher breeding synchrony rates (lower SD values), compared to Gara (One-way ANOVA,  $F=17.16$ ,  $P < 0.05$ , d.f. =3). This is explained by the longer egg laying period at Gara relative to the other colonies.

Table 2.4 Colony size, mean date of clutch initiation and synchrony of egg laying period for Lesser Crested Tern colonies in Libya

Breeding season	Colony	Number of breeding pairs	Mean laying date	SD of mean Egg laying
2009	Jeliana	68	24 May	2.74
2010	Jeliana	37	19 May	2.73
2012	Jeliana	210	24 May	3.03
2009	Elba	23	29 June	2.45
2010	Elba	10	29 June	1.58
2012	Elba	14	27 June	2.16
2010	Fteha	12	28 June	2.45
2009	Gara	1063	22 June	4.47
2010	Gara	1093	22 Jun	3.89

Synchrony was strongly correlated with colony size (Spearman's  $\rho = 0.89$ ,  $n=10$ ,  $P < 0.001$ ). The larger the colony the more days spent in egg laying, compared to smaller colonies that conclude the egg laying period in a few days (Figure 2.15).



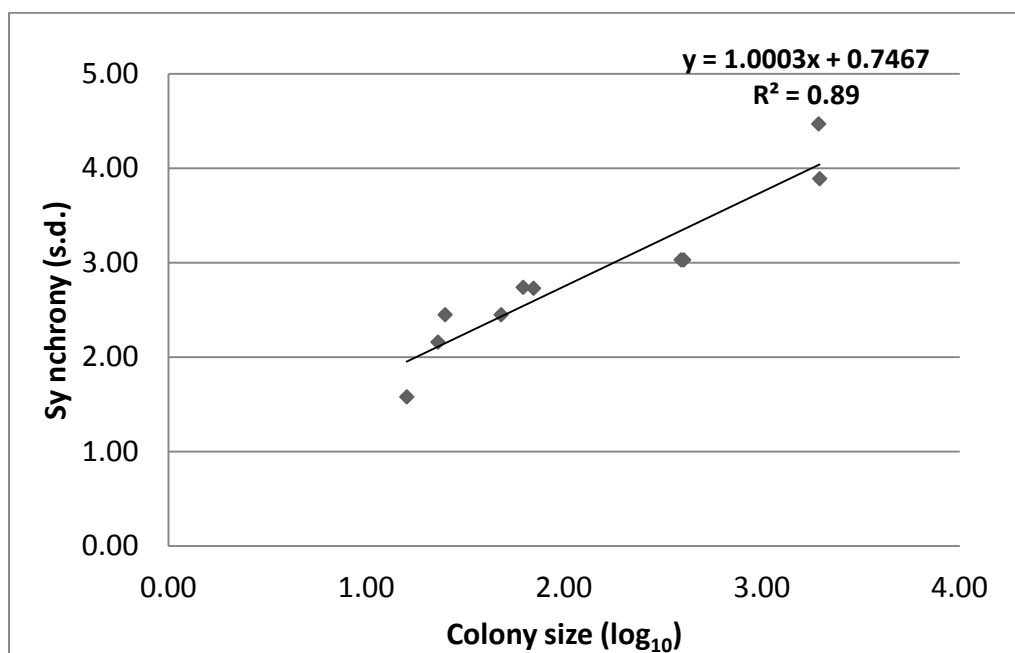


Figure 2.15 Relationship between breeding synchrony and colony size.

#### 2.5.4 Egg volume, nesting density and inter-nest distance

Mean length, breadth and volume ( $\pm$ SD) of 187 eggs (fresh first or second day laid only) from the four breeding sites in Libya and one breeding site in the Red Sea, Egypt, were 52.24 mm ( $\pm$ 2.4 mm), 36.30mm ( $\pm$ 1.88 mm) and 35.22 cm<sup>3</sup> ( $\pm$ 4.55 cm<sup>3</sup>) respectively. The mean fresh mass of an egg was 34.84g ( $\pm$ 0.49g, n=166). The mean clutch size was 1.07 (range =1.0-1.18). Data published in the literature for Libya 1993 (Meininger et al., 1994), and for the Gulf region population (Ghasemi et al., 2011) have been included for comparison (Table 2.5). Egg volume differences between sites (regardless of sampling year) show overall significant differences (One-way ANOVA,  $F(4, 1) = 63.7$ ,  $p < 0.01$ ). Multiple comparisons of egg volume (using the Tukey HSD test) indicate a significant difference between both Fteha and Ashrafi Islands and eggs from the other three sites, but no significant difference was found among eggs from Elba, Gara and Jeliana (Figure 2.16).

Two-egg clutches were found at all sites, except Elba in the 2012 and Fteha in the 2010 seasons. The proportion of two-egg clutches was highest at Gara in 2010 at 12% (similar figures in the 1993 season by Meininger et al., 1994) whereas it was lowest at Jeliana in the 2010 season at 2.8%. At Ashrafi it was only 0.7% (Table 2.5).

Table 2.5 Weight (g) and measurements (mm) of fresh Lesser Crested Tern eggs at the breeding sites.

Site	N	Measurement	Mean $\pm$ SD	Range	Data Source
<b>Jeliana 2012</b>	21	Length	52.3 $\pm$ 1.27	50.3-55.7	This study
		Width	37.6 $\pm$ 0.84	36.5-39.3	
		Weight	n/a	n/a	
		Volume	37.67 $\pm$ 2.14	34-3-42.9	
<b>Elba 2009</b>	10	Length	52.7 $\pm$ 1.92	50.2- 53.4	Meininger et al. (1994)
		Width	36.7 $\pm$ 1.31	36.4-38.8	
		Weight	n/a	n/a	
		Volume	36.14 $\pm$ 2.82	32.8-41.0	
<b>Elba 2010</b>	66	Length	53.03 $\pm$ 1.95	47.6 - 57.8	This study
		Width	36.95 $\pm$ 1.00	34.4 - 39.1	
		Weight	37.17 $\pm$ 2.80	29.6 - 44.8	
		Volume	37.29 $\pm$ 2.84	31.3-43.8	
<b>Fteha 2010</b>	20	Length	48.33 $\pm$ 2.31	45.4 - 53.1	This study
		Width	32.53 $\pm$ 1.80	29.2 - 36.1	
		Weight	34.48 $\pm$ 3.14	29.2 - 43.1	
		Volume	26.2 $\pm$ 3.95	19.8-35.2	
<b>Gara 1993</b>	30	Length	53.1 $\pm$ 2.09	49.4-56.9	Meininger et al. (1994)
		Width	37.6 $\pm$ 1.21	35.0-39.0	
		Weight	n/a	n/a	
		Volume	38.38 $\pm$ 3.29	31.7-43.8	
<b>Gara 2010</b>	59	Length	52.92 $\pm$ 2.19	47.6 - 58.7	This study
		Width	36.66 $\pm$ 1.46	28.3 - 39.4	
		Weight	34.03 $\pm$ 5.73	20.6 - 42.7	
		Volume	36.28 $\pm$ 3.39	22.2-43.9	
<b>Ashrafi 2009</b>	20	Length	51.44 $\pm$ 1.53	48.4 - 55.1	This study
		Width	35.44 $\pm$ 0.99	33.6 - 37.4	
		Weight	31.86 $\pm$ 2.83	27.3 - 38.8	
		Volume	32.91 $\pm$ 2.16	29.6-37.7	
<b>The Persian Gulf region</b>	229	Length	49.33 $\pm$ 0.53	n/a	Ghasemi et al. (2011)
		Width	33.93 $\pm$ 0.38	n/a	
		Weight	32.33 $\pm$ 0.79	n/a	
		Volume	27.78 $\pm$ 0.91	n/a	

Table 2.6 Breeding biology parameters of Lesser Crested Tern colonies in Libya (2009, 2010 and 2012 seasons)

Colony	Gara		Elba			Fteha	Jeliana			Ashrafi
Year	2009	2010	2009	2010	2012	2010	2009	2010	2012	2009
<b>Mean clutch size</b>	1.13	1.07	1.04	1.19	1.0	1.0	1.09	1.05	1.04	1.01
<b>No. of single-egg clutches</b>	1935	1023	42	16	28	24	62	70	377	967
<b>No. of two-egg clutches</b>	190 (8.9%)	140 (12%)	2 (4.5%)	2 (11.1%)	0 (0%)	0 (0%)	6 (8.8%)	2 (2.8%)	25 (6.2%)	7 (0.7%)
<b>No. of breeding pairs</b>	1063	1093	23	10	14	12	68	37	210	1934
<b>No. of eggs</b>	2126	1163	46	20	28	25	74	74	420	981
<b>No. of nestlings hatched</b>	1525	861	25	12	15	?	4	0	269	?
<b>Hatching success</b>	72%	74%	52%	63%	56%	?	5.70%	0%	64%	?
<b>No. of nests hatching young</b>	1493	820	23	9	15	?	4	0	228	?
<b>No. of chicks fledged</b>	1196	832	20	8	13	?	4	0	250	?
<b>Fledging success</b>	78.40%	96.60%	80%	67%	86%	?	100%	0.00%	93%	?
<b>Breeding productivity</b>	0.56	0.72	0.43	0.40	0.46	?	0.05	0.00	0.60	0.0
<b>Mean breeding productivity</b>	0.64		0.43			0.0	0.22			0.0

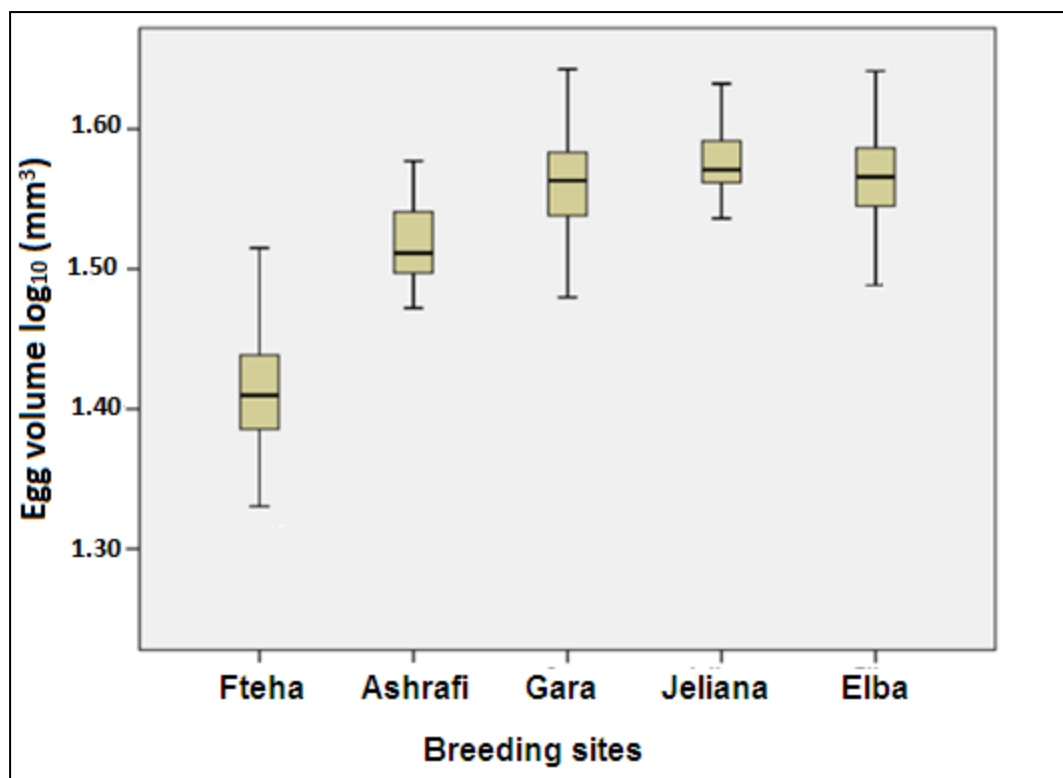


Figure 2.16 Mean egg volume ( $\log_{10}$ ,  $\text{mm}^3$ ) of Lesser Crested Tern at the study sites.

Between-season comparison of egg volumes was possible only at Elba, as data for two years (2009-2010) were available. There was no significant difference in mean egg volume (t-test,  $t(64) = 1.65$ ,  $P > 0.05$ ) at Elba between the 2009 season ( $M = 1.57$ ,  $SD = 0.032$ ) and in 2010 ( $M = 1.55$ ,  $SD = 0.038$ ).

The mean lengths of eggs from the centre of the Gara Island colony were significantly larger than those at the edges of (One-way ANOVA  $F = 4.60$ ,  $p < 0.05$ ). However, no significant differences were found between either the egg breadth (One-way ANOVA,  $F = 1.12$ ,  $p > 0.05$ ), nor the egg volume (One-way ANOVA,  $F = 3.4$ ,  $p > 0.05$ ) of the same samples.

Egg masses (weights) were obtained for all sites for at least one season (in Elba for the two successive seasons 2009 and 2010, data pooled for this comparison). There were significant differences in mean egg weight between the study sites (One-way ANOVA,  $F(4, 1) = 8.87$ ,  $p < 0.001$ ). Tukey HSD tests for multiple comparisons revealed that the significant differences in egg weights are between eggs from Gara and each of Elba and Jeliana, also there was a difference between eggs of the Red Sea site and both Jeliana and Elba (Table 2.7).

Table 2.7 Multiple comparisons (using the Tukey HSD test) of egg mass at the study sites

	<b>Jeliana</b>	<b>Elba</b>	<b>Fteha</b>	<b>Ashrafi</b>
<b>Gara</b>	0.003*	0.005*	0.99	0.24
<b>Jeliana</b>	-	0.72	0.06	0.001*
<b>Elba</b>		-	0.26	0.001*
<b>Fteha</b>			-	0.25

\* The mean difference is significant at the 0.05 level.

The nesting density (nests/m<sup>2</sup>) ranged between 2.6 nests/m<sup>2</sup> at Elba to 8.8 nests/m<sup>2</sup> at Gara (Table 2.8). Inter-nest distance -the distances (cm) between a nest and the neighbouring nests- the minimum inter-nest distance was at Gara Island colony (12.0 cm) whilst the maximum distance was at Fteha colony (88.0 cm), where the inter-nest distances greater than 40 cm represent 64.5% of the data. Comparable figures were only 6%, 3.2% and 3.1% for Elba, Jeliana and Gara colonies respectively, due to the dispersed nest layout over larger area at Fteha compared to the other three colonies.

Table 2.8 Inter-nest distance (cm) and nest density (nests/m<sup>2</sup>) at the breeding sites of Lesser Crested Tern in Libya

	<b>Gara Island</b>	<b>Jeliana</b>	<b>Elba</b>	<b>Fteha</b>
<b>Nest density (nests/m<sup>2</sup>)</b>	8.8	5.5	2.6	3.2
<b>No. of quadrates used</b>	21	20	8	5
<b>Mean inter-nest distance (cm)</b>	18.1	22.6	27.8	48
<b>±SD</b>	6.8	6.9	6.43	15.7
<b>Maximum inter-nest distance (cm)</b>	40	42	50	88
<b>Minimum inter-nest distance (cm)</b>	12	13	21	25

Mean inter-nest distance differed significantly between the study sites (One-way ANOVA  $F(3, 1) = 57.24, p < 0.001$ ). Comparisons between individual breeding sites, showed no significant difference in mean inter-nest distance, neither between the Gara and Jeliana colonies nor between the Elba and Jeliana colonies ( $P > 0.05$ ). The main significant difference was between Fteha and the

other three sites (Table 2.9), due mainly to the wider spatial dispersal of the nests within this colony.

Table 2.9 Multiple comparisons (Tukey HSD) of mean inter-nest distance for the breeding sites

	<b>Jeliana</b>	<b>Elba</b>	<b>Fteha</b>
<b>Gara</b>	0.26	.000*	.000*
<b>Jeliana</b>	-	.11	.000*
<b>Elba</b>	-	-	.000*

\*. The mean difference is significant at the 0.05 level.

### 2.5.5 Effect of colony size on mean egg laying initiation date

Pooling the data from all Libyan breeding colonies across the study years, showed no evidence of significant correlation between colony size and egg laying date (Spearman Rank Order Correlation  $r = -0.26$ ,  $Z = -0.78$ ,  $p > 0.05$ ,  $n = 10$ ).

### 2.5.6 The incubation period

The incubation period of the species has been stated to range between 21-26 days (Cramp, 1985). This period reported from the study sites in Libya, during the 2009-2010 and 2012 seasons has ranged from 19 days at Jeliana in 2010 to 26 days at the Elba colony during the 2009 season (Figure 2.17). The incubation periods at the Jeliana colony in 2009 and 2010 were one and two days (respectively) shorter than the known lower limit of the species incubation period (21 days), this was also the case at the Elba colony in 2009. Changes in incubation period length have been detected only at the Jeliana and Elba sites, where predation (at Jeliana) and egg poaching (at Elba) are the two main threats for the colonies during the preceding breeding seasons.

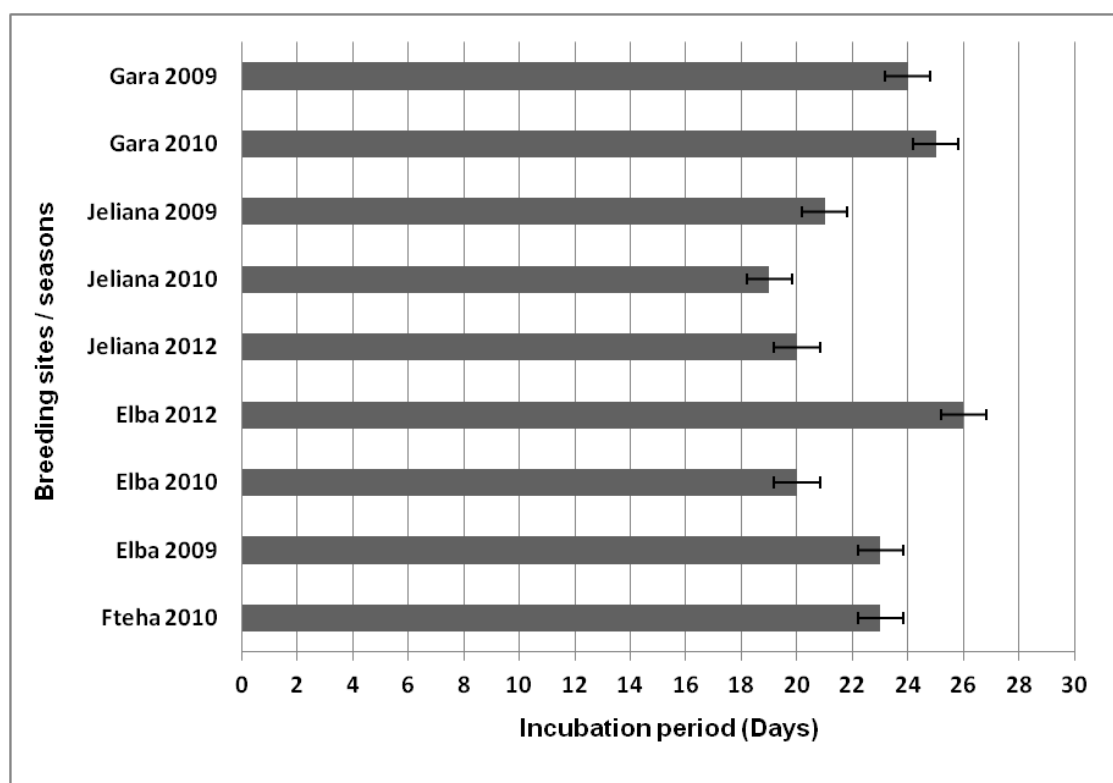


Figure 2.17 The incubation period length (Mean  $\pm$  Standard Error) of Lesser Crested Tern at the study sites.

### 2.5.7 Hatching, fledging success and breeding productivity

Hatching success is the proportion of hatched eggs relative to all laid eggs at each colony in each season. Hatching success was highest during 2009 and 2010 at Gara, with 72% and 74% of eggs hatched respectively. The lowest hatching success was in 2010 at Jeliana with no hatched eggs, as they were all predated by feral dogs, who reached the islet site due to the dewatering of the Lake for the development works. The hatching success at Elba ranged from 52% during 2009 season to 63% during the 2010 season (Table 2.6 Table 2.10).

Fledging success (number of fledged chicks/ number of nestlings) was highest at Gara in 2010 (96.6%), the lowest fledging success being at Jeliana in 2010 with no nestlings fledged with that of Elba in 2010 at 67%. Breeding productivity (#of fledged chicks/breeding pair) ranged from complete colony failure at Jeliana during 2010 to 0.05 in 2009 to a maximum of 0.72 chicks/breeding pair.

It was not possible to obtain similar breeding parameters at Fteha and the Ashrafi archipelago. At Fteha five nestlings had hatched during the first visit to the site on July 9<sup>th</sup> 2010, but on July 20<sup>th</sup> the colony was deserted and only four adult pairs were seen on the Island. After a thorough search on the Island and the surrounding waters, no trace of nestlings or adults were found. It became apparent later on the day that local poachers camped on the Island two days before the visit (July 18<sup>th</sup>), and collected the nestlings. During the field visit two egg shells were present a few metres from the colony site and 20 Yellow-legged Gulls were found close to the colony site. Although no trace of predation by gulls was observed on either eggs or nestlings, this cannot be excluded as another cause of colony loss. At Ashrafi the field visit was conducted during the second week of incubation and it was subsequently not possible to obtain further data on the site. However, egg predation by White-eyed Gull *Larus leucophthalmus* was reported, taking six eggs during 30min visit (personal observations), which suggests that incubating terns at this particular colony were under imminent predation stress by this gull species, which is in turn a protected species in the Red Sea region (Baha El Din, 2003), presenting a management issue for both species there.

#### **2.5.8 Nest failures**

The nest is considered to have failed if the egg did not hatch by the end of the expected incubation period, or the egg hatched but the nestling did not survive, due to starvation, predation or nest abandonment by the parent birds (Table 2.10).

At the Gara colony, nest abandonment and un-hatched clutches were the main causes of nest failure during both 2009 and 2010 seasons. It was not possible to attribute specific percentages to each cause, because of the limited number of visits during the latter part of the breeding season. At Elba, nest desertion was responsible for most of the nest failures (80%), while 20% were related to nestling starvation, which found dead near the colony perimeter



Table 2.10 Nest failures of Lesser Crested Tern during the study period

	2009	2010	2012	Total by site
<b>Gara</b>	601	302	n/a	903
<b>Elba</b>	21	8	13	42
<b>Jeliana</b>	70	74	151	295
<b>Fteha</b>	n/a	25	n/a	25
<b>Total by season</b>	692	409	164	1,265

While at Jeliana the flooding of the lake destroyed the whole colony in 2009, while dewatering in 2010 allowed feral dogs to access and to destroy the colony in 2012 approximately 53% of failed nests were due to un-hatched clutches (infertile) while the remaining 47% were nestlings lost because of starvation or loss of parent birds. At Fteha, poaching by local hunters and campers in addition to potential predation by Yellow-legged Gulls are believed to have caused the failure of the whole colony in 2010. No data were collected at this site in 2012 as well as at Gara because of adverse sea condition and security concerns.

## 2.6 Discussion

The breeding ecology of Mediterranean Lesser Crested Tern *T. b. emigrata* has not been detailed in previous surveys (Moltoni, 1938, Meininger et al., 1994, Bundy, 1976, Baker, 1984). The Mediterranean Action Plan on Seabirds relating to this population highlights the study of breeding ecology as one of its priorities (UNEP-MAP-RAC/SPA, 2003). Although previous surveys have reported on the start of breeding (based on calculation of the incubation period of eggs), no details was known on rates of nest failure, the breeding success, the breeding productivity or the fledging success (Hamza et al., 2007, Azafzaf et al., 2006), because the previous surveys were conducted during the early stages of nestling growth, mainly for census and ringing purposes. Reporting on long-term breeding ecology data is a fundamental requirement for monitoring population growth, conducting population modelling and preparation of management plans for each

of the breeding colonies. This study documents the breeding phenology of Lesser Crested Terns in Libya, based on three years of field data.

Breeding dates differed between the Jeliana colony (centrally located) and both the Gara colony (to the southwest) and the Elba colony (to the northeast). The initiation of breeding at Jeliana was approximately one month earlier than at the other two sites, which could indicate differences in departure from the wintering areas in West Africa. The population breeding at Jeliana, may start the journey to their breeding site (s) earlier than the other populations, and consequently arrive earlier to breeding site.

The cues that trigger birds to migrate and initiate breeding at a different area are well documented in temperate zone breeding species (Hau et al., 1998, Hau, 2001), but for those wintering in a tropical region (e.g. West Africa), there are less seasonal and climatic variations compared to those at temperate zones. Most temperate and arctic animals rely to a high degree on photoperiodic stimuli for controlling reproduction, by activation of a certain set of hormones that stimulate the reproduction process, whereas in tropical zones these contrasting differences in photoperiod do not have the same magnitude (Hau, 2001, Wingfield et al., 1992) The timing of breeding in animals inhabiting environments with only slight seasonal fluctuations such as the tropics is poorly understood (Wingfield et al., 1992), although some bird species were found to utilize minor changes in photoperiod in the tropics to start migration (Hau et al., 1998). Sympatric tern species in West Africa such as the Sandwich tern *Thalasseus sandvicensis* and the Arctic terns *Sterna paradisaea* may have with Lesser Crested Tern common cues that trigger the instinct of migration northwards.

The migration pattern of Lesser Crested Terns as small groups of 2-4 individuals (Jacob, 1983), along the west and north-western African coast (Isenmann and Moali, 2000, Isenmann et al., 2005, Cramp, 1985), making them not particularly notable, because of both the small size of migrant groups and their mixing with other seabird species. They stop at certain sites to feed and rest as shown by sightings of ringed terns, e.g. in the Straits of Gibraltar and at Ceuta (Paracuellos and Jerez, 2003), at Tangir and Sousse Massa in Morocco (Thévenot et al., 2003). This pattern of migration may contribute to the asynchronous arrival at the breeding sites. Field observations in southern Italy (the Island of Sicily) showed

that, in late spring Lesser Crested Terns do associate with later breeding Sandwich Tern then move southwards towards their breeding sites in North Africa (Brichetti and Fracasso, 2006). This suggests that adult tern groups migrate along the North African coast and then split somewhere in northern Tunisia, where one group crosses to southern Italy, then move southwards to the Libyan coastline, arriving earlier than those followed the whole coastline of Tunisia and Libya (1,500-2,000 km). This may explain the earlier arrival of breeding birds at Jeliana islet, several weeks before others arrive at both Elba and Gara. One pair of Lesser Crested Terns found breeding within a sandwich tern colony at the Venice lagoon, northern Italy (Scarton, 2000), the estimated laying date of the single nest being 20-23 May 1999, the same date of breeding initiation as at Jeliana. This supports the suggestion here, that Italy is used as the final part of a migration route by these terns on the way south to Libya. Increasing ring sightings and monitoring of passing groups of Lesser Crested Tern in southern Italy and northern Tunisia could further support this assumption.

The other indication that there is a temporal segregation in migration between birds from Jeliana and the other colonies, is that adult birds are being sighted in northern Algeria and Morocco during the second half of August, when the first wave of small groups of Lesser Crested Tern migrate back from breeding to wintering sites (Thévenot et al., 2003, Isenmann and Moali, 2000). These birds could originate from Jeliana or be non-breeding individuals from Gara or Elba populations, because at that time of the season, breeding birds would be still be rearing their chicks at both Gara and Elba.

Another possible explanation for the asynchronous arrival and colony initiation is the significant differences of diet species diversity and quantity at Jeliana compared to the other sites. Abundance of food both inshore and offshore at Jeliana site may trigger the early arrival of breeding terns to this site. Diet diversity in the present study was highest at Jeliana, with 18 different prey species consumed by the young terns, while at both Elba and Gara number of prey species found in the young regurgitations were 8-10 species. Lesser Crested Terns breeding at Jeliana can be relatively considered as generalist feeders compared to their conspecifics at both Elba and Gara. Faria et al. (2010) found that generalist feeding birds are more able to find sufficient food during

migration and after arrival to breeding areas, than those with specialized -limited diversity- diets. This can be another cause of the early arrival at Jeliana breeding site, where prey diversity in the diet plays an important role in arrival and breeding initiation date.

At Elba, the population size fluctuated across the study years, inter-annual fluctuations accounted for 57% variability in breeding population size, as the more than half of the colony members moved to Fteha Island in 2010 and 2012. Human-related disturbance at this colony is considered the major limiting factor for population growth, in addition to mortality at wintering sites. The only ringed bird sighted outside breeding range was a dead recovery found on the beaches of National Park of Souss Massa, Agadir, Morocco in March 2013.

Most “crested tern” species have similar colony formation characteristics, starting with a small founding group, which selects nest sites, initiate nest formation and the egg laying. These individuals would be followed, perhaps daily, by other immigrating breeding adults who then lay their eggs around the first group of nests (Cramp, 1985, Gochfeld and Burger, 1996). This pattern was found in many other colonial seabirds, e.g. Common tern *Sterna hirundo* (Tims et al., 2004), Audouin’s gull *Larus audouinii* (Oro and Ruxton, 2001), Manx shearwaters *Puffinus puffinus* (Storey and Jon, 1985) and Kittiwakes *Rissa tridactyla* in the Atlantic Ocean (Lashko, 2004).

The duration of the egg laying period in terns depends on colony size (Burger and Gochfeld, 1991). In this study we found that the egg laying period at the Gara colony was significantly longer than at the other colonies. This pattern of colony formation has also been observed in the Persian Gulf region breeding population at Shidvar Island, Iran (Ghasemi et al., 2011), the Egyptian Red Sea and the Gulf of Aden colonies (PERSGA/GEF, 2003, Baha El Din, 2003) and in the Australian breeding population (Nicholson, 2002, Hulsman, 1977).

Early breeding pairs that occupy the colony’s central territory were found in this study to have higher breeding success compared to later breeding pairs at the colony edges. This has been documented for several seabird species, e.g. Black-headed Gull *Larus radibundus* (Patterson, 1965), Sandwich Tern *Thalasseus sandvicensis* (Langham, 1974) and the South American Common tern *Sterna*

*hirundinacea* (Fracasso et al., 2010). Lower breeding success caused mainly by predation, because the colony edge nests are detected easily by predators, while the rest of the colony members attempt to deter predators by group mobbing defence and predator swamping (Wittenberger and Hunt, 1985). Such predation avoidance can be explained by the selfish-herd hypothesis (Hamilton, 1971) where peripheral nests only have neighbours on one side. This hypothesis predicts that individuals breeding at the edge of a colony should suffer higher losses due to predation than individuals breeding near the centre, being surrounded by other nests. In contrast other research suggests that central nests may suffer more predation pressure in some species, e.g. the Least Tern *Sterna antillarum*, where Black-crowned Night Heron *Nycticorax nycticorax* targets the colony centre rather than the edges, attacking the colony centre with higher probability of obtaining eggs and nestlings as food (Brunton, 1997). The relationship between nest location, predation and colony size is complex and varies in different systems depending on predator numbers and type, colony age and size (Hunter, 1991).

Although no evidence of avian predation on Lesser Crested Tern colonies has been observed during past surveys or in this study, such predation threat cannot be excluded. This is because of sympatric coexistence of breeding Lesser Crested Terns with potential predators such as Yellow-legged Gull *Larus michahellis* (on Gara, Elba and Fteha), as well as the occasional presence of other bird species such as Pomarine Skua *Stercorarius pomarinus* observed at Gara Island, during the 2010 season, chasing and trying to kleptoparasitise (stealing food from other species) adult Lesser Crested Terns carrying fish in their bills. This has been documented for other “crested terns” e.g. Sandwich Terns in Malaga were chased by the Pomarine Skua *Stercorarius pomarinus* where 22% of the latter species attempted to steal the former’s food (Paterson, 1986). On the other hand the presence of roosting Grey Heron *Ardea cineria* at Jeliana islet, used by breeding Lesser Crested Terns, may pose potential predation impact on nestlings, as that species was reported to predate similar sized chicks of breeding White-throated Rail *Dryolimnas cuvieri* (Pistorius, 2008). Mammalian predation was found to be the main cause of nest failure at Jeliana, as feral dogs consumed all eggs during the 2010 season. Dogs were also observed repeatedly chasing the crèche at the same site, causing direct predation and disturbance to

the colony members. The accumulation of house-hold waste and building operations waste on the edges of Jeliana Lake may provide a suitable habitat for Rats in the near future, especially as the lake is connected to the sewer channels of the city, which in turn may threaten the survival of the Lesser Crested Tern colony at this site, and it would be a difficult process to eradicate such predators once they establish their population, utilizing the food resources available at the site, including tern eggs and other birds breeding on the site. Potential spread of Rats in the vicinity of Jeliana area, due to the dumping of house-hold waste, can also represent a threat on the breeding success of the study species and other bird species breeding during spring and summer. Immediate actions need to be taken to control this threat.

The breeding habitat rehabilitation at Jeliana islet, the availability of newly-formed islet within the same lake and the fencing of the whole lake area has positively contributed to the tripling of breeding population size at this site. It would be essential to provide other alternative breeding areas (islets) at the Jeliana Lake or other Benghazi's northern lakes that might be used by terns and other sea- and waterbirds in the future if development works caused deterioration of habitat quality at the current site. Further monitoring in future seasons would show the potential usage of these other islets by breeding terns, if appropriate habitat quality is provided at these sites, in terms of vegetation density and soil type, it will positively enhance the Lesser Crested Tern population size, as the diet species diversity was the highest at Jeliana (See Chapter 3 on Feeding Ecology), however the increase of islets surface area would in turn increase the potential of breeding space competition with other sympatric bird species, mainly the Black winged Stilt *Himantopus himantopus* that breeds within the same period.

Disturbance at the Elba colony is notably affecting the future of the colony, as for three successive seasons, where almost 50% of the population have moved to Fteha Island. The small and stable size of the Elba colony (ca. 35-40 pairs), since its discovery in 1993 (Meininger et al., 1994), can be resulted from high mortality and low recruitment rates at wintering or migration staging areas. The split of the breeding population is seen as a response to direct disturbance by visitors to Elba Island. Some information obtained during field visits indicates that illegal bird

hunters may use tern nestlings to trap birds of prey during summer months in this region of Libya.

Population size at the breeding sites is considerably variable; at Gara the initial population estimated by Moltoni (1939) was 1,000 breeding pairs. Since then no data have been available until the early 1990's, when Meininger et al., (1994) estimated the population size at 1,700 breeding pairs at Gara and discovered the second breeding site at the Elba Island, with 35 breeding pairs. Regular monitoring of the sites then started in 2006 and provided annual estimates of population size at both sites and added in 2007 the new breeding site of Jeliana (Hamza et al., 2007). The most important breeding site in terms of population size is Gara Island followed by Jeliana islet. The populations at Elba and nearby Fteha Island are significantly smaller and have not showed any change since Elba was discovered in summer 1993 (Meininger et al., 1994). Available data on population size from the literature and this study have showed some degree of stability, however additional future monitoring is needed to assess long-term trends in population size.

The increase in breeding pairs at Jeliana during 2012 season was exceptional, perhaps because of the reduced human access and habitat rehabilitation activity conducted during that spring, prior to the breeding season start. On the other hand no visit to the nearby Gara colony was possible in 2012 because of security concerns, and a proportion of the breeding terns at Jeliana may have originated from Gara, because of local disturbance or other threats. If this is the case it would indicate an exchange of population groups between the two sites, increasing the number of breeding pairs at Jeliana. For instance, one adult tern with a colour ring (AA) ringed as a nestling at Gara in August 2008 was observed breeding at Jeliana in 2012 (See Chapter 4). In addition, nine newly recruited breeding individuals returned to Jeliana colony after being ringed as nestlings in 2007 (1 tern) and 2008 (8 terns). Future monitoring and ring reading at Jeliana is needed as more ringed individuals would start to breed, in order to quantify this further for the study of population dynamics of the species at these sites.

Synchrony can be identified as "an expression of the tendency of individuals to cluster in time" (Gochfeld, 1980). Therefore synchronous individuals enjoy the greatest reproductive success and centripetal selection (a type of natural

selection that operates in stable environmental conditions and in a short span of time, when species living in particular environmental conditions are perfectly adapted to them) would maintain synchrony by eliminating asynchronous phenotypes (Findlay and Cooke, 1982).

There is a positive relationship between colony size and egg laying period. For example in the Common Tern *Sterna hirundo*, smaller colonies were more synchronous than larger colonies (Burger and Gochfeld, 1991). This was also found to be the case for other colonial seabird species, such as the Herring Gull *Larus argentatus* (Burger, 1979) and Adelie Penguins *Pygoscelis adeliae* (Ninnes et al., 2011).

The close association between spatial (colonial breeding) and temporal (synchrony) aspects of breeding ecology suggests some evolution pattern in synchrony that should not be considered independent from the evolution of breeding in colonies (Gochfeld, 1980, Findlay and Cooke, 1982). Synchrony is suggested to provide an adaptive selective advantage for some individuals breeding together, which may take the form of reduced predation impact or increased efficiency of food utilization. In contrast asynchronous broods encounter reduced food availability and are more susceptible to starvation than their synchronous counterparts (Burger, 1979). The observed temporal clustering of individuals may result from selection pressures acting on the timing, rather than the synchronization of reproduction. As individuals use an unpredictable changing environment they need to use the shortest time and resources available to raise healthy offspring (Findlay and Cooke, 1982).

The timing of reproduction is often considered one of the major life history traits reflecting the adaptation of birds to local environmental conditions, and selection of breeding initiation timing controls the synchrony of breeding chronology (Lambrechts et al., 1997).

Early-season environmental cues are used by birds to predict feeding conditions weeks to months later, when they are raising chicks (Forbes and Mock, 1996), however several studies investigated the relationship between laying date and environmental variables and found that, some seabird species are able to lay and



re-lay their eggs regardless of the environmental change experienced during the breeding season (Mark Hipfner et al., 2008).

Seabird species shows contrasting patterns of their reaction with in environmental variables change, some species with arrival and/or first egg dates becoming earlier in Arctic Terns *Sterna paradisaea*, Common Terns *Sterna hirundo* and Sandwich Terns *Sterna sandvicensis*, whilst other species such as auks (Common Guillemot *Uria aalge*, Razorbill *Alca torda* and Atlantic Puffin *Fratercula arctica*) and Black-legged Kittiwake *Rissa tridactyla*, the trend was in the opposite direction towards later breeding (Wanless et al., 2009), the delayed start of breeding is contradicting the general tendency for early breeding shown in many species, e.g. An analysis of 65 bird species in the UK showed that 20 species have tended to lay their eggs 4-17 days earlier in the year over the past 25 years which clearly indicate the climate change impact on breeding dates.(Crick, 1999),

In the present study, breeding synchrony was significantly correlated with colony size, i.e. the larger the colony the greater the chance to become reproductively successful. This is explained by the concept of safety in groups, where the larger the group the better its anti-predator behaviour, with better foraging skills, which all lead to higher breeding success (Brunton, 1997).

At Gara, the overall egg laying period was much longer (lower synchrony) compared to the other colonies, mainly due to the colony size. The longer laying period at Gara shows also the gradual establishment of nests by breeding adults that arrive later than the founding group which select and initiates colony establishment. High significant correlation between synchrony and colony size was found to increase reproductive fitness of the colony in other similar seabirds (Emlen, 1975) by determining intra-specific competition for resources such as food, nest sites or mates (Wittenberger and Hunt, 1985) which in turn means that breeding density and synchrony may directly influence breeding success.

There were no significant differences of egg volume at Gara, Elba and Jeliana colonies, although egg size from both the Ashrafi archipelago, Egypt and the newly formed colony at Fteha was significantly smaller in terms of volume compared to the other sites.

Studies on avian egg size variation have been reviewed by several authors (e.g. Williams, 1994, Christians, 2002, Krist, 2011, Kvalnes et al., 2013). Egg mass (size) with clutch size are reproductive traits with strong links to fitness in natural bird populations (Krist, 2011) The causes of such variation are debatable and found to be attributed to a set of factors as the following:

It has been suggested that intra-clutch variation in egg size may simply be due to physiological or nutritional constraints. Egg size variation in herring gulls was proposed as a result of food availability (Kilpi et al., 1996), however Saino et al. (2010) further studied this phenomenon in the Yellow legged Gull, and found that there was an increase in third laid egg mass, when females supplemented with food after clutch initiation, and that increase was in albumen mass. Furthermore other researchers found that nutritionally constrained females lay low quality clutches, in a support of the relationship between food intake and egg quality and viability (Rubolini et al., 2011). On the other hand, some research indicated that energetic constraints on breeding female were responsible for intra-clutch egg mass variation. Nilsson and Svensson (1993)

Variations in the physiological systems involved in egg production (e.g. the production of yolk precursors by the liver and their uptake at the ovary) as well as the intake quality and quantity of food and its metabolism during egg formation can cause variation in egg size (Bernardo, 1996), however Kozłowski and Ricklefs (2010) studied the relationship between egg size and yolk steroids and found differences in their concentration across laying order. More recently Chin et al. (2012) found no sex related difference in egg size, but eggs with male embryos contained more albumen, while eggs with female embryos contained more yolk, lipid and non-lipid (protein and carbohydrate), and egg mass or size may not necessarily reflect nutrient allocation strategies mothers use in ovulation, indicating that the intra-clutch variation in egg mass is an adaptive selection favouring one chick on other siblings for survival reason, and not simply a result of nutritional constraints.

Egg size and clutch size were also attributed to genetic factors, a significant positive correlations was found between egg size and heterozygosity (Wetzel et al., 2012), however this relationship was found to be more complex, resulting from multiple loci effect on this trait. This effect is believed to be in form of small

contributions of several loci spread throughout the genome, which are subject to continued input of variation by mutation and migration (Santure et al., 2013). In a meta data analysis of 283 studies on egg size relationship with fitness, Krist (2011) Egg size was found to be positively related to nearly all studied offspring traits, the relationship was strongest at hatching but persisted until the post-fledging stage. Morphological traits were the most closely related to egg size but significant relationships were also found with hatching success, chick survival, and growth rate.

Other authors attributes egg size variation to anatomical causes related to female body size and mass (Figuerola and Green, 2006) female body mass was found to be positively correlated with egg size but not with clutch size, and that clutch size decreased as egg size increased (Kolm et al., 2007). Environmental factors, such as ambient temperature before and during egg laying, Kvalnes et al. (2013) has found that egg size of house sparrow has increased with maternal body mass, and positively affected by spring temperature during the two weeks prior to egg laying. The relationship between environmental factors (mainly temperature) and egg size in birds were extensively studied, with different outcomes, where in some studies a clear positive correlation were found between egg size and temperature (see above) other studies have reported negative correlation (Williams and Cooch, 1996)

Female age was also found to significantly affect egg size in many species (Saether, 1990). For example, the female Wandering Albatross *Diomedea exulans* change in size with age (Croxall et al., 1992). The size and mass of the female body and its condition explained less than 20% of the variation in avian egg size (Christians, 2002), while food quality and quantity can affect egg composition but not egg size in Lesser Black-backed Gull *Larus fuscus* (Hiom et al., 1991). Similarly, other studies found no relationship between egg size, clutch size and laying date e.g. for common terns (Moore et al., 2000), but some studies found that a decrease in egg size with laying date may be due to younger birds laying smaller eggs later, rather than the effect of laying date itself (Hipfner et al., 1997, Vinuela, 1997, Nisbet and Cohen, 1975)

Egg size increased with maternal body mass, was positively affected by spring temperatures and curvilinearly related to temperature during the 2 weeks prior to

egg laying. Some 46.4 % of variation in egg size was due to differences between females, and 21.9 % was explained by variation between broods by the same female. The heritability of egg size was low ( $h^2 = 0.26$ ) compared to estimates from other studies ( $h^2 > 0.6$ ). The present study challenges the recent idea that egg size is an inflexible maternal characteristic with very high additive genetic variance, and suggests that females are subject to both intrinsic and extrinsic constraints prior to and during egg formation, leading to the observed plasticity in egg size. In a general sense, propagule size could be expected to be both limited by and adaptively adjusted in accordance to prevailing environmental conditions. (Kvalnes et al., 2013) Although each of the above-mentioned factors does explain a small proportion of the variation in avian egg size, it is possible that all of these factors together explain a substantial amount of the variation between females. The evolutionary significance of much variation is not well understood. For instance, while variation could be due to differences in optimum egg size between individuals, there is no evidence as to what aspects of female phenotype would determine this optimum, or why optima would vary greatly between females but not between breeding attempts for a given female (Christians, 2002).

On the other hand, a recent review of 283 published studies (Krist, 2011) showed that egg size is positively correlated to all studied offspring traits across all stages of their life cycle, the relationship being strongest at hatching but persisting until the post-fledging stage. A matrix of the above factors may have affected egg size at both Fteha Island and Ashrafi archipelago colonies. However, further studies investigating this variation should be undertaken, in order to understand both short-term (hatchability) and long-term (population survival) effects at these two sites, compared to the other breeding sites where egg size was more uniform.

Lesser Crested Terns bred at Fteha in 2010 season as a result of human disturbance at the nearby Elba Island. The significantly smaller eggs laid at Fteha might be a direct result of the physiological and behavioural state of the adult females, that selected this alternative site to lay their eggs. Time spent by these terns to find this alternative suitable habitat instead of foraging to increase their egg formation viability, may explain the smaller (and possibly low yolk content) eggs. At the Red Sea colony, inter-population differences might cause such

difference in egg volume, when compared to the Libyan colonies of Gara, Elba and Jeliana. No study of diet structure in this Red Sea population is available to provide what quantity and quality of food is used by the species there. Being characterised with physical conditions similar to tropical conditions, the Red Sea may offer lower energy content food compared to the Mediterranean.

These speculations of potential causes of differences in egg volume could be tested using stable isotope analysis, to find whether the Lesser Crested Terns generally are “income breeders” relying on local conditions to develop their eggs or “capital breeders” accumulating energy reserves while in migration to the breeding sites (Schmutz et al., 2006). This technique can provide insights into the role of endogenous reserves (capital) vs. recently acquired nutrients (income) breeding in egg formation of these terns, (see review by Inger and Bearhop, 2008).

The density-dependent habitat selection theory states that “individual fitness declines in high density populations because of decreased habitat quality (Morris, 1989)”. The population fitness declines due to an increased intra-specific interaction (resource competition), predation, parasite load and disease spread (Rosenzweig, 1991). Several advantages of colonial breeding and high population density have been proposed as outweighing the costs of high density, including use of limited high-quality habitat, optimal location, foraging success and predation avoidance (see review by Coulson, 2001). Nest density is governed by several factors, namely the quality and availability of nesting space (i.e. the carrying capacity of the site) and the presence or absence of other pressures such as predation, and has been identified as a factor affecting reproductive success in many seabird colonies (e.g. Anderson and Hodum, 1993, Stokes and Boersma, 2000, Savoca et al., 2011, Buckley and Buckley, 1977).

Nest density varied with colony size in this study where large colonies (Gara) had significantly more nests/m<sup>2</sup>, compared to the other colonies, which were substantially smaller in size. Density is known to affect the growth rate of young birds; (e.g. Savoca et al., 2011) found that herring gull chicks reared in dense sub-colonies grew at a rate significantly higher than that of those reared in more loose colonies (lower density nesting).

For Lesser Crested Terns (other than *T. b. emigrata* population), nest density and hence inter-nest distance, vary considerably, with nests sometimes being rather loosely spaced and of other times being tightly packed (Hulsman, 1977). Some colonies have much larger inter-nest distance when the colony is shared, e.g. at Abutilon Island, Australia the colony is composed of Lesser Crested Terns and Greater Crested Terns *Thalasseus bergii*, where 27% of inter-nest distance was  $\geq 60$  cm (Nicholson, 2002). Inter-nest distance in the present study was inversely correlated with colony size, i.e. large colonies (e.g. Gara) had a significantly smaller mean inter-nest distance compared to the other smaller colonies (Table 2.8). In a related crested tern species, (Shealer, 1999) found that mean inter-nest distance for sandwich terns was  $24.8 \pm 12.6$  cm, close to that found at the Jeliana colony ( $22.68 \text{ cm} \pm 6.99$ ) in the present study, but much smaller than that at Fteha Island ( $48.03 \text{ cm} \pm 15.72$ ) and much greater than at the Gara colony ( $18.16 \text{ cm} \pm 6.81$ ). These contrasting inter-nest differences are consistent with those in other Lesser Crested Tern colonies, e.g. in Australia (Hulsman, 1977) and the Persian Gulf (Ghasemi et al., 2011).

Colony species composition can also affect inter-nest distance being larger in mixed species colonies, e.g. Lesser Crested Terns breeding with greater crested terns in Australia had significantly larger inter-nest distance ( $51.6 \pm 16.4$  cm;  $n = 58$ ) in mixed species colonies, compared to single species colonies (Nicholson, 2002). Lesser Crested Terns in Libya breed in single-species colonies although in the Red Sea the species is usually associated with other tern species, such as greater crested terns and/or White Cheeked Tern *Sterna repressa*. Unfortunately it was not possible to sample inter-nest distance at the Ashrafi colony in the Red Sea, due to time constraints and the exposure of clutches to predation by the White-eyed Gull *Larus leucophthalmus*, when incubating adults were flushed from the colony.

In colonial seabirds, a compact nesting pattern serves as an anti-predator strategy. Such pattern in both Royal *Thalasseus maxima* (Buckley and Buckley, 1977) and Elegant Tern *Thalasseus elegans* (Collins and Taylor, 2008) has been suggested to maximise colony nest density and to resist predation pressure, as each nest is in direct contact with six other nests surrounding it in a hexagonal shape.

Colony size can affect egg-laying date (Burger 1979). Larger colonies have an earlier laying date compared to smaller colonies and in turn, earlier breeding pairs are expected to have more healthy offspring than those who lay their nests later in the season (Burger, 1979, Krist, 2011) mainly due to experience and nest site selection skills (e.g. Votier et al., 2009). The present study shows a negative relationship between colony size and mean egg-laying date; mainly caused by asynchrony of egg-laying date at Jeliana compared to the other three colonies, starting one month earlier.

A similar pattern has been reported for other tern species, e.g. common terns in larger colonies starting to breed first then later breeding documented for smaller colonies (Burger and Gochfeld, 1991). The colony at Jeliana in this study is not the largest, although as mentioned above it is hypothesised that terns arriving at Jeliana before the other sites may use different migration routes and stop-over sites compared to those breeding at the Gara colony, or they may start their journey from West Africa before individuals of the other colonies. This topic needs more research and monitoring migration patterns along the Tunisian coast and southern Italian Islands in addition to offshore monitoring, using either selected site intensive surveillance telemetry tracking technologies.

The incubation period of clutches at the study sites was within the presently known maximum for the species, 30 days (Cramp, 1985, Gochfeld and Burger, 1996). However, the incubation period at both the Jeliana and Elba colonies was shorter than the known minimum range (21 days). These shorter incubation periods in birds have been suggested to be a natural selection in response to high predation levels during the early part of the breeding season, especially for small ground nesting birds, such as terns (Schreiber and Burger, 2002). In tropical and subtropical habitats, higher air temperature may trigger a more development of embryos resulting in a shorter incubation period. However, (Ardia et al., 2006) found that the complex interaction of environmental conditions contributes to this phenomenon, not just the effect of increased air temperature *per se*. Other researchers claim that differences in parent bird age and experience can cause intra-specific differences in incubation period, i.e. older females have longer reproductive capacity and shorter incubation periods than yearling females (Joyce et al., 2001).

The relatively shorter incubation period at Jeliana, if continued, could be considered as a long-term adaptation due to breeding disturbance at this site for several generations, being located within an urban area and exposed to similar threats during previous breeding seasons. At Elba, the poaching of chicks by local bird hunters, using them as baits to trap passing birds of prey may have also induced the same effect. A long-term data set of incubation period at the breeding sites in Libya is essential to test the hypothesis of shorter incubation period due to disturbance and predation at both Elba and Jeliana. The hatching ratio and fledging success were lower at both Elba and Jeliana compared to Gara, the later with no mammalian predation and low human disturbance (Table 2.6).

The number of failed nests varied among this study's breeding sites. It is notable that the lowest proportion of failed nests/nestlings was at Gara, with 903 (26.5%) nests during the study period (2009-2010 seasons), while at Elba 44 (44%) of nests had failed during the three seasons (2009, 2010 and 2012). At Jeliana 295 (78.2%) nests had failed (2009, 2010 and 2012). Clearly the most affected colony was at Jeliana, due to both the flooding of nests in 2009 and predation by dogs in 2010 and 2012. Special measures should be taken to protect the breeding population of Lesser Crested Tern at this site (Chapter 6).

The optimal diet theory predicts that when prey are abundant, predators should consume primarily the most valuable prey type, and the inclusion of other prey types in the diet should depend on the abundance of the more profitable prey (MacArthur and Pianka, 1966). A higher diversity of prey species in the diet in certain seasons may thus indicate lower abundance of the preferred prey, allowing birds to feed less on that resource proportionally. The present study shows a positive relationship between diet diversity (Shannon-Weaver index) and breeding success, when data from all breeding sites are pooled together. However, the low breeding success at Jeliana in particular did not affect the correlation relationship. If only Jeliana data were compared with prey species diversity, there would be a negative correlation due to low breeding success there, caused by extrinsic factors (predation and habitat degradation) not related to food quality nor breeding physiology of the terns at this site.



To conclude this chapter has updated the basic breeding, incubation, hatching and fledging data for the Mediterranean race of the Lesser Crested Tern (*T. b. emigrata*) breeding in Libya. Timing of breeding has been confirmed for each site, with data on colony structure and clutch size as well as other information regarding predation and nest failure, in addition to estimation of hatching and fledging success for each colony. There was a significant positive relationship between colony size and synchrony, with larger colonies having more days spent in egg-laying compared to smaller colonies. Colony disturbance by both humans and feral dogs caused nest failures in most of the colonies except at Gara Island, possibly because of its offshore location compared to the other three sites which were either at inshore (Jeliana) or near-shore locations (Elba and Fteha). Systematic and continuous monitoring of the sites will allow the conservation authorities in Libya to obtain more information on population dynamics, threat levels and trends, leading to long-term conservation breeding and feeding habitats.

Detailed information on migration routes, stop-over and wintering sites as well as threats during migration and at wintering sites are all scarce (Chapter 4). The threats faced by the limited size of the Mediterranean population of Lesser Crested Tern such as impacts from unregulated hunting and fisheries interactions with prey population, on feeding terns and habitat degradation at breeding, wintering and stop-over sites will shape the future of this localised population of terns in the coming decades. Hence conservation actions should be taken by both Libya and other countries in Northwest and West Africa to ensure the healthy status of this breeding subspecies.

**Chapter3 Feeding ecology of  
Mediterranean breeding  
Lesser Crested Tern young**



## **Chapter.3 Feeding Ecology of Mediterranean breeding Lesser Crested Tern nestlings**

### **3.1 Introduction**

Food is considered to be one of the three requirements for successful exploitation of habitat resources (McLeay et al., 2010). Seabirds are long-lived animals with high adult survival rates, relatively long maturation periods and low reproductive output. These features are believed to reflect adaptation for maintaining the survival of their populations against fluctuations in prey availability (Boyd et al., 2006). Any decrease in prey would cause significant consequences for the survival of the parent seabirds and their offspring unless the parents increased foraging activity investment to maintain a positive energy budget (Furness and Tasker, 1999). Optimal foraging theory states that “parent seabirds should optimize prey delivery rate by minimizing travelling distances between colonies and food sources (MacArthur and Pianka, 1966)”. However, when food resources are distant the adults may spend more time in foraging, which poses the challenge of meeting their own dietary requirements, as well as those of their young (Drent and Daan, 1980). Such a decision-making process controls the survival of the whole breeding population over a certain area.

Generally, seabirds are classified according to feeding habitat into two major categories: inshore and offshore. The former includes species that breed on the mainland or on inshore Islands, feeding within sight of land, such as terns (Sternidae), gulls (Laridae) and cormorants (Phalacrocoracidae), incubating their clutches for periods of a day or less and feeding their young several times per day (Diamond, 1978, Oro and Martinez-Abraín, 2004). Offshore feeders such as albatrosses (Diomedidae), shearwaters and petrels (Procellariidae) breed on oceanic Islands and feed out of sight of land (Oro and Martinez-Abraín, 2004) incubating their eggs for periods of several days, feeding their young once a day or less (Diamond, 1978). Inshore feeders are usually small seabirds (e.g. terns) with larger reproductive effort, early maturity and shorter life spans. They inhabit less isolated habitats and are exposed to a variety of predators (other birds, mammals and poachers) which makes their reproductive success unpredictable

over time (Oro and Martinez-Abraín, 2004). Living under the pressures of predation, disturbance and limitation of food availability not only influences the number of eggs laid by inshore feeders, but also the possible number of young that can be reared per season, which might be compensated, at least in part by higher growth rates, over a shorter period, sustained by a greater number of feeding events per day.

Diet diversity in seabirds is linked to latitude as in higher latitudes, they acquire fewer prey species making up diet volume, being composed of clupeid fish and sandeels in addition to crustacea (krill) in northern and southern oceans (Soave, 1996, Furness and Todd, 1984). In tropical seabirds that breed in the subtropics there is a greater prey diversity, dominated by fish available throughout the breeding season (Dunlop, 1997). In the Mediterranean Sea the distribution of epipelagic fish communities is highly heterogeneous in terms of hydrography, bathymetry and productivity (Cardinale et al., 2010), which results in a variable spatial and temporal distribution of fish species, depending on the species' requirements and the availability of suitable habitat features (physical, chemical and biological). This makes the Mediterranean more similar to the tropical model of patchy fish distribution than for temperate areas. Several factors are found to control the structure and size of seabird diet (Table 3.1).

Table 3.1 Factors that control the composition of seabird diet

<b>Factor</b>	<b>Reference</b>
Seabird morphology and mass	Hulsman, 1988
Weather conditions	Shealer, 2002
Prey distribution and availability at foraging areas used by seabirds	Carty, 2009
Differences in prey size	Hulsman, 1981; McLeay et al., 2009;
Differences in energetic efficiency between parent and young	
Position of prey with respect to the water depth, correlated to prey size.	Dänhardt and Becker, 2011

Studying the feeding ecology of a seabird depends also on interaction between intrinsic and extrinsic factors: intrinsic factors could include the time of foraging (within the day hours) and the adult travel distance from and to the colony site (Nicholson, 2002), in addition to physiology in combination with their foraging regime which will determine which prey items encountered can be caught by them (Ashmole and Ashmole, 1967). In contrast extrinsic factors include spatial and temporal distribution of potential prey species in the foraging areas used by the adult terns (Nicholson, 2002).

Studying seabird's diet can provide a tool for assessing the type, size, mass and quality of prey available to them within their foraging areas during the breeding season (Dänhardt et al., 2011). Diet composition is a major limiting factor in breeding productivity through its effect on growth and survival, as the energy and nutrients acquired are affected by the quality and quantity of food consumed which in turn affects growth rate (Wanless et al., 2005, Golet et al., 2000). Diet composition can also indicate the feeding regime of the parent bird as a generalist or specialist feeder.

### **3.1.1 Feeding in Lesser Crested Terns**

Lesser Crested Terns use two methods to obtain their food: plunging at the water surface (i.e. the bird dives in to the water but does not submerge completely) and/or feeding by aerial diving, which take place when the bird dives and submerges completely, relying on momentum to carry it to catch its prey (Ashmole and Ashmole, 1967). The preferred food of these terns is epipelagic and nektonic fish, with a smaller proportion of benthic species such as crustaceans and cephalopods (Cramp, 1985, del Hoyo et al., 1996, Hulsman et al., 1989, Nicholson, 2002).

As there are few previous studies on feeding ecology and diet structure in Lesser Crested Terns, there is a need to update existing information and to document data from poorly studied populations (Horn et al., 2010) as most published information on this subject is restricted to the Australian populations (Hulsman, 1981, Hulsman, 1977, Hulsman, 1988, Hulsman et al., 1989, Nicholson, 2002). Information regarding diet structure and foraging of the Mediterranean breeding population (*T. b. emigrata*) in Libya is scarce, limited to seven fish species

reported during a short visit to breeding sites in summer 1993 at the Elba and Gara colonies (Meininger et al., 1994), possibly due to the fact that that study aimed at documenting breeding but not the species diet structure at both colonies. No information was previously available on diet structure at the Jeliana breeding site (Benghazi) as it was discovered later (Hamza et al., 2007). Obtaining detailed information on spatial and temporal aspects of foraging ecology of the parent birds would help in demarcation of future marine protected areas for the population at and around the known breeding sites. The use of tracking technologies has helped to clarify the uncertainties of when and where individual seabirds forage, however the costs and potential effects on bird's behaviour because of the use of tracking devices usage (GPS or satellite) still need to be investigated for different seabird species.

Epipelagic fish play a key ecological role in coastal ecosystems i.e. transfer of energy from primary producers (phytoplankton) and primary consumers (zooplankton) to the upper trophic levels (Cury et al., 2000). They are short-lived, low in the food chain, and their reproductive strategy is to produce large amounts of eggs. Their populations are dependent on oceanographic conditions especially during the planktonic stages. The differences in oceanographic conditions are reflected in the biotic patterns of those epipelagic fish communities (Bonanno et al., 2012) the oceanographic elements including hydrology, water temperature regime and primary productivity which contribute to shape the distribution of such species (Patti et al., 2005), that in turn represent main food for several seabird species, among them the terns.

### **3.1.2 Hypotheses**

As Lesser Crested Terns breed during the summer in the Mediterranean area, they are expected to select the timing, foraging strategy and hence the choice of breeding site(s) depending on abundance and diversity of food resources available within reach of those sites. The following hypotheses are made here:

- As they breed at different sites off the Libyan coast, it is hypothesized that the terns will forage on different fish species found relatively in close distance from the breeding sites, although several species might be

common in the tern diet, some prey species would be more dominant in terms of mass and/or frequency being more accessible to the adults (epipelagic species) or with proportionally a more nutritional value to the young (active prey selection).

- When there is a difference in diet structure between breeding sites, it would be attributed to reciprocal replacement of ecological niches by different prey species at different sites.
- Prey size and species will vary throughout the provisioning period according to the growing chicks dietary and energetic requirements, as reported in other tern species.

### **3.1.3 Objectives and research questions**

The principal aim of this Chapter is to document the diet of Lesser Crested Tern young, and to identify any differences in diet structure between the colonies at Gara, Elba and Jeliana, as a basis for preparing conservation measures for the species, in respect of dietary requirements. In particular it addresses the following questions:

- What prey species are consumed by Lesser Crested Terns during the breeding period and what species are the most important in terms of frequency and mass?
- What are the similarities in diet structure among the breeding sites?
- Do adult terns employ active selection for larger prey with the progression of the provisioning period, or do they increase the frequency of foraging trips but select the same prey size.

## **3.2 Methods**

### **3.2.1 Introduction**

The diet structure of Lesser Crested Tern young was studied using regurgitated food collected from the young during field visits to the Gara, Jeliana and Elba

colonies. This provided data on the abundance and diversity of prey species during the breeding period, at locations around the colony sites or at the foraging grounds used by the parent birds.

During field visits to breeding sites in July and August 2008, 2009, 2010 and 2012, regurgitated food was collected by hand from chicks aged 1-3 weeks. No estimates of individual meal size were made. Study sites and maps are included in Chapter 2. Visits were made between either 0600 - 1000 hrs or after 1700 hrs to avoid disturbing the colonies during the hottest part of the day. Regurgitation occurred spontaneously at the end of each visit, after being handled for ringing. Although this method may not always empty the proventriculus of the chicks (Nicholson, 2002, Shealer, 1998a, Surman and Wooller, 2003), it is the method of minimal intrusion commonly used for the study of seabird diet (Cooper and Klages, 1995, Croxall, 1987, Barrett et al., 2007), including terns (Nicholson, 2002, Shealer, 1998a, Surman and Wooller, 2003). Food discarded near nests was also collected and included in the data analyses. All samples were preserved in plastic bags and then kept in 70% alcohol, prior to identification at the Fish Biology laboratory of Tripoli University, and at Omar Al-Mokhtar University in the 2012 season. Whole prey items (fish) and partially digested fish/cephalopods (where length could still be estimated) were included in the analyses.

### **3.2.2 Identification of food items**

In the laboratory, the prey samples were rinsed with tap water, then each sample (containing single or multiple prey items) was identified to the highest possible taxonomic level (species) using published fish identification keys (Whitehead et al., 1984–1986). Sample identification was also confirmed by fish taxonomist Dr. A. Ben Abdalla of the Zoology Department, Tripoli University. Prey total length was measured using a digital calliper to the nearest 0.01 mm from the tip of the snout to the posterior edge of the caudal fin fold. The body biomass (g) of each fish was reconstituted using allometric length-weight equations published at [www.fishbase.org](http://www.fishbase.org) (Froese and Pauly, 2000). The sample size used to extract the equations and proximity to Libyan waters were considered in allometric equation choice, as equations may differ according to intercept value of the equation, for



the same species, caused by sample size used and the fish body sizes used to extract that equation. The general formula used to estimate mass (g) as a function of the total length (TL) was:

$$W = a (TL^b) \dots \dots \dots (1)$$

Where W = fish weight (gm); a = intercept value; TL= fish total length (cm),  
b = slope value.

For example, the *b* variable used in an equation to estimate Round Sardine (*Sardinella aurita*) weight =2.272 based on 16 individual fish, with total length ranging from 9.9-16.8 cm, whereas *b* = 3.120 based on 645 individual fish with total length ranging from 10 to 17 cm. Therefore the equation extracted using a larger sample size and comparable length range was selected and used in this study to estimate wet weight (mass).

### 3.2.3 Data analysis

The descriptive statistics of prey samples were calculated using the statistical package IBM -SPSS ver.19, the overall importance of each diet taxon was assessed using three indices: frequency of occurrence, the percentage by number and by reconstituted mass for each prey species. The overall importance of each diet taxon was ranked using the index of relative importance (IRI), which is a combination of occurrence, numerical abundance and reconstituted mass of the prey specimens (Pinkas, 1971), according to the following formula:

$$IRI = (N + V) F \dots \dots \dots (2)$$

Where N = Numerical percentage of each prey species in the diet  
V = Volumetric percentage of each prey species in the diet  
F = Frequency of occurrence percentage

Index of Relative Importance (IRI) was calculated through the following steps:

1. Summation of the numerical and volumetric percentage values
2. Multiplying the value for each species at each site by the frequency of occurrence percentage value.

Frequency of Occurrence was calculated by adding all same taxon specimens from the three sampling sites (Gara, Jeliana and Elba), then the proportional percentage per taxon at each site were calculated, by dividing the original number of specimens at each site by the total of that species. Since IRI varies according to data input, the values have been normalized for each prey species in the diet at each site, as a percentage of the total IRI value for that species (%IRI). This index has been used to quantify the effect of numerous small fish samples that may overshadow the importance of a few large fish in the diet at each site (Cortes, 1997).

Normality distributions for collected data (fish length and weight) were checked using the Shapiro-Wilks test on SPSS. Only data collected at Elba in 2008 and 2010, and data at Gara in 2009 were normally distributed, hence the homogeneity of variance was tested to determine statistical test choice, the variance being not homogeneous across the data set. In this case a non-parametric test that assumes neither normal distribution nor homogeneity of variance was selected (Mood's Median Test), to compare fish total length between years for the same site, or between sites. This test is a non-parametric equivalent of One-way ANOVA, does not require normality and homogeneity of variance in comparison to the Kruskal-Wallis test and Mann-Whitney U test. This test is a special case of Pearson's chi-squared test, a non-parametric test that checks the null hypothesis that the median (of fish length or mass in the present study) from which two samples are drawn is identical. The data in each sample are assigned to two groups, one consisting of data whose values are higher than the median value in the two groups combined, and the other consisting of data whose values are at the median or below. The two-sample Kolmogorov-Smirnov test was used to find which pair-wise comparisons were responsible for the significance of the relationship (as a *post hoc* test).

Non-parametric analyses of similarity (ANOSIM) on a Bray-Curtis similarity index (PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer, 2001) ,  $p \leq 0.05$ ) were used to assess variation in prey structure at each of the three study sites. ANOSIM is a hypothesis-testing procedure that generates a probability value and test statistic (R) which lies between +1 and -1. Higher positive R-values indicate greater variation between

groups than within groups and negative values indicate high levels of within-group variation compared to between-group variation (McLeay et al., 2010). Similarity percentages (SIMPER) were used to identify which prey taxa were responsible for inter-group differences, comparing the average contribution of individual diet species (as regurgitation) to the average dissimilarity between sampling years for a specific colony (Catalan et al., 2006).

To compare the number of species found in the three breeding sites, as the sampling effort differed between years and within each year, being dependent on collection of regurgitated fish, it is expected that greater sampling effort would yield a larger sample and more species. Therefore the comparison of, so you can't just compare the number of species found in each region. Rarefaction uses the data from the larger sample to answer the question "How many species would have been found in a smaller sample?" .Rarefaction takes hypothetical subsamples of  $n$  organisms from the more-sampled region, and calculates the average number of species in such subsamples. This average can be compared to the number of species actually found in the less-sampled region. Rarefaction calculation was conducted using an online application hosted by San Diego State University, USA. This tool can be accessed on [http://fastgroup.sdsu.edu/cal\\_tools.htm](http://fastgroup.sdsu.edu/cal_tools.htm)

The relationship between breeding productivity and diet species diversity was investigated using data on fledglings per breeding pair, at each site, wherever these data was being able to be collected, to compare it with the corresponding Shannon-Wiener Index calculated for diet species diversity. Regression coefficient was then calculated using SPSS, and a trend line was added to the relationship graph.

### **3.3 Results**

#### **3.3.1 Nestlings diet structure in terms of frequency and mass**

The total of 422 diet samples belonging to 18 families of fish and one family of cephalopods were collected during the 2008-2010 and 2012 breeding seasons (Table 3.2). Sampling occurred 1-4 times per season, although it was not possible to collect diet samples during the incubation period, due to high nest

abandonment risk when handling incubating birds. At Gara Island, nine species of fish and a cephalopod species (*Loligo sp.*) belonging to 10 families were collected from regurgitated food and adjacent nest discards of nestlings. 13 fish species belonging to nine families were collected from the Elba Island colony and 18 species of fish belonging to 13 families were collected at the Jeliana islet, most of them during the 2008 and 2012 seasons (Table 3.2)

Table 3.2 Taxa constituting the diet of Lesser Crested Tern young at their breeding sites

	Family	Species	Gara	Elba	Jeliana
1	Atherinidae	<i>Atherina boyeri</i>			1
2	Belonidae	<i>Belone belone</i>		1	
3	Blenniidae	<i>Lipophrys trigloides</i>			2
		<i>Salaria pavo</i>			3
4	Carangidae	<i>Seriola dumerili</i>		2	
5	Centranchidae	<i>Spicara smaris</i>	1		4
6	Cichlidae	<i>Tilapia zilli</i>			5
7	Clupeidae	<i>Sardinella aurita</i>	2	3	6
8	Coryphaenidae	<i>Coryphaena hippurus</i>		4	
9		<i>Lichia amia</i>			7
10	Engraulidae	<i>Engraulis encrasicolus</i>	3		8
11	Exocoetidae	<i>Cheilopogon heterurus</i>	4	5	
12	Hemiramphidae	<i>Hemiramphus far</i>	5	6	
13	Labridae	<i>Coris julis</i>			9
14	Loliginidae	<i>Loligo vulgaris</i>	6		
15	Pomacentridae	<i>Chromis chromis</i>	7	7	10
16	Scaridae	<i>Sparisoma cretense</i>	8	8	11
17	Scombridae	<i>Scomber scombrus</i>	9		12
18	Siganidae	<i>Siganus luridus</i>			13
		<i>Siganus rivulatus</i>			14
19	Sparidae	<i>Boops boops</i>	10	9	15
		<i>Lithognathus mormyrus</i>			16
		<i>Sarpa salpa</i>			17
		<i>Diplodus vulgaris</i>		10	18
		<i>Diplodus sargus</i>		11	
		<i>Oblada melanura</i>		12	
		<i>Pagellus erythrinus</i>		13	

The depth range for those prey species within the water column varies greatly (Figure 3.1) ranging from a few metres to as deep as 360m (Whitehead et al., 1984–1986). This reflecting prey taxa diversity available for Lesser Crested Terns (pelagic and demersal) during the breeding season (late May to early September) coinciding with the spawning season of most of the prey species. The majority of regurgitated fish falls within the Ichthyoplanktonic to juvenile or sub-adult size classes, indicating the preference of terns to locate their food in shallow lagoons and coastal waters, being also the preferred habitat for fish nurseries. This does not exclude the fact that some terns may travel longer distances from the breeding sites seeking food in deeper waters (reported during own field observations, at locations up to 9 nautical miles from Gara Island in 2009 season). Advances in tracking systems may help to map the actual proportions of time budgets between coastal and oceanic foraging activities for this subspecies

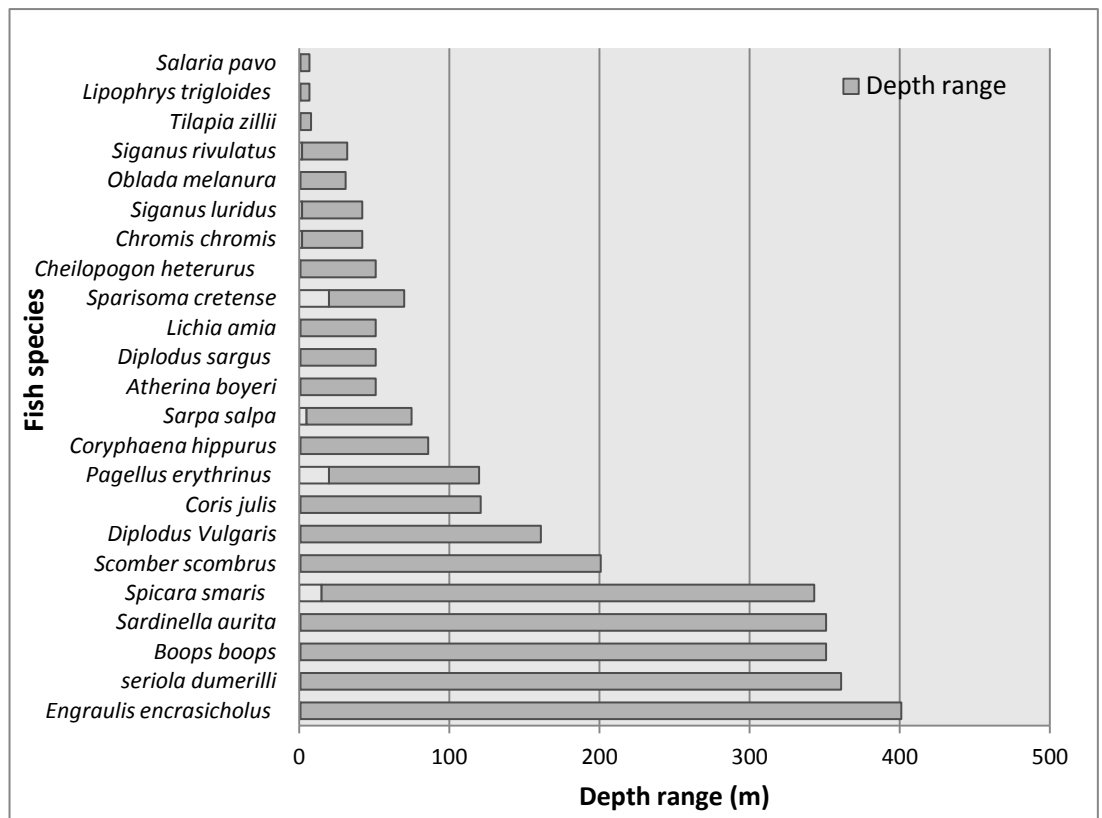


Figure 3.1 Depth ranges of fish species (adult range) found in diet of Lesser Crested Tern nestlings at Elba and Gara Islands, Libya (source: [www.fishbase.org](http://www.fishbase.org))

The number of regurgitated samples that contained several prey taxa during the whole of the study period ranged from 2-147 (mean = 27.33, SD  $\pm$  41.7), whereas the number of diet specimens (individual items) at those samples ranged from 4-216 (mean=54.27, SD $\pm$ 61.3). The highest mean number of prey individual per sample was obtained at Gara during the 2010 season, the lowest being at Elba during 2010 (Table 3.3). Meal size was not quantified when collecting the diet samples, as the aim was to create only the species list. The number of prey species was almost identical at Gara and Elba (11 and 13, respectively). At Jeliana it was 5 species in 2008 (limited sampling effort), 13 new species being added to the diet list at this site during the 2012 fieldwork.

The Index of Relative Importance (IRI) using pooled data from all sampling seasons at each breeding site showed that, the three most important fish families in the diet of Lesser Crested Tern young at Gara were Clupiedae, Exocoetidae and Hemiramphidae, while at the Elba Island colony they were Exocoetidae, Carangidae and Hemiramphidae and at the Jeliana they were Sparidae, Blennidae and Atherinidae (Tables 3.4, 3.5 and 3.6)

It is of note that, there is a clear variation in sampling effort and the collected sample size at each of the study sites, e.g. Jeliana sample size is 2.5 times more than that of Gara and 3.5 times of what's collected at Elba. More sampling at the former two sites might alter the ranking resulted from the index of relative importance, by adding either new individuals of the same species (change of relative mass or frequency) or by adding some new species not present in study seasons.

Table 3.3 Main characteristics of the regurgitated diet samples of Lesser Crested Tern young at the breeding sites (2008-2010 and 2012).

Breeding site	Gara				Elba					Jeliana		
Seasons	2008	2009	2010	Total	2008	2009	2010	2012	Total	2008	2012	Total
Sampling days	1	2	3	<b>6</b>	1	2	1	1	<b>4</b>	1	4	<b>5</b>
number of samples	3	8	25	<b>36</b>	5	9	2	6	<b>22</b>	18	25	<b>43</b>
Mean wet mass $\pm$ SD (g)	5.03 ( $\pm$ 4.06)	2.18 ( $\pm$ 1.31)	3.08 ( $\pm$ 2.98)	<b>n/a</b>	4.9 ( $\pm$ 1.97)	3.26 ( $\pm$ 2.98)	3.81 ( $\pm$ 3.76)	2.71 ( $\pm$ 1.54)	<b>n/a</b>	6.44 ( $\pm$ 6.31)	6.16 ( $\pm$ 5.94)	<b>n/a</b>
Number of prey individuals	7	39	58	<b>104</b>	17	33	4	16	<b>70</b>	33	216	<b>249</b>
Cumulative number of prey species	4	4	2	<b>10</b>	6	6	0	1	<b>13</b>	5	13	<b>18</b>
Number of families	4	8	8		4	8	3	4		5	14	

Table 3.4 Diet composition of Lesser Crested Tern young at the Gara breeding site (pooled data of regurgitated food: 2008-2010 and 2012 seasons) ranked by the Index of Relative Importance (IRI)

Prey Family	Prey species	Occurrence			Mass		IRI	
		n	%N	F	M (g)	%M	Rank	% Rank
Clupeidae	<i>Sardinella aurita</i>	44.0	42.7	62.0	65.3	21.7	3990.4	44.9
Exocoetidae	<i>Cheilopogon heterurus</i>	21.0	20.4	37.5	69.7	23.1	1631.3	18.3
Hemiramphidae	<i>Hemiramphus far</i>	9.0	8.7	42.9	41.6	13.8	965.8	10.9
Scombridae	<i>Scomber scombrus</i>	4.0	3.9	80.0	9.6	3.2	565.9	6.4
Loliginidae	<i>Loligo vulgaris</i>	2.0	1.9	100.0	10.0	3.3	525.9	5.9
Centracanthidae	<i>Spicara smaris</i>	3.0	2.9	21.4	39.3	13.0	341.5	3.8
Pomacentridae	<i>Chromis chromis</i>	6.0	5.8	26.1	16.3	5.4	293.2	3.3
Sparidae	<i>Boops boops</i>	5.0	4.9	17.9	24.3	8.1	230.6	2.6
Siganidae	<i>Siganus luridus</i>	4.0	3.9	30.8	3.6	1.2	156.6	1.8
Engraulidae	<i>Engraulis encrasicolus</i>	4.0	3.9	17.4	9.0	3.0	119.6	1.3
Scaridae	<i>Sparisoma cretense</i>	1.0	1.0	14.3	12.7	4.2	74.0	0.8
TOTAL		103.0			301.5			

Where Number = number of samples per taxon; Frequency = frequency of taxon specimens in the site compared relative to other sampling sites; Relative abundance = abundance percentage within each the sampling site; Mass = total weight by taxon species, %M= Relative contribution of taxon mass within the site, IRI = Ranking based on the overall frequency and mass of each taxon (similar abbreviations apply for Table 3.5 and 3.6).



Table 3.5 Diet composition of Lesser Crested Tern young at the Elba breeding site (pooled data of regurgitated food (2008-2010) ranked based on the Index of Relative Importance (IRI).

Prey Family	Prey species	Occurrence			Mass		IRI	
		n	%N	F	M (g)	%M	Rank	% Rank
Exocoetidae	<i>Cheilopogon heterurus</i>	35.0	50.0	62.5	197.2	55.7	6608.9	50.2
Carangidae	<i>seriola dumerilli</i>	3.0	4.3	100.0	32.1	9.1	1334.5	10.1
Hemiramphidae	<i>Hemiramphus far</i>	12.0	17.1	57.1	18.1	5.1	1271.1	9.7
Sparidae	<i>Oblada melanura</i>	3.0	4.3	100.0	24.9	7.0	1132.7	8.6
Sparidae	<i>Pagellus erythrinus</i>	3.0	4.3	100.0	14.9	4.2	849.5	6.5
Coryphaenidae	<i>Coryphaena hippurus</i>	2.0	2.9	100.0	18.1	5.1	796.8	6.1
Belonidae	<i>Belone belone</i>	3.0	4.3	100.0	4.6	1.3	557.5	4.2
Scaridae	<i>Sparisoma cretense</i>	3.0	4.3	42.9	12.4	3.5	334.3	2.5
Sparidae	<i>Diplodus sargus</i>	1.0	1.4	100.0	2.2	0.6	205.0	1.6
Sparidae	<i>Boops boops</i>	1.0	1.4	3.6	19.0	5.4	24.3	0.2
Sparidae	<i>Diplodus Vulgaris</i>	1.0	1.4	7.7	3.2	0.9	18.0	0.1
Clupeidae	<i>Sardinella aurita</i>	2.0	2.9	2.8	4.2	1.2	11.4	0.1
Pomacentridae	<i>Chromis chromis</i>	1.0	1.4	4.3	3.0	0.9	9.9	0.1
<b>Total</b>		70.0	100.0		353.8	100.0		

Table 3.6 Diet composition of Lesser Crested Tern young at the Jeliana colony (pooled data of regurgitated food from 2008 and 2012) ranked based on the Index of Relative Importance (IRI).

Prey Family	Prey species	Occurrence			Mass		IRI	
		n	%N	F	M (g)	%M	Rank	% Rank
Sparidae	<i>Lithognathus mormyrus</i>	39.0	15.7	100.0	319.2	20.9	3655.7	21.7
Blenniidae	<i>Lipophrys trigloides</i>	34.0	13.7	100.0	166.9	10.9	2458.4	14.6
Sparidae	<i>Boops boops</i>	22.0	8.8	78.6	212.5	13.9	1787.4	10.6
Atherinidae	<i>Atherina boyeri</i>	26.0	10.4	100.0	58.0	3.8	1423.6	8.5
Engraulidae	<i>Engraulis encrasicolus</i>	19.0	7.6	82.6	77.9	5.1	1051.5	6.2
Centracanthidae	<i>Spicara smaris</i>	11.0	4.4	78.6	112.5	7.4	925.9	5.5
Siganidae	<i>Siganus rivulatus</i>	7.0	2.8	100.0	67.6	4.4	723.6	4.3
Sparidae	<i>Diplodus Vulgaris</i>	12.0	4.8	92.3	44.4	2.9	713.4	4.2
Pomacentridae	<i>Chromis chromis</i>	16.0	6.4	69.6	53.8	3.5	692.2	4.1
Siganidae	<i>Siganus luridus</i>	9.0	3.6	69.2	83.4	5.5	628.3	3.7
Clupeidae	<i>Sardinella aurita</i>	25.0	10.0	35.2	117.4	7.7	624.3	3.7
Sparidae	<i>Sarpa salpa</i>	10.0	4.0	100.0	30.9	2.0	603.8	3.6
Coryphaenidae	<i>Lichia amia</i>	6.0	2.4	100.0	50.2	3.3	569.4	3.4
Cichlidae	<i>Tilapia zillii</i>	5.0	2.0	100.0	34.9	2.3	429.4	2.5
Scaridae	<i>Sparisoma cretense</i>	3.0	1.2	42.9	66.9	4.4	239.2	1.4
Labridae	<i>Coris julis</i>	2.0	0.8	100.0	14.6	1.0	176.2	1.0
Blenniidae	<i>Salaria pavo</i>	2.0	0.8	100.0	6.3	0.4	121.2	0.7
Scombridae	<i>Scomber scombrus</i>	1.0	0.4	20.0	10.1	0.7	21.2	0.1
<b>Total</b>		249.0			1527.4			

### 3.3.2 Dissimilarity ratios in diet structure of Lesser Crested Tern young

One-way analysis of similarity (ANOSIM) based on the Bray-Curtis index revealed significant differences in diet structure between the Gara, Elba and Jeliana sites ( $R=0.088, p < 0.001$ ), the significant dissimilarities ( $p$ -values) being shown in bold font (Table 3.7). The positive  $R$ -value (0.088) indicates that there was more variation between sites than within sites.

Table 3.7 Analysis of similarity (ANOSIM) Bonferroni-corrected  $p$  values for dissimilarity in diet structure (regurgitated food) of Lesser Crested Tern nestling at the Gara, Elba and Jeliana colonies (2008-2010 and 2012): values in bold font refer to a significant dissimilarity.

	Gara 2008	Gara 2009	Gara 2010	Elba 2008	Elba 2009	Elba 2010	Elba 2012	Jeliana 2008	Jeliana 2012
Gara 2008		1	1	1	0.777	1	1	1	0.349
Gara 2009			0.118	1	<b>0.003</b>	1	0.158	0.082	1
Gara 2010				0.061	0.108	1	<b>0.021</b>	<b>0.039</b>	<b>0.003</b>
Elba 2008					1	1	1	<b>0.010</b>	1
Elba 2009						1	0.633	<b>0.010</b>	<b>0.003</b>
Elba 2010							1	1	0.302
Elba 2012								<b>0.003</b>	1
Jeliana 2008									<b>0.003</b>
Jeliana 2012									

Furthermore the cluster analysis of the data, for all years and sites (Figure 3.2), showed a significant dissimilarity of diet structure at the Jeliana site during the 2012 season. This is the result of the additional new nine fish species to the diet list in 2012 which were not reported at any other breeding site. The grouping of Elba 2010 and Gara 2008 is a result of low sampling effort in both years as only three and four species respectively were sampled.

The presence of 42% and 50% of samples at the two sites, belonging to the same species *Cheilopogon heterurus* is the main reason for such grouping, therefore is not representative of the actual prey structure at these sites, being biased and based on small sample sizes.

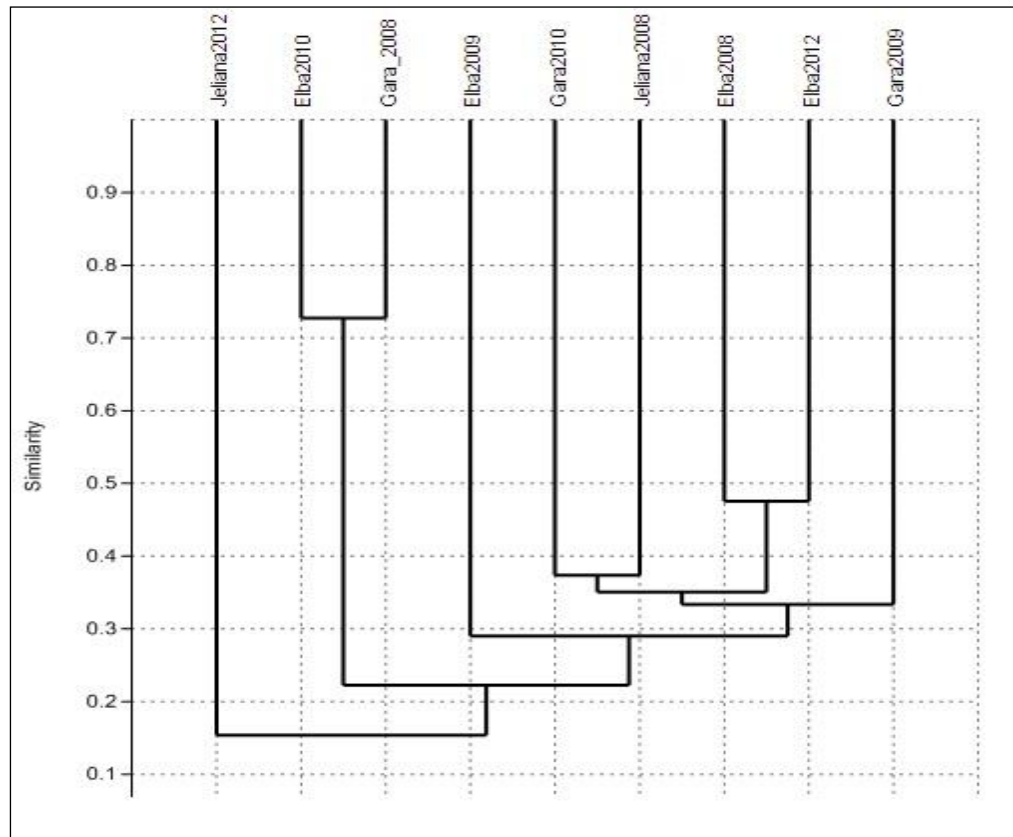


Figure 3.2 Analysis of similarity using cluster analysis on the Bray-Curtis similarity index of diet structure (regurgitated food) of Lesser Crested Tern young at the Gara, Elba and Jeliana colonies (2008-2010 and 2012).

Similarity percentages (SIMPER) were used to identify the species with the most contribution to dissimilarity in diet within and between sites. Within the Gara site, 47.25% of the dissimilarity was caused by both the Round Sardine *Sardinella aurita* (26.35%) and the Mediterranean flying-fish *Cheilopogon heterurus* (20.9%) while the remaining nine species shared the rest of the effect (Table 3.8). The relative contribution of Round Sardine in the diet might be considered an indicator of actual population density of these species in the waters around the three breeding sites during the study seasons. Round Sardine is known to spawn in Libyan waters from May to August (Pinaud and Weimerskirch, 2007), coinciding with the breeding season of terns. Looking at the pooled data from each site for all sampling seasons, we notice that this species represents 42% of the fish caught by terns at Gara, 10% at Jeliana and only 2.4% at Elba, which may support the apparent gradual decrease of Sardine abundance from the central region (Gulf of Sirte, i.e. Gara and Jeliana) to the

east of the country (Elba), as also indicated by smaller sardine catches in eastern coastal Libya (Lamboeuf et al., 2000) possibly due to better habitat availability in the western region (Tripolitania and the Gulf of Sirte) compared to the eastern region.

Sardines (*Sardinella aurita* and *Sardina pilchardus*) and Anchovies *Engraulis encrasicolus* comprise the bulk of small pelagic fish caught in the Mediterranean Sea (Giannoulaki et al., 2011). Sardines constitute 19% of the fish caught in the Mediterranean (Begon et al., 1986), the suitable habitats of these species being found to be variable annually and dependent on a combination of several limiting factors including sea surface temperature, Chlorophyll-a, active radiation, sea surface anomaly and sea surface salinity. Habitat suitability maps based on models that include the contribution of the above parameters have indicated variable annual probabilities for suitable sardine habitat (Giannoulaki et al., 2011). By looking at the maps of the Libyan coast, we note the variation of habitat suitability probability from year to year, which in turn govern the actual population density for Round Sardine in consecutive seasons (Figure 3.3).

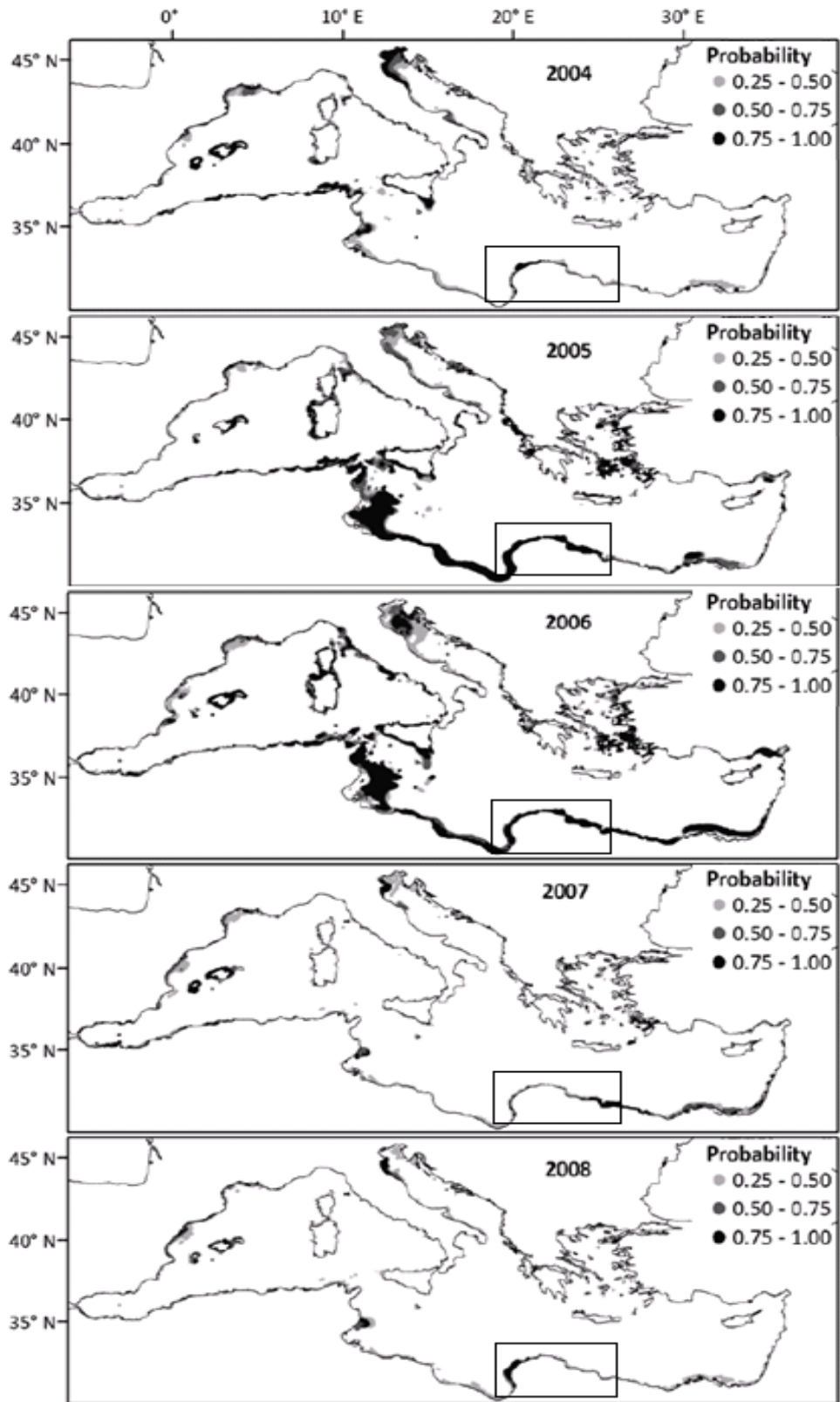


Figure 3.3 Annual habitat suitability maps for Sardine probability presence on the Mediterranean coastal areas during June, based on the selected GAM model. Study area in black rectangle. The scale indicates probability range (after Giannoulaki et al., 2011)

Table 3.8 Similarity percentages (SIMPER) of the Bray-Curtis index for the relative contribution to dissimilarity in diet structure of Lesser Crested Tern young at the Gara colony (2008-2010) ranked by decreasing discriminating power (%).

Taxon	Average dissimilarity	Contribution (%)	Cumulative (%)
<i>Sardinella aurita</i>	16.7	27.3	27.3
<i>Cheilopogon heterurus</i>	12.7	20.7	48.0
<i>Hemiramphus far</i>	8.1	13.2	61.2
<i>Chromis chromis</i>	5.2	8.5	69.7
<i>Boops boops</i>	3.7	6.0	75.6
<i>Spicara smaris</i>	3.2	5.3	80.9
<i>Diplodus vulgaris</i>	2.7	4.4	85.3
<i>Scomber scombrus</i>	2.6	4.2	89.5
<i>Loligo vulgaris</i>	2.2	3.5	93.0
<i>Siganus luridus</i>	1.8	3.0	96.0
<i>Sparisoma cretense</i>	1.2	2.0	98.0
<i>Engraulis encrasicolus</i>	1.2	2.0	100.0

At Elba, 57.47% of the dissimilarity was due to Mediterranean Flying fish *Cheilopogon heterurus* (22.5%) and the Black-barred halfbeak *Hemiramphus far* (13.54%). The remaining ten species shared the remaining effect. (Table 3.9).

Table 3.9 Similarity percentages (SIMPER) of the Bray-Curtis index for the relative contribution to dissimilarity in diet structure of Lesser Crested Tern young at the Elba colony (2008-2010) ranked by decreasing discriminating power (%).

Taxon	Average dissimilarity	Contribution (%)	Cumulative (%)
<i>Cheilopogon heterurus</i>	16.6	28.1	28.1
<i>Hemiramphus far</i>	13.2	22.4	50.6
<i>Sardinella aurita</i>	4.5	7.7	58.3
<i>Oblada melanura</i>	3.9	6.7	64.9
<i>Pagellus erythrinus</i>	3.7	6.3	71.2
<i>Sparisoma cretense</i>	3.6	6.2	77.4
<i>Belone Belone</i>	3.4	5.7	83.1
<i>Seriola dumerili</i>	2.5	4.2	87.4
<i>Coryphaena hippurus</i>	2.0	3.4	90.8
<i>Diplodus sargus</i>	1.5	2.6	93.4
<i>Chromis chromis</i>	1.3	2.2	95.6
<i>Boops boops</i>	1.3	2.2	97.8
<i>Diplodus vulgaris</i>	1.3	2.2	100.0



At Jeliana, based on two sampling seasons, about 51% of dissimilarity was caused by Round Sardine and European Anchovy (25.06% and 50.52% respectively). The other sixteen species shared the remaining effect (Table 3.10).

Table 3.10 Similarity percentages (SIMPER) of the Bray-Curtis index for the relative contribution to dissimilarity in diet structure of Lesser Crested Tern young at the Jeliana colony (2008 and 2012) ranked by decreasing discriminating power (%).

Taxon	Average dissimilarity	Contribution (%)	Cumulative (%)
<i>Lithognathus mormyrus</i>	11.3	15.3	15.3
<i>Lipophrys trigloides</i>	9.0	12.2	27.5
<i>Sardinella aurita</i>	8.0	10.8	38.3
<i>Engraulis encrasicolus</i>	6.5	8.8	47.2
<i>Atherina boyeri</i>	6.4	8.7	55.9
<i>Boops boops</i>	5.9	7.9	63.8
<i>Chromis chromis</i>	5.0	6.7	70.6
<i>Diplodus vulgaris</i>	3.7	5.0	75.6
<i>Spicara smaris</i>	3.4	4.6	80.1
<i>Sarpa salpa</i>	3.0	4.1	84.2
<i>Siganus luridus</i>	2.8	3.8	88.0
<i>Siganus rivulatus</i>	2.2	2.9	90.9
<i>Lichia amia</i>	2.1	2.9	93.8
<i>Tilapia zilli</i>	1.8	2.4	96.2
<i>Sparisoma cretense</i>	1.2	1.6	97.8
<i>Coris julis</i>	1.0	1.3	99.1
<i>Salaria pavo</i>	0.7	0.9	100.0

### 3.3.3 Temporal distribution of reconstituted mass in the young's diet at Elba colony

Nine families of fish composed the diet of Lesser Crested Terns at Elba Island during the 2008-2012 breeding seasons (Table 3.11). The temporal distribution of species and their relative mass in the total mass of diet samples varied across the sampling years. In 2008 the most dominant species was Mediterranean Flying fish *Cheilopogon heterurus* (Exocoetidae) with 56.7% of the diet followed by three species of Sea Bream (Sparidae) responsible for 30% of the mass, the remaining two families (Carangidae and Pomacentridae) comprising 9.68 and 3.61% of the diet respectively.

In 2009 five new species were added to the diet list, but Flying fish continued to dominate the mass, with 30.2%, followed by the Dolphin fish (Coryphaenidae) with 21.1% and Blackbarred halfbeak *Hemiramphus far* (Hemiramphidae) with 21% (a new established alien species originated from the Red Sea). Carangidae accounted for 16.8% of the total mass followed by Garfish *Belone belone* (Belonidae), Parrot fish *Sparisoma cretense* (Scaridae) and the Round Sardine *Sardinella aurita* (Clupiedae) with 4.7%, 3.9% and 2.3%, respectively. The sampling effort in 2010 was not optimal due to the division of the colony between the two Islands (Elba and Fteha) and breeding failure of the new colony at Fteha Island due to a sea storm on July 21<sup>st</sup> 2010, leaving only five nestlings which later were either predated by gulls or collected by local poachers.

At Elba, egg collection by some local poachers disturbed the colony resulting in nest desertion for 7 out of the 19 nests. Only three species of fish were collected from regurgitated food at Elba during the 2010 season; Greater amberjack (Carangidae) with 63.1% of the total mass, followed by Mediterranean Flying fish *Cheilopogon heterurus* (Exocoetidae) with 36.3% and the Black-barred half beak *Hemiramphus far* (Hemiramphidae) with 0.6%.

In 2012, regurgitation collection only occurred on one day; Mediterranean Flying fish was dominant species with over 87% of total mass, followed by two Sea Breams species *Oblada melanura* and *Diplodus sargus* together with over 8%,

and 4.4% by *Sparisoma cretense*. The values of 2010 and 2012 at Elba should be considered with caution due to the small sample size (Table 3.11).

Table 3.11 Percentage reconstituted mass (g) of regurgitated food collected at the Elba breeding site (2008-2010).

Taxon	Family	2008		2009		2010		2012		Total mass
		mass		mass		mass		mass		
		(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	
<i>Belone belone</i>	Belonidae			4.56	4.6					4.56
<i>Seriola dumerili</i>	Carangidae	8.08	6.6	14.93	15.1	9.04	63.1			32.05
<i>Sardinella aurita</i>	Clupiedae			4.16	4.2					4.16
<i>Coryphaena hippurus</i>	Coryphaenidae			18.08	18.2					18.08
<i>Cheilopogon heterurus</i>	Exocoetidae	71.8	58.7	17.3	17.5	5.2	36.3	102.9	87.2	197.2
<i>Hemiramphus far</i>	Hemiramphidae			17.96	18.1	0.09	0.6			18.05
<i>Chromis chromis</i>	Pomacentridae	3.01	2.5							3.01
<i>Sparisoma cretense</i>	Scaridae			7.19	7.3			5.24	4.4	12.43
<i>Boops boops</i>	Sparidae	18.9	15.5							18.99
<i>Diplodus vulgaris</i>		3.24	2.6							3.24
<i>Diplodus sargus</i>								2.2	1.9	2.2
<i>Oblada melanura</i>		17.2	14.1					7.69	6.5	24.91
<i>Pagellus erythrinus</i>				14.89	15.0					
Total mass (g)		122.3		99.07		14.33		118.03		353.73

By pooling data from all survey years at Elba by prey taxon frequency, fish belonging to the Exocoetidae and Hemiramphidae represent 67% of the diet structure at this site, followed by the Sparidae (13%), while the other families share the remainder (Figure 3.3). In terms of prey taxon mass, 56% of the diet was composed of Flying fish (Exocoetidae), while Sparidae and Carangidae represent 18 and 9% of the total mass, respectively (Figure 3.4).

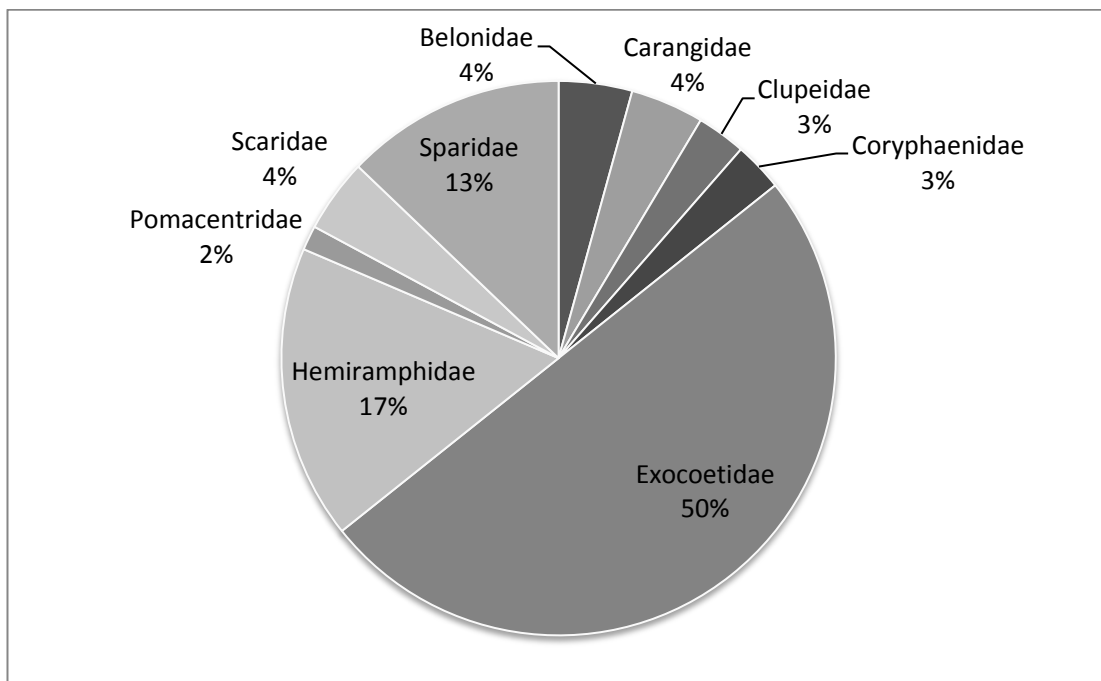


Figure 3.3 Relative abundance of fish families in the diet of Lesser Crested Tern young at Elba, Libya (pooled data 2008-2010 and 2012).

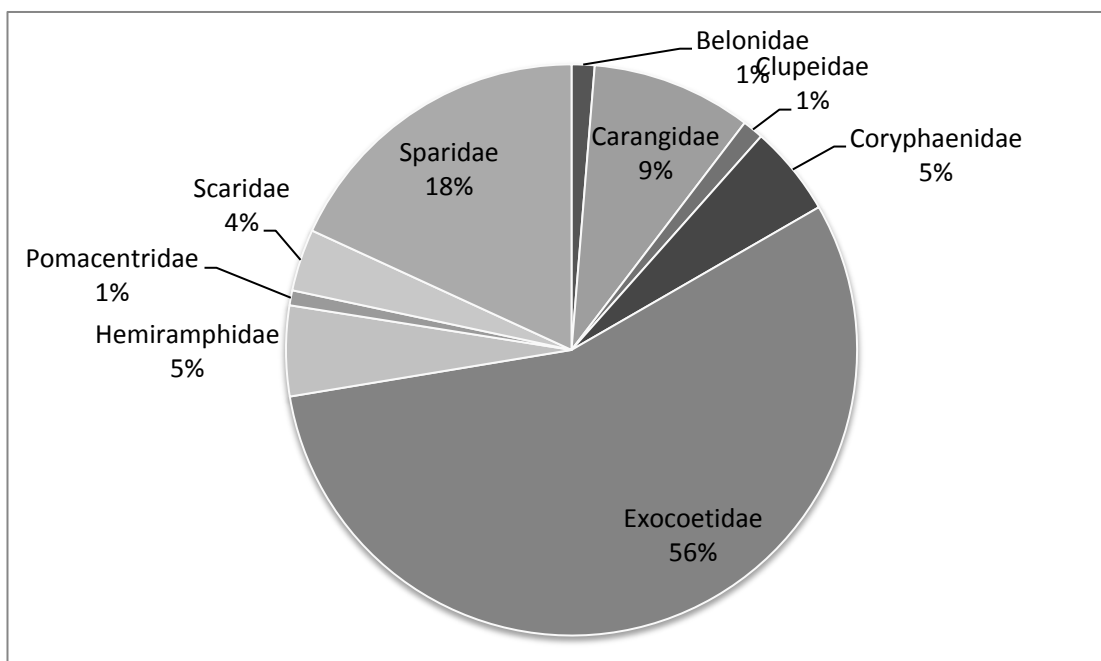


Figure 3.4 Relative mass of fish families in the diet of Lesser Crested Tern young at Elba, Libya (pooled data 2008-2010 and 2012).

### 3.3.4 Temporal distribution of reconstituted mass in the young's diet at Gara colony

Ten species of fish and one cephalopod species were found in the diet of young at Gara from 2008 to 2010 (Table 3.12). In 2008, Blotched Picarel *Spicara maena*, (Centracanthidae) contributed 44% of the total mass, followed by Mediterranean Flying fish *Cheilopogon heterurus* (Exocoetidae) with 24.5%, Round Sardine *Sardinella aurita* (Clupiedae) with 20.1% and finally 11.3% of the diet mass consisting of European Anchovy *Engraulis encrasicolus*, (Engraulidae).

In 2009, four species were added to the diet list due to sampling effort and frequency of visits to the colony site. Mediterranean Flying fish were the highest in diet weight, with 37.2%, followed by the Round Sardine (*Sardinella aurita*, Clupiedae) with 26.3%, six other species sharing the remaining weight, with 10% for Atlantic Mackerel *Scomber scombrus* (Scombridae), 7.4% for Bogue *Boops boops* (Sparidae), 6.7% for European Anchovy *Engraulis encrasicolus* (Engraulidae), 5.8% for Mediterranean Chromis *Chromis chromis* (Pomacentridae) and finally 2.2% for Blotched Picarel *Spicara maena* (Centracanthidae).

In 2010, the invasive Black-barred halfbeak *Hemiramphus far* (Hemiramphidae) seemed to be well established in the Gara area, indicating of its spread from the east towards the western Mediterranean waters of Libya. It shared with Round Sardine about 45% of the diet (23.9% and 21.4%, respectively), while Mediterranean flying fish were at 19%. Bogue *Boops boops* (Sparidae) was responsible for 10.4 % of the diet. The other three species mass ranged from 7-7.3% each (Table 3.12).

During 2010, remains of Squid *Loligo sp.* (Loliginidae) were found near the nests. Indicating that although Lesser Crested Terns utilise this species as part of their diet, the source of this prey item being either from discarded fish or actively fished by terns was not able to be confirmed (Figure 3.5). Lesser Crested Terns have been observed to forage nocturnally during the several overnight sea observation periods at the Elba and Gara colony sites, this also being recently confirmed at other breeding sites of the species in the Persian Gulf region (Symens and Evans, 1993), which may explain the presence of squid which is active during

night. However the size of adult squid probably excludes this assumption unless it was a small juvenile or fish discarded.

Table 3.12 Percentage reconstituted mass (g) of regurgitated food collected at the Gara breeding site (2008-2010).

Taxon	Family	2008		2009		2010	
		mass		mass		mass	
		(g)	(%)	(g)	(%)	(g)	(%)
<i>Sardinella aurita</i>	Clupeidae	6.08	20.15	22.08	25.95	37.18	21.43
<i>Sparisoma cretense</i>	Scaridae					12.70	7.32
<i>Hemiramphus far</i>	Hemiramphidae					41.60	23.97
<i>Cheilopogon heterurus</i>	Exocoetidae	7.40	24.52	31.12	36.57	31.06	17.90
<i>Engraulis encrasicolus</i>	Engraulidae	3.40	11.27	5.63	6.62		
<i>Spicara smaris</i>	Centracanthidae	13.30	44.07	1.87	2.20	11.40	6.57
<i>Siganus luridis</i>	Siganidae			3.64	4.28		
<i>Chromis chromis</i>	Pomacentridae					11.45	6.60
<i>Scomber scombrus</i>	Scombridae			9.62	11.31		
<i>Boops boops</i>	Sparidae			6.17	7.25	18.12	10.44
<i>Loligo vulgaris</i>	Loliginidae					10	5.76
Total mass (g)		<b>30.18</b>		<b>80.13</b>		<b>173.51</b>	

Round Sardine *Sardinella aurita* and Mediterranean Flying fish *Cheilopogon heterurus*, the recently reported Red Sea migrant Black-barred halfbeak *Hemiramphus far* (Shakman and Kinzelbach, 2006) and Sea Bream (Sparidae) were the most common species.

Generally by pooling data on abundance and mass of all the survey seasons at Gara, it is clear that Clupeidae (sardines) represented the most abundant species (42%) in the diet of the young followed by Exocoetidae (Flying fish) with 20% (Figure 3.5). In terms of prey mass, the family Exocoetidae represented 23% of diet mass followed by the Clupeid at 22%, with the Hemiramphidae and Centracanthidae at 14% and 13%, respectively (Figure 3.6).

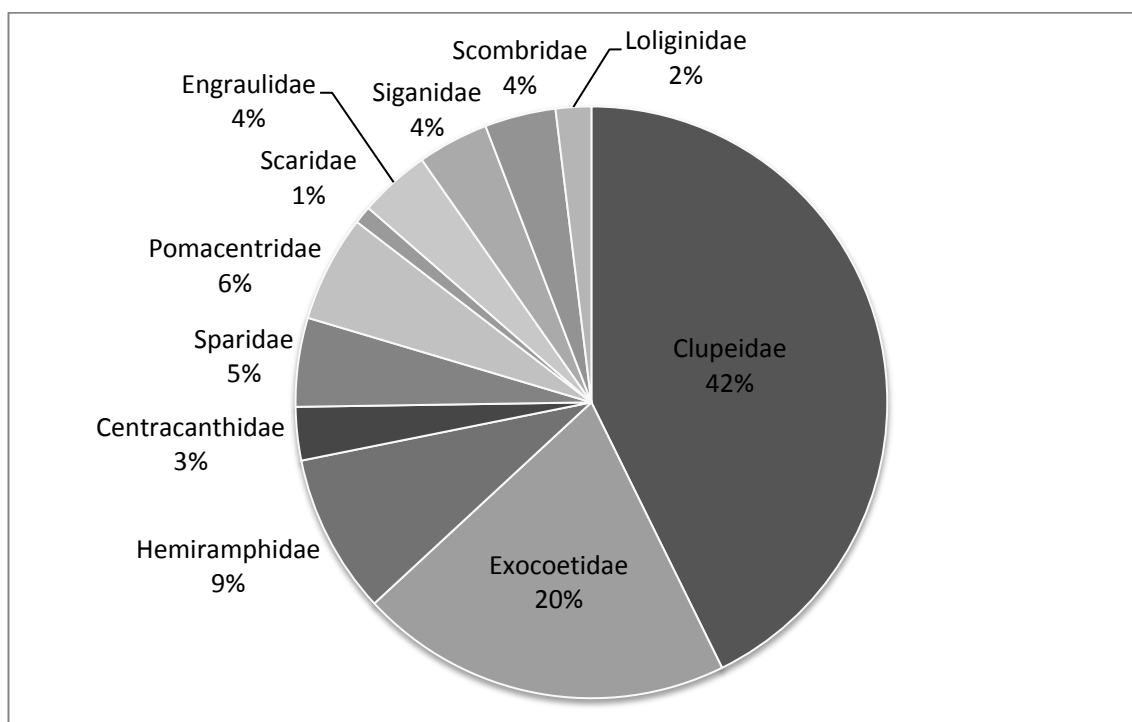


Figure 3.5 Relative abundance of fish families in the diet of Lesser Crested Tern young at the Gara, Libya (pooled data 2008-2010).

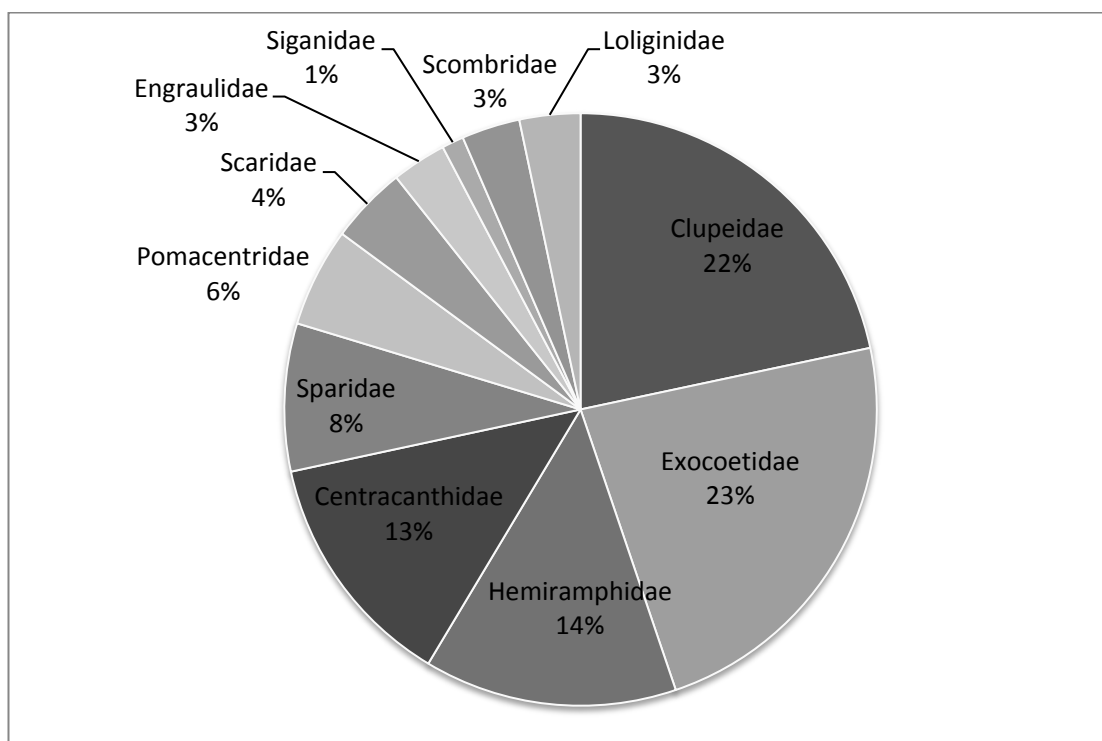


Figure 3.6 Relative mass of fish families in the diet of Lesser Crested Tern young at the Gara, Libya (pooled data 2008-2010).

### 3.3.5 Temporal distribution of reconstituted mass in the young's diet at Jeliana colony

Eighteen fish species belonging to eleven families composed the diet of Lesser Crested Tern young at Jeliana in the 2008 and 2012 breeding seasons (Table 3.13). The temporal distribution of species and their reconstituted mass in the diet samples varied across years. In 2008 the most dominant species was Round Sardine *Sardinella aurita* with 46% of the diet followed by European Anchovy *Engraulis encrasicolus* at 40%, the least dominant three families (Centracanthidae, Blenniidae and Scaridae) comprising 3.2 %, 4.96 % and 5.4% respectively. In 2012 thirteen new species were added to the diet list, 43.9% of the total mass relating to four Sparidae species, Sand steenbras *Lithognathus mormyrus* with 23% followed by 15.3% for Bogue *Boops boops*, 3.2% for Two-banded Sea Bream *Diplodus vulgaris* and Salema *Sarpa salpa* at 2.2%. Two Blenniidae species *Lipophrys trigloides* and *Salaria Pavo* shared 12.1% of the total mass, the remainder being composed of other fish species.

In terms of fish frequency, pooling the data of both years revealed that fish belonging to the family Sparidae represented more than 33%, followed by the Blenniidae at 15% and 11% for the Atherinidae which is solely recorded only from Jeliana (Figure 3.7).

A similar trend has been noticed with regards to fish mass at Jeliana, species belonging to the Sparidae representing 40% of the total fish mass collected, followed by the Blenniidae at 11% although Atherinidae were replaced by the Siganidae at 10%. The other families shared the remaining 39% (Figure 3.8).



Table 3.13 Percentage reconstituted mass (g) of regurgitated food collected at the Jeliana breeding site 2008 and 2012.

Taxon	Family	2008		2012	
		mass		Mass	
		(g)	(%)	(g)	(%)
<i>Boops boops</i>	Sparidae			212.53	15.34
<i>Diplodus vulgaris</i>				44.44	3.21
<i>Lithognathus mormyrus</i>				319.15	23.03
<i>Sarpa salpa</i>				30.89	2.23
<i>Lipophrys trigloides</i>	Blenniidae	7.10	4.96	161.34	11.64
<i>Salaria pavo</i>				6.25	0.45
<i>Spicara smaris</i>	Centracanthidae	4.58	3.20	107.93	7.79
<i>Siganus luridus</i>	Siganidae			83.41	6.02
<i>Siganus revulatus</i>				67.58	4.88
<i>spraisoma cretense</i>	Scaridae	7.74	5.40	59.12	4.27
<i>Atherina boyeri</i>	Atherinidae			57.96	4.18
<i>Chromis chromis</i>	Pomacentridae			53.83	3.88
<i>Sardinella aurita</i>	Clupeidae	65.88	45.98	51.56	3.72
<i>Lichia amia</i>	Carangidae			50.16	3.62
<i>Tilapia zillii</i>	Cichlidae			34.92	2.52
<i>Engraulis encrasicholus</i>	Engraulidae	57.98	40.47	19.90	1.44
<i>Coris julis</i>	Labridae			14.64	1.06
<i>Scomber scombres</i>	Scombridae			10.05	0.73
<b>Total mass (g)</b>		143.28		1,385.66	

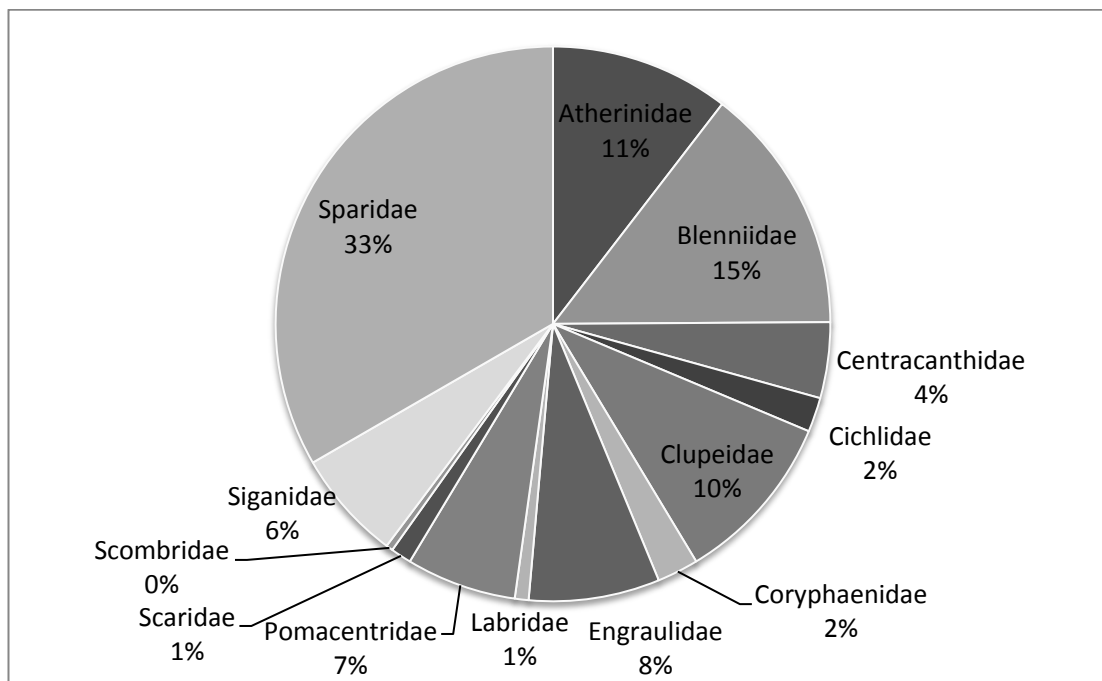


Figure 3.7 Relative abundance of fish families in the diet of Lesser Crested Tern young at Jeliana, Libya (pooled data 2008 and 2012)

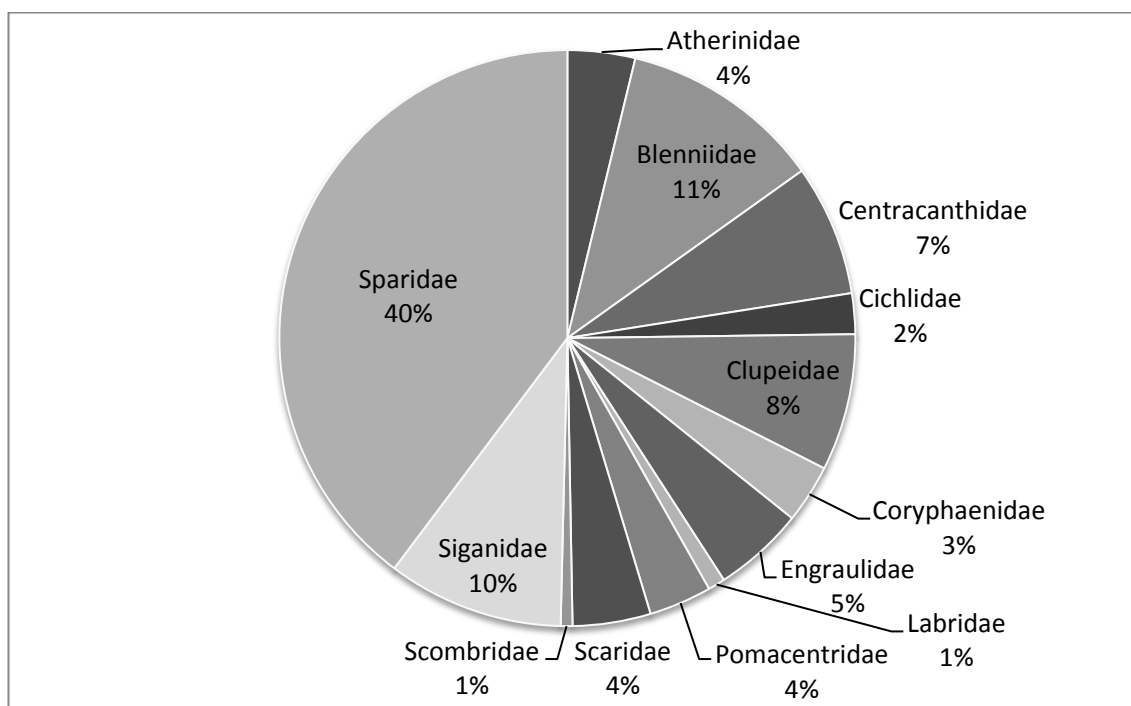


Figure 3.8 Relative mass of fish families in the diet of Lesser Crested Tern young at Jeliana, Libya (pooled data 2008 and 2012)

### 3.3.6 Distribution of prey total length (TL) at the three breeding sites

The total length of regurgitated fish specimens (whole and partially digested fish) collected at the Lesser Crested Tern colonies ranged from 26.8-150 mm (mean =  $77.34 \pm 21.8$ ) at Jeliana, from 32-140 mm (mean =  $77.7 \pm 25.1$ ) at Elba and 33-109 mm (mean =  $63.4 \pm 20.0$ ) at Gara. The percentage frequency distribution of fish length shows some variable trends between the sites. At Jeliana, most fish ranged from 50 to 100 mm while at Elba, about 25% of the fish sampled were in the 100-140 mm category, and at Gara about 70% of fish length was in the 40-70 mm category (Figure 3.9). Prey total length in the samples represents either planktonic or juvenile fish stages, as these terns actively select prey that suit the maximum gape opening of both adult and young terns (Hulsman, 1981).

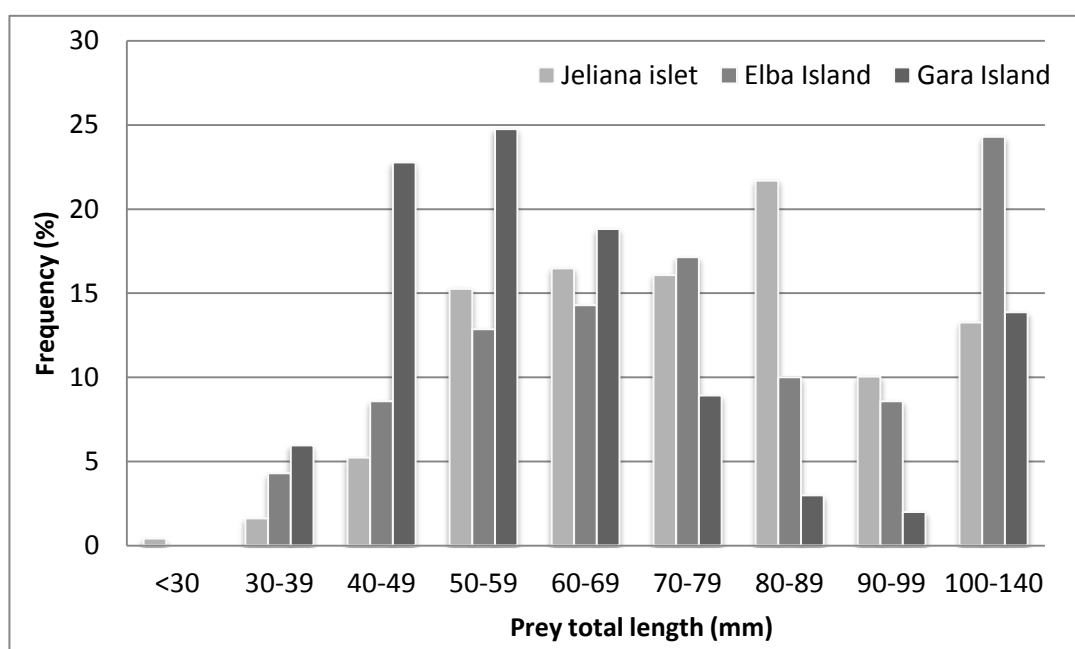


Figure 3.9 Percentage frequency distribution of prey total length (mm) collected from Lesser Crested Tern young at the Gara, Jeliana and Elba colonies, Libya.

The frequency of fish total length departing from the median at the Elba colony is shown in (Table 3.14). There was no significant difference in median total length of prey at the Elba colony among the sampling years (2008, 2009, 2010 and 2012). Mood's Median Test (Pearson  $\chi^2 = 1.90$ , d.f. = 3, n = 70,  $P > 0.05$ ) indicating the terns may actively select their prey in a specific length range during the provisioning period.

Table 3.14 Frequency of fish total length departing from median at the Elba colony

Elba fish TL (mm)	Sampling years			
	2008	2009	2010	2012
<b>&gt; Median</b>	8	13	1	9
<b>≤ Median</b>	9	20	3	7

The frequencies of fish total length departing from the median at the Gara colony are shown in Table 3.15). Mood's Median Test produce this table to present the data in each of the four groups tested (one consisting row of data whose values are higher than the median value in the four groups (years) combined (> Median in Table 3.15), and the other consisting of data whose values are at the median or below (< Median in Table 3.15)

There was a significant difference in median total length of prey sampled at the Gara colony between sampling years (2008, 2009 and 2010). Mood's Median Test (Pearson  $\chi^2 = 9.22$ ,  $n = 101$ ,  $d.f = 2$ ,  $P < 0.01$ ) indicates some year to year temporal change in fish length across the sampling seasons. Pair-wise comparison shows that there were significant differences in fish total length between the years 2008-2009 and 2008-2010 (Two-sample Kolmogorov-Smirnov Test,  $Z = 1.84$ ,  $P < 0.05$ ) but not between 2009 and 2010 (Two-sample Kolmogorov-Smirnov Test,  $Z = 0.856$ ,  $P > 0.05$ ). This difference is due to the sample size collected in 2008 with a relatively larger median value (105 mm) compared to the medians for 2009 and 2010 (60 and 57mm, respectively).

Table 3.15 Frequency of fish total length departing from median at the Gara colony

Gara fish TL (mm)	Sampling years		
	2008	2009	2010
<b>&gt; Median</b>	6	16	20
<b>≤ Median</b>	0	23	36

The frequency of fish total length departing from the median at the Jeliana colony is shown in (Table 3.16). At this colony, fish total length was significantly different between the two seasons (2008 and 2012) as given by the Mood's Median Test

(Pearson  $\chi^2 = 9.7$  n = 101, d.f. = 2,  $P < 0.01$ ). This result should be taken with care as the samples collected during 2008 represent only 6.4% of the total samples collected at this site; therefore a bias in dataset size is possible cause of this high significant difference.

Table 3.16 Frequency of fish total length departing from median at the Jeliana colony

Jeliana fish TL (mm)	Sampling years	
	2008	2012
> Median	14	110
≤ Median	2	123

### 3.6.7 Prey mass distribution at the three breeding sites

Terns fed on larger fish at the Jeliana and Elba colonies compared to the Gara colony. The pooled reconstituted mass for regurgitated fish at Jeliana ranged from 0.3-41.2g (mean = 6.1 ±5.9, n = 249), at Elba it ranged from 0.1-19 g (mean = 5.1 ±4.3, n = 70), while at Gara it ranged between 0.3-25.7g (mean = 3.3, ±2.6, n =103). As the fish mass data are not normally distributed, the median and range of minimum and maximum fish weight are shown in Figure 3.10.

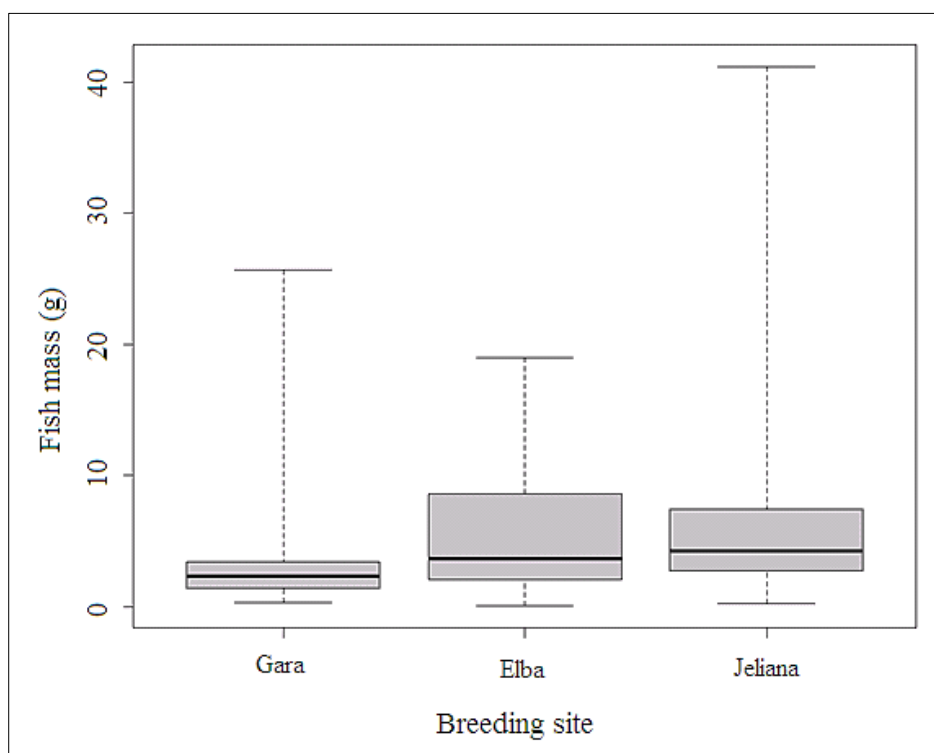


Figure 3.10 Distribution of pooled median fish mass (g) of regurgitated food collected from Lesser Crested Terns nestlings at three breeding sites, Libya

When pooled data of reconstituted prey mass from all sites and years were compared, significant differences in mass were found between sites (Mood's Median Test: Pearson  $\chi^2 = 42.09$ , d.f.= 2, n = 420,  $P < 0.01$ ).

### 3.3.8 Temporal distribution of prey mass at the three colonies

Using Mood's Median test (Mood, 1950 cited in (Zar, 1996)), assuming that there is no significant difference in median values of fish weight across sampling years for a single site, the median value for reconstituted fish mass at Elba was significantly different between sampling years (2008-2012) (Mood's Median Test: Pearson  $\chi^2 = 27.07$ , d.f.=3, n =70,  $P < 0.01$ ). The same was noticed for the Gara samples (2008-2010) (Mood's median Test: Pearson  $\chi^2 = 7.16$ , d.f = 2, n=101,  $P < 0.05$ ) and at Jeliana (2008-2012) (Mood's median Test: Pearson  $\chi^2 = 74.88$ , d.f =1, n = 249,  $P < 0.05$ ).

### 3.3.9 Prey sizes during the later provisioning period

It is expected that with the growing energetic requirements during the later provisioning period, adult seabirds need to increase foraging effort either by increasing foraging hours or by actively selecting larger prey to feed their offspring (McLeay et al., 2010). Therefore the total length of regurgitated fish was divided according to sampling date in relation to the total provisioning period (hatching to fledging). Regurgitated fish during the middle and later provisioning periods were sub-sampled, total length medians being obtained and plotted against sampling date (or provisioning period). At Elba, there was a trend of larger fish being caught during the later provisioning period in 2008, 2009 and 2012 compared with the middle provisioning period for those years (Figure 3.11). When all samples from both years were pooled as three classes (early, middle and late) there was a significant difference in median fish total lengths (Mood's Median test: Pearson  $\chi^2 = 26.0$ , d.f = 2, n =249,  $P < 0.05$ ). At Gara a similar trend was also noticed (Figure 3.12), fish length increasing between middle and later provisioning periods (Mood's Median test: Pearson  $\chi^2 = 10.47$ , d.f = 4, n =100,  $P < 0.05$ ).

At Jeliana (Figure 3.13), fish length was larger during the 2008 and 2012 later provisioning periods; there appears to be no difference between the early and middle provisioning periods during the 2012 season; and a difference can be seen between the first and second 2012 middle provisioning periods.

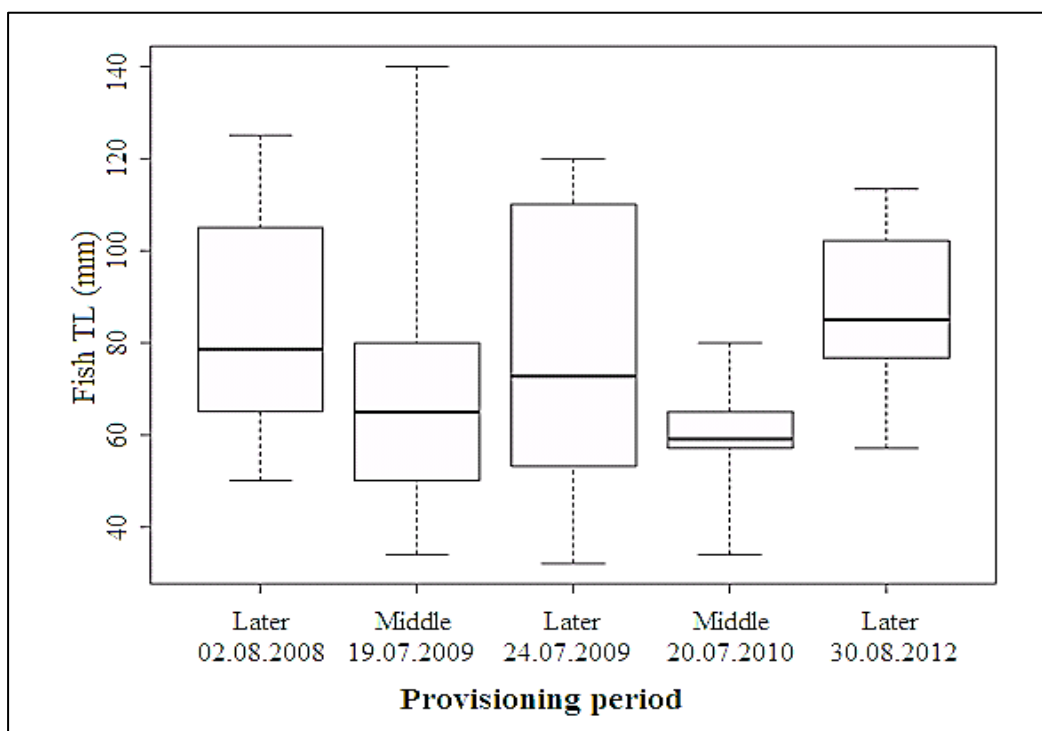


Figure 3.11 Mean prey total length during the middle and later provisioning periods at Elba colony.

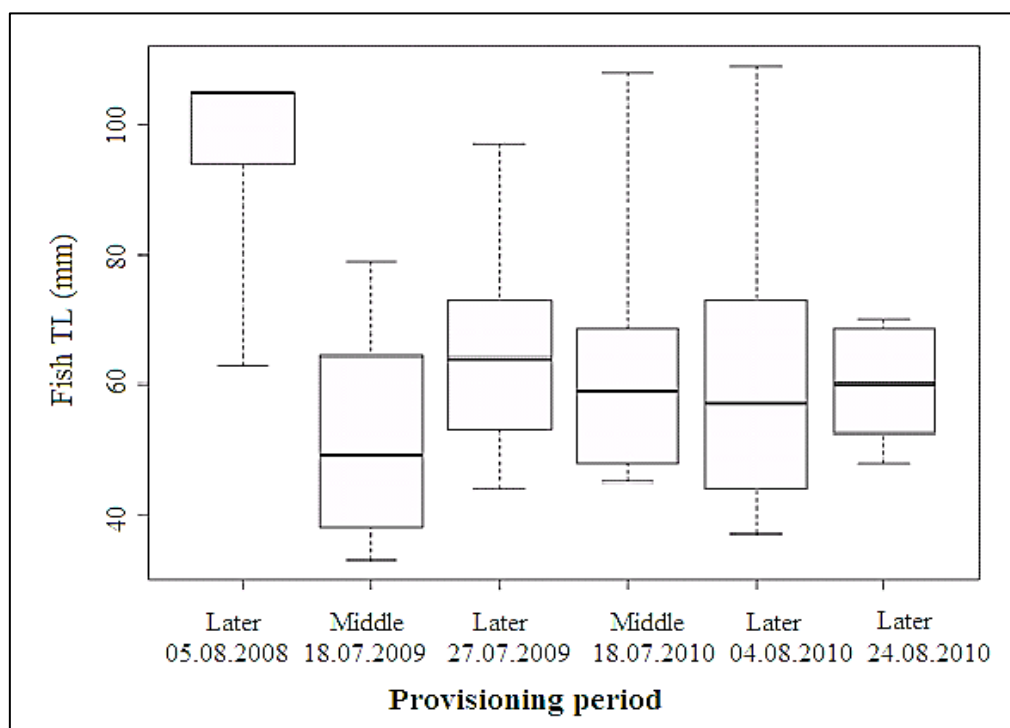


Figure 3.12 Mean prey total length during middle and later provisioning periods at Gara colony.



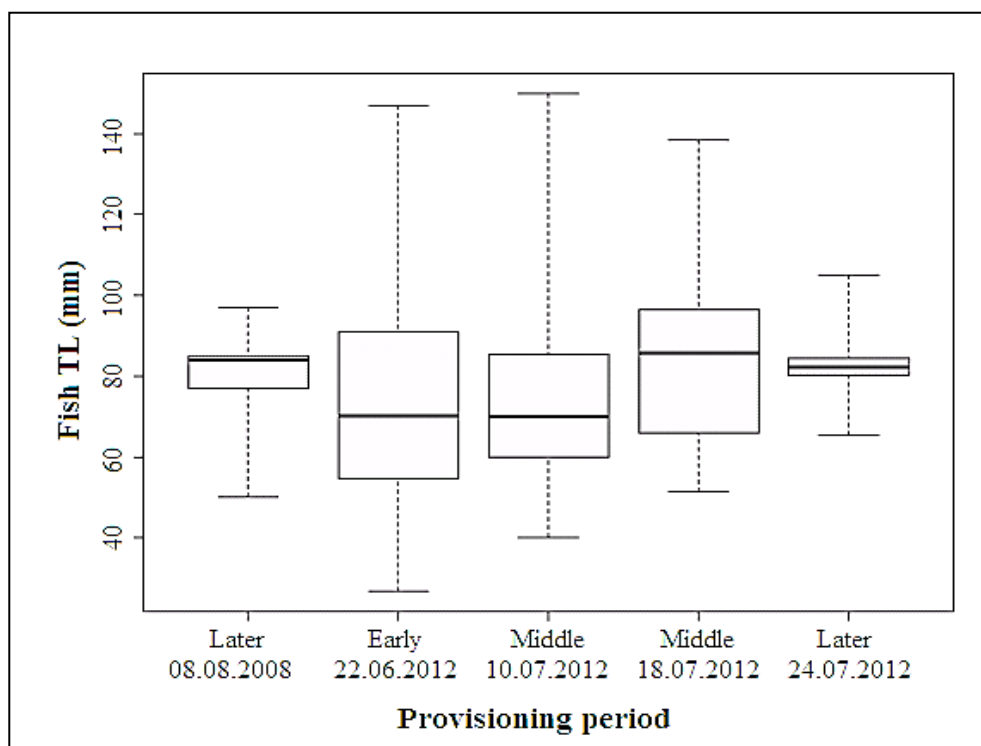


Figure 3.13 Mean prey total lengths during middle and later provisioning periods at the Jeliana colony.

### 3.3.10 Species richness at each sampling site (Rarefaction method):

Rarefaction curves were consistent with the coverage values found and reached saturation for Jeliana sampling site (Figure 3.14), indicating that the sampling effort was sufficient to reveal all species present in the diet of lesser crested terns at this site. However, for the other two sites of Gara and Elba, it is clear from the curve shape that Gara is close to saturation stage, while more sampling would reveal more species in the diet of the terns at this site.

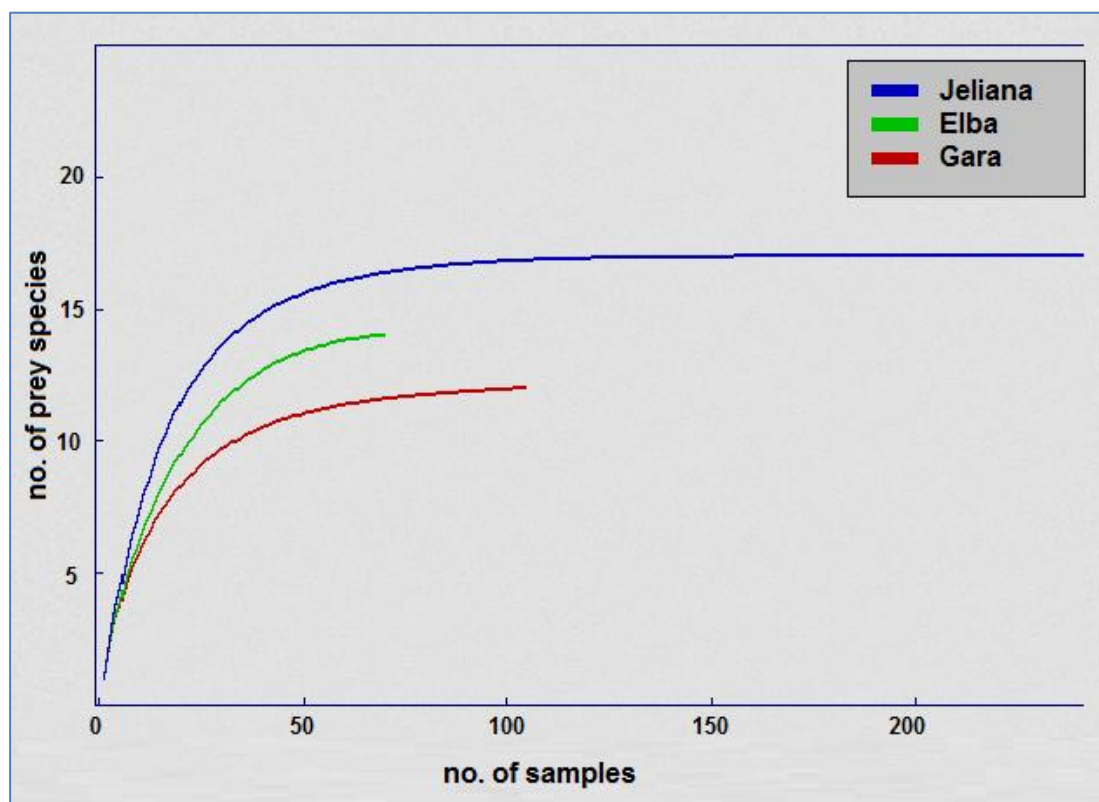


Figure 3.14 Rarefaction accumulation curves for the diet sampling at three breeding sites, Libya.

### 3.3.11 Breeding productivity and diet species diversity

Diet species diversity (Shannon-Wiener index) was positively correlated to breeding productivity (Spearman's  $\rho = 0.77$ ,  $P < 0.05$ ) with higher nest success in years when diet was most diverse (Figure 3.15).

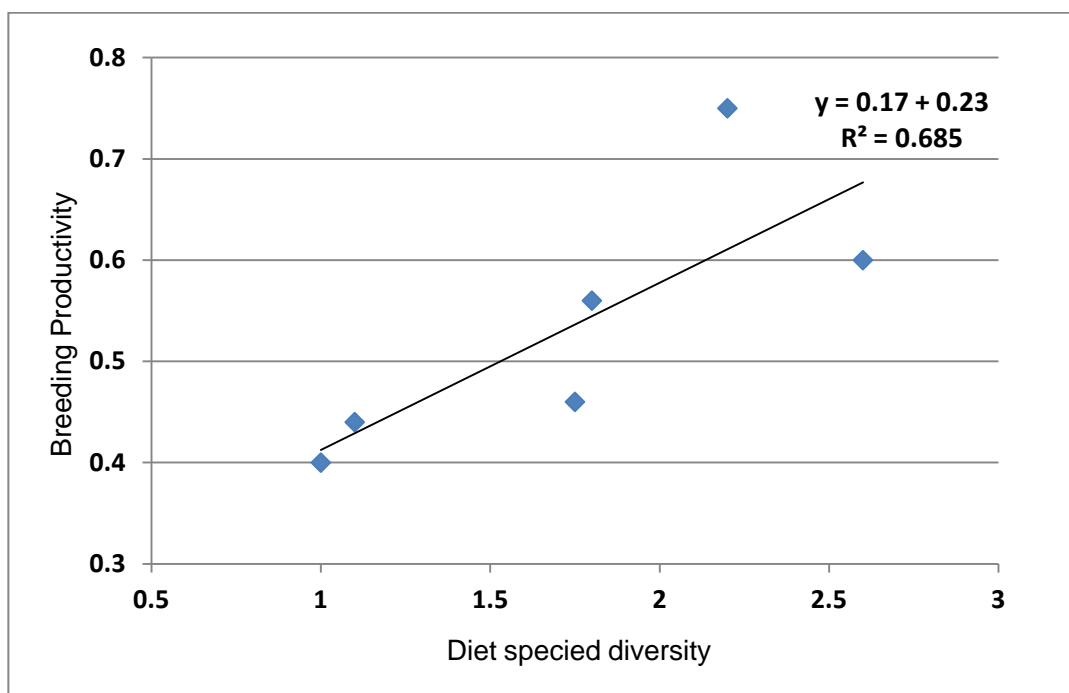


Figure 3.15 Effect of diet species diversity of the Lesser Crested Tern young on breeding productivity (fledged chicks/ breeding pair).

### 3.4 Discussion

This chapter assesses one of the ecological requirements for the Mediterranean population of Lesser Crested Tern *T. b. emigrata* breeding along the Libyan coast. The results of this study are discussed according to the chapter objectives and research questions stated in section 3.4 above:

Crested Terns utilize a wide range of epipelagic fish species and to a lesser extent some Cephalopods and Crustaceans (Blaber et al., 1995, Cramp, 1985, Gochfeld and Burger, 1996, Nicholson, 2002). Epipelagic fish play an important role in coastal ecosystems by transferring energy from primary producers to upper trophic levels (Cury et al., 2000, Giannoulaki, 2010, Burger, 1988). The diet composition of the Lesser Crested Terns varies spatially, for instance in Sri Lanka they feed exclusively on Herrings (Clupeidae) while Prawns are the main food in Pakistan waters (Cramp, 1985). The diet structure resulted from the present study show similar prey population structure utilized by the terns with those in Australian waters at prey family level (Nicholson, 2002), indicating similar niche structure.

The foraging system of Lesser Crested Tern close to their colonies, on shallow and/ or inshore waters, provide a potential for feeding on diversity of prey species, due to the availability of such species at these habitats, compared with offshore foraging seabirds that may depend on a smaller number of prey species, and expend more time and energy to deliver the food to the young in colonies (Hulsman et al., 1989). This type of near-colony foraging is ultimately influence tern life history traits and their broader ecology.

Ten fish species and one cephalopod at Gara, 12 fish species at Elba and 18 fish species at Jeliana were found to make up the diet of Lesser Crested Tern young at the three main breeding sites of the species in Libya. From the sample sizes collected, the majority of fish are either planktonic (larval) or the juveniles of demersal species. During this phase, many of these species tend to occupy the upper layer of the water column, searching for food (mainly zooplanktons and phytoplankton) and use seagrass meadows of *Posidonia oceanica* as a shelter from predators. Some Mesopelagic and Benthopelagic fish species have daily vertical migration patterns and predatory fish species can drive Epipelagic and other pelagic fish to the water surface (Burger, 1988). In some cases these fish have adapted to leave the water surface for some distance (e.g. Mediterranean flying fish *Cheilopogon heterurus*) to avoid predation. All of these factors allowed Lesser Crested Terns to use this variety of food resources available in the waters around breeding colonies (Table 3.2). There is an overlap with the plunge-dive depth range of adult birds, which is known to depend on air dive angle and the mass of the bird (Hulsman, 1988) in order to achieve the appropriate dive depth to capture its prey. In fact the large area covered by foraging Lesser Crested Terns, represents a way to understand the general structure of fish community that is usually poorly known, being not targeted by commercial fishing. Such fish communities occupying very shallow waters may also reveal signs of a changing environment that could be missed without the action of marine samplers (seabirds) that depend on them as a food (Horn et al., 2010), accumulating data on seasonal seabird food communities structure can be used as bio-indicators of the marine ecosystem health (Furness and Tasker, 1999).

Meininger et al., (1994) mentions four fish species in the diet of Lesser Crested Tern young at Elba colony: Bogue *Boops boops*, Garfish *Belone belone*;

Sandeel *Gymnamodytes cicerelus*; and a probable Grey Mullet (Mugilidae). No sandeel specimens were found in the Elba diet samples in this study which may indicate changes in structure of the local fish assemblage around this breeding site (although one specimen sandeel was recorded in Meininger's study, and it may not be a preferred food for the terns in Libya). At Gara all the prey species mentioned by Meininger (1994) were also found in the species list in this study. However, he did not mention either of the Black-barred halfbeak nor the Mediterranean Flying fish, which might be explained the later establishment of Black-barred halfbeak populations in Libya (first reported 13 years later by Shakman and Kinzelbach, 2006) and possibly due to the low sampling effort for diet composition in the 1994 survey.

Statistical analysis using the index of relative importance (IRI) has shown that some prey species are more dominant in the diet compared to others, either because of active selection by adult terns due to the ease and profitability of captured prey (caloric value), or as a result of a proportional higher abundance of such species at the foraging areas. Baseline information on ichthyoplankton distribution and diversity in Libya are limited, however it is well documented for the central Mediterranean that both European Anchovy *Engraulis encrasicolus* and Round Sardine *Sardinella aurita* spawn from May to September, with maximum population density during June and July (Palomera et al., 2007, Pinaud and Weimerskirch, 2007). An acoustic survey conducted in 2007 in Libyan coastal waters found that European Anchovy biomass was the highest, followed by Round Sardine, Bogue and Blotched Picarel (Harding et al., 2011, Bonanno et al., 2012), which in agreement with the findings of this study, regardless of the difference in sampling method.

Our results show some clear dissimilarity in structure of prey species between the three sampling areas, which may reflect the local abundance and fish assemblage structure at each site. For example at the Gara colony, Round Sardine and Mediterranean Flying fish represent about 63% of species frequency and about 46% of total food mass for the young. Similar proportions were obtained at Elba except that the prominent two fish species were Mediterranean Flying fish and Black-barred halfbeak, the later appearing to replace the Round Sardine niche.

The highest number of prey species was found at Jeliana (19 species) possibly due to the large sample size (249 items) compared to the other two sites. However, the location of this colony at Benghazi with its many inshore bays and coastal lakes (Ain Ziana lagoon and Benghazi Lake) appears to show a Lesser Crested Tern preference for foraging at these inshore shallow water sites, a lower proportion being observed to using deeper offshore waters. Taking into account the importance of these inshore sites as fish nurseries (and a former fish aquaculture site at the Ain Ziana lagoon), the adult terns have an easy and proximal area for provisioning their young.

Significant differences in pooled prey mass may indicate a preference for capturing a range of fish of different mass at each of the three sites. This would also reflect differences in fish recruitment and growth rate over the period of young tern provisioning at each site. The contribution of Mediterranean Flying fish with 56% and 23% of total prey mass at Elba and Gara, respectively, indicates the vulnerability of such fast moving surface fish to predation by terns as they emerge from the water it gives the foraging adults a stimulus as to both shoal size and location, making it easier to locate and caught.

The bathymetry and bottom structure can also limit fish diversity and their size range. Underwater observation by the author around both of Gara and Elba Islands has shown differences in bottom physical and biological components. At Elba the bottom is shallow and characterized by isolated circular structures of sea grass meadows, interrupted with a bare muddy bottom (Pergent et al., 2007), making fish movement between these meadows more visible to aerial predators (terns). The terns may also exploit the contrasting colour of discrete sea grass meadow patches, which are rich in small sized fish and where they concentrate their foraging effort to gain an energy profitable foraging activity. This situation explains a complex relationship between the physical and biological structure of the sea bottom, where fish communities inhabiting these structures are consumed by an aerial predator to fulfil its own energetic requirements, and requirements of their growing young.

At Gara the offshore deeper waters have a rocky/sandy bottom habitat, covered also by patches of *Posidonia* meadow, with a more disturbed water column compared to the relatively shallow water around the Elba Island, therefore the

majority prey collected at Gara were shoaling epipelagic species (Sardine and Flying fish), which are readily accessible to foraging terns than the other species occurring in deeper waters. At Jeliana, field observations indicate that most terns using the shallow lagoons and lake complex around the breeding site (Benghazi port marina, elthama/Essabri Lakes and Ain Ziana lagoon), with smaller proportion of terns foraging at coastal sea waters. This indicated by the high presence of two Blennidae *Lipophrys trigloides* and *Salaria pavo* in addition to the Cichlidae species *Tilapia zilli* which all occur in shallow marine and/or brackish waters.

In conclusion, Lesser Crested Terns have been found to use a wide range of fish size, with more preference for certain species being both easier to locate and of higher energetic value, thus fulfilling the high food demand for the young, with an active selection of larger prey during the later provisioning period as their gape becomes more suitable for receiving larger food items.

Seabirds in terms of feeding systems are either specialists who depend exclusively on particular prey species; e.g. Sandwich tern on herring and sand eel (Stienen et al., 2000), and the Roseate tern *Sterna dougallii* on sand eels (Safina et al., 1990), or generalists which are more common and successful breeding species because they can alternate between different prey type, depending on availability and foraging strategy (Begon et al., 1986). The selection of small number of prey species may also indicate an adult bird preference for utilizing high caloric food (Aygen and Emslie, 2006) in order to maximize the amount of energy delivered per trip (Jaquemet et al., 2008).

This ability to alternating prey choice can subsequently have some costs to the adults and their offspring (Hulsman et al., 1989), because each prey species varies in its energy content which may imply foraging on low calorie food, thus require increased foraging effort by the adults, to compensate for energy loss especially in the case of population decline of high-quality prey (Hamer et al., 1991, Pinaud and Weimerskirch, 2007). Population decline can be caused by overfishing (McLeay et al., 2009, Tuck et al., 2011, Furness and Tasker, 2000) and/or changes in climatic conditions during the critical provisioning period during the breeding season (Jones et al., 2002).

Switching between prey species varies among seabirds depending on foraging mode and prey type (Hulsman, 1988). Hence surface feeders such as terns might be more sensitive to changes in prey availability, compared to other species that obtain their food from deeper waters, because of the physical restrictions of their diving abilities (Stienen et al., 2000, Dänhardt and Becker, 2011, Hulsman, 1988) which may govern their conservation status because of depletion in food resources (e.g. the breeding failure of Arctic tern *Sterna paradisaea* in the North Sea breeding sites (Enstip et al., 2006)).

The present study of diet structure of Lesser Crested Tern population show that these terns are generalist opportunistic birds, with a tendency to increased foraging effort on a small number of high quality fish species, namely European Anchovy, Round Sardine, Mediterranean Flying fish and Black-barred halfbeak, which form the prominent diet species at the three breeding sites with differing proportions. Any change in prey population structure may adversely affect the breeding success of colonies with small number prey species (Gara and Elba), whereas terns at the Jeliana colony having more prey species.

Perrins (1970) models the relationship between food supply and timing of breeding, basically stating that: “food abundance should be sufficient to permit birds to meet their increased nutritional and energy needs during the breeding periods”. Therefore the timing of the breeding season is controlled by food availability needed to form eggs (Ramos, 2001).

The information on biology of fish species that comprise most of the Lesser Crested Tern young diet collected in the present study (and presumably a large proportion of adult tern food as well), most of these prey species are present throughout the year in Libyan waters (Whitehead et al., 1984–1986), their relative abundance increases steadily due to spring spawning with favorable oceanographic conditions, especially the Sea Surface Temperature (SST) during April-May (D'Elia et al., 2009, Pawson and Giama, 1985), which increases up to 10°C, compared to March. This in turn would trigger the increase in primary productivity of these waters, thus initiating the spawning of fish species, when the terns using this increase in prey population in May as a cue to start breeding.



Breeding is also separated from molting, as the species starts pre-breeding molt at wintering sites during late November, continuing in the head and body feathers from late February and completed by mid-March to early April when they are migrating back to their breeding sites (Cramp, 1985). Breeding then starts in late May at Jeliana or by mid-June at the Gara and Elba colonies (See Chapter 2). A simplified model of interaction between physical, biological and temporal parameters in Lesser Crested Tern breeding initiation was designed based on data collected from both this study and the literature (Chapter 6, Figure 6.1).

The four prominent prey species as mentioned above are shoal-forming species (Bahri and Freon, 2000, Whitehead et al., 1984–1986). The preference of adult terns to feed on shoaling fish seems to be facilitated by the conspicuousness of such aggregations from an aerial prospective (Erwin, 1977) that may move towards the water surface due to encountering another predation threat from underwater species, such as the Bottle-nose Dolphin *Tursiops truncatus* and the Atlantic Blue-fin Tuna *Thunnus thynnus* (Burger, 1988). These two latter species are common in the Libyan waters from May to July or August in synchrony with the Lesser Crested Tern breeding season (El-Kebir et al., 2002, Bearzi, 2006, Rooker et al., 2007). Some Bottle-nose dolphins were observed during the marine crossing to Gara Island during all the study years with shoals of Mediterranean Flying fish escaping underwater predation (personal observation).

To increase their foraging success, predators should seek out areas with high prey density while prey, in order to reduce the risk of mortality, should move away from areas with high density of predators (Sih, 2005). Predator-prey interaction between seabirds and fish shoals were controlled by several constraints, including spatial distribution of resources, interspecific competition, the location of spawning and breeding areas, and limitations on diving depth for seabirds to effectively utilize the fish resources available. Interaction between seabirds and prey should be viewed as a two-way spatial game where the outcome depends on how the participants are spatially constrained (Fauchald, 2009)

Spatial pattern is formed through self-organizing behavior that includes schooling, local enhancement and area-restricted search (reviewed by Weimerskirch, 2007).

Recently modeling has been employed to understand the complex spatial distribution relationship between predators and prey aggregations (Abrams, 2007), predators were found to aggregate in patches with high input of resources, while prey are also aggregated in the most productive patches; however, as a consequence of increased predation risk, they should have a more uniform distribution among resource patches (Sih, 2005, Fauchald, 2009). On the other hand, others found that habitat use by predators was found to be predicted most strongly by prey patch characteristics such as depth and local density within spatial aggregations (Benoit-Bird et al., 2013)

Predators are also dynamic in altering their foraging strategy accordingly, for example Northern Gannets *Morus bassanus* were found to change their diving and foraging behavior when there are changes in prey distribution and density. Diving depth and foraging ranges increased in seasons where prey density and distribution change (Garthe et al., 2011).

Field observations during this study showed some evidence of Lesser Crested Tern feeding in flocks around Gara Island in certain areas, which may indicate the presence of occasional fish shoals, in open waters. The number of terns participating in these flocks increase rapidly with elapsing time, as other birds become attracted via visual and aural cues from conspecific individuals. This shows a type of cascade feeding activity development, although limited by the maximum number prey in the tern's bill at the time. This limitation would require multiple foraging trips per day in an area where the distribution of prey species is sparse and unstable (D'Elia et al., 2009). The terns thus are challenged to locate, capture, transfer and deliver food to their young, which could be subjected to heat and hunger stress at the colonies. Therefore the amount of time they spend at sea may exceed the time period spend in caring for their young, hence collaborative parental care represents a strategy of managing time and effort when raising their offspring. Furthermore, during the second week after hatching, when a crèche is formed, personal observations indicate

cooperative care by other terns than parents, who dedicated themselves to the young, similar to Sandwich Tern adults (Stienen et al., 2000).

On the Great Barrier Reef of Australia, only 0.8% of Lesser Crested Terns have been documented feeding in flocks (Hulsman, 1988) which may pose some intra-specific differences in foraging behavior, possibly due to differences in spatial distribution prey fish, between Australian and Mediterranean waters. Based on own field observations of feeding behavior of the species in Libya, the terns are individually attracted to fish shoals, not as a cooperative foraging arrangement. There could be individual terns feeding independently on epipelagic fish below the water surface.

The accumulative effect of energy acquiring, through the food chain is of note. During the coupling and nest formation phases, seabirds need to restore their physiological condition, in order to be ready for the reproduction process, after a long migration journey. Spawning fish shoals can provide an enriched source of nutrients, with relatively lower energy expenditure during capture. While shoaling fish (e.g. Round Sardine) is preparing to spawn, they invest a great effort in building their protein and lipid reserves needed to produce their eggs to reproduce. A few weeks later, this food will be needed to nourish the recently hatched young. The terns opportunistically utilize the growing range of fish larvae to feed their nestlings, the growth rate in the fish working to favour prey type and size needed by the growing young (Chapter 2). Although the time synchrony of breeding tern with the increase of food abundance has been proved in many other tern species (e.g. the common terns), the continuity of food supply may decline when most needed during the post-hatching and fledging period (Safina et al., 1988). In this case it is noted that common tern breeding phenology is physiologically constrained, which could imply early breeding to achieve optimal timing and exploitation of the pattern of prey dynamics, which may not always be possible (Safina and Burger, 1985)

Diet structure indicated in this study was not significantly different across sampling years within each site, which may indicate some degree of stability in prey availability. However, it is important here to emphasize that the foraging decision-making process by adult terns plays a key role in selecting prey type and size, largely reflecting the structure of their diet rather than a simple

reflection of proportional abundance of prey species at the foraging area(s) (Elliott et al., 2008, Pinaud and Weimerskirch, 2007). Two expressions should be clearly differentiated here, the *preferred prey size* which the predator eats in preference to other sizes, and the *optimal prey size* which provides the predator with the highest net gain of energy per unit of handling time (Hulsman et al., 1989). The best prey item is that which falls between these two expressions. The decision-making process to catch the right prey species of the appropriate size is controlled by the dietary needs of the seabird (or its young) and the adult bird's foraging experience (Limmer and Becker, 2009) that in some conditions could constrain the individual to utilize certain prey species and size classes (McLeay et al., 2009, McLeay et al., 2010, Watanuki et al., 2008). The bill length of foraging adults would control prey size that can be physically caught i.e. the longer the bill the smaller capable of being obtain the prey (Hulsman, 1988).

The analysis of similarity (ANOSIM) revealed some weak but significant differences in diet structure of young at the three colonies. This is largely attributed to the presence of some species at Gara but not at Elba or Jeliana, and vice versa, which indicate differences in prey population structure due to differences in local hydrological properties between these areas. Bottom bathymetry differences are also known to control fish species diversity, spawning and recruitment (D'Elia et al., 2009). The main difference in food available at Elba is the established and commercially exploited Black-barred halfbeak *H. far*, population, as one of other 17 fish species reported in Libya waters to date (Shakman and Kinzelbach, 2006, Spanier and Galil, 1991, Shakman and Kinzelbach, 2007, Ben Abdallah et al., 2005). Black-barred halfbeak was recorded at Gara during the 2010 season only, while it was present at Elba being reported in 2005, which could have implications in coming decades, if its population becomes well established there, affecting other fish species populations occupying the same niche at the Gulf of Sirte, due to the westwards gradual colonization of these alien species from both eastern Mediterranean and the Red Sea (Spanier and Galil, 1991). The combination of sea water temperature and thermal tolerance of fish species controls fish distribution with an area (Wootton, 1992) and the increasing trend of Red Sea species colonization at the central and western Mediterranean basins might be explained by adaptability of those species to tolerate cooler sea temperatures

(Golani, 1998). This phenomenon is in addition to overexploitation of fisheries stocks in the region could lead to easier establishment and niche replacement of some depleted fish populations by invasive species originating from both the Atlantic Ocean and the Red Sea.

When prey samples were pooled by site and compared between sampling years, there was a significant difference in prey mass between sampling years at the three study sites. This could be a result of differences in the recruitment stage of prey populations, controlled by several factors such as changes in annual oceanographic conditions and/or local primary productivity levels, in addition to changes in population size for some species caused by commercial fisheries. Generally, fish in the eastern and southern Mediterranean tend to be smaller in size, have low longevity, mature at an earlier age and size and may suffer high adult mortality (Stergiou, 2000) because of the thermal and oligotrophic conditions, when compared to similar species in temperate seas (Lloret and Lleonart, 2002). Breeding cycles for most prey species do not follow specific seasons, although with distinctive peaks, except for some migratory species, such as Common Dolphin fish *Coryphaena hippurus* that have certain spawning periods. Most Mediterranean fish populations therefore follow the changes in local currents and temperature regime, which induce blooming of phytoplankton and trigger local meta-populations of certain fish species to spawn. Whilst this may not be the case for the same species at different zones of the same marine area, it makes the determination of key parameters for fish population dynamics (e.g. year-class strength, growth and maturity) and modeling (e.g. establishment of stock-recruitment relationships and yield per recruit analyses) difficult if not impossible to achieve (Lloret and Lleonart, 2002), hence difficulty in estimating fish stocks for such species.

Foraging experience and efficiency in terns are found to differ between adults and recruits (first-time breeding individuals), the latter being less proficient foragers obtaining higher proportion of low-energy yielding prey compared to experienced older common terns (Limmer and Becker, 2009). This would impact breeding performance and overall breeding success of the colony.

The selection of prey size when foraging, depending on the physical capabilities of the young has been documented for several tern species (Hulsman et al.,

1989, McLeay et al., 2009, Aygen and Emslie, 2006, Bugoni and Vooren, 2004). The present study has shown a trend of selecting increased prey size with the advancement of the breeding season (middle to later provisioning periods) which might be explained by the growing demand by the young for increased caloric content of larger prey. This has been noticed in feeding patterns of other tern species e.g. Royal Tern (Hulsman et al., 1989); Roseate Tern (Shealer, 1998b) and Common Tern (Dänhardt et al., 2011) as well as in other seabird species (e.g. adult Great Skua *Catharacta skua* showing an increase in foraging effort in order to maintain consistent rate of energy delivery to its young (Hamer et al., 1991).

The results of Chapter 3 can be used as baseline data on feeding aspects of the Lesser Crested Tern young at the Mediterranean breeding sites. It shows that although several prey species are consumed by the chicks and nestlings (and possibly other prey species are being utilized by the adult terns), only a few species are of greater importance in terms of occurrence and mass. Any change in population dynamics of these preferred few prey species, whether being due to fisheries or changing oceanographic/climatic conditions (e.g. sea surface temperature, circulation) over the foraging areas would have a severe consequences on tern population dynamics (Wanless, 2006) because of the direct effects on prey populations which could lead to, changes in breeding initiation dates, lower breeding success, changes in survival and recruitment levels in the following seasons.

The above mentioned aspects were well documented for many tern species and other seabirds (Anderson et al., 2005, Croxall, 1992, Furness and Tasker, 1999, Hamer et al., 1991, Votier et al., 2009, Safina et al., 1988, Safina and Burger, 1985). The consequences for tern populations can be also caused by a shortage or instability of the food supply, due to either fish disease, e.g. sardine population collapse affecting survival and recruitment of crested terns in Australia (McLeay et al., 2008), or because of stock depletion by overfishing (e.g. North Sea sand eel depletion affecting breeding seabirds in northern Scotland (Monaghan et al., 1989, Monaghan, 1992, Furness and Tasker, 1999, Furness and Tasker, 2000, Furness, 1990).

Marine pollution accidents are another major population threat (e.g. oil spills) can have a direct destructive impact on breeding, feeding habitats of the seabird, for example, the Persian Gulf oil spill during the 1990's war had affected breeding tern species and survival of coral Islands in Saudi Arabia (Symens and Evans, 1993, Evans et al., 1993) and needed a huge effort to minimize the impact of oiling on seabirds and other marine species/habitats. The geographic location of Gara Island (the largest and most important Lesser Crested Tern colony in the Mediterranean) at a short distance of the Zouitina oil terminal and the new offshore oil and gas concessions within the Gulf of Sirte (Whidden et al., 2011) both poses a high threat to breeding and feeding terns and other seabirds/organisms in that area, giving the fact that Oil pollution may move using the marine currents to distant areas from the source spill point, causing a long-term impact on the marine and coastal environments not only in Libya but it can affect the neighboring countries to the north, west and east of Libya. Strict oil spill prevention and mitigation measures must be enforced to oil industry in this area to minimize the probability of oil spill disaster.

Fishing in Libyan waters is described in the literature as underdeveloped compared to neighboring countries (Laurent, 1997, Lamboeuf et al., 2000) hence there is potential for investment in this sector both commercial and artisanal in the coming years, in the Gulf of Sirte and north-eastern Libya waters, representing about 45% of the current national fishing fleet (Lamboeuf et al., 2000). The illegal practice of blast fishing in several coastal areas along the Libyan coast would adversely impact on spawning fish populations, decreasing their viability and recruitment, thus deteriorating ecosystems in some areas (Hamza et al., 2011). In particular, the waters near the Gara and Benghazi coast where these practices have been more frequently observed in recent years.

Habitat degradation and urbanization poses threat to Jeliana islet, being located within the Benghazi City center, particularly the Benghazi lakes deepening project, which would change the natural salt marsh habitat of the area to concrete built pools, affecting the breeding and feeding habitats of the terns in this site. The status of the Lesser Crested Tern breeding population at this site might become critical due to potential habitat degradation as no other Island

habitat (inshore or offshore) is available nearby. The population might relocate to Gara some 150 km south west, however as available information on migration routes (specifically which route Jeliana terns take when returning from wintering areas) can make the prediction of new breeding habitat location difficult, especially with the level of site fidelity observed in the 2012 breeding population at Jeliana (93.3%). More on conservation aspects of the population are discussed at the final discussion of this thesis.

The data collected on Lesser Crested Tern diet during the four study seasons can be used as baseline information to preparing a management plans for the known breeding sites at national and sub-regional levels. Both the Gara and Elba Islands and their surrounding waters were shortlisted as proposed marine protected areas by the Libyan government (Hamza et al., 2011) not only due to their importance as Important Bird Areas (IBAs) listed by Birdlife International (Fishpool and Evans, 2001), but also to preserve a wide range of marine and terrestrial species and their respective habitats including endangered species of marine turtles (Cheloniidae), marine vegetation (Posidoniaceae, Cymodoceaceae) and as fish species nurseries (Badalamenti et al., 2011).

The knowledge of the foraging ranges of terns at their breeding sites in Libya is an essential prerequisite for not only maintaining healthy breeding populations of the terns and their prey species, but also for delineating borders of future marine protected areas to ensure survival of the Mediterranean breeding population of Lesser Crested Terns.



**Chapter 4 Ringing and migration of  
Mediterranean Lesser Crested Terns  
*Thalasseus bengalensis emigrata***



## Chapter.4 Ringing and migration of Mediterranean breeding Lesser Crested Terns *Thalasseus bengalensis emigrata*

### 4.1 Introduction

Bird ringing with metal and/or colour rings is a technique which has been used for long time to collect information on the dispersal and migration of birds (Balmer et al., 2008). The first ringing for scientific purposes dates back to 1889 when Hans Christian Mortensen ringed European Starlings *Sturnus vulgaris* using zinc metal rings (Brown and Oschadleus, 2009 ). The first organised ringing scheme was established in Germany by Johannes Thienemann in 1903 at the Rossitten Bird Observatory on the Baltic Coast of East Prussia. In the following years ringing, schemes started in many other European countries (Spencer, 1985). Bird ringing has provided valuable information through not only ring sightings of live birds and ring recovery from dead birds, but also by clarifying migration routes and patterns (Wernham et al., 2002). The monitoring of population dynamics (Balmer et al., 2008) as well as becoming a tool for several animal behavioural studies (see review by Sharp, 2009).

Organised ringing will provide better information than individually-conducted ringing, especially in seabird colonies, as these birds as expected may return in waves of post-breeding migration to their non-breeding ranges, the probability of encountering the newly ringed birds decreasing the distance from their breeding grounds (Spencer, 1985).

Long-term ringing efforts provide valuable data on a particular species or population. For example, the ringing programme of Black-tailed Godwit *Limosa limosa* has data from multiple re-sightings made by a network of active volunteers, resulting in the tracking of several individuals over successive years between breeding grounds in Iceland and the wintering sites in the UK. However, irrespective of the number of ringed individuals released, monitoring is important for data collection and analysis from sightings. The extensive network of birdwatchers and ring detection/reading in Western Europe is far better organised and equipped than counterparts in Africa, for example, making data flow limited to individual observations of ringed birds in Africa, especially when there may be confusion with other similar species (for example, Lesser Crested Tern and

young Royal Tern *Thalasseus maximus* in West Africa in the case of this study (Clive Barlow, *pers. com.*).

Libya is one of the least known countries in terms of bird studies (see literature review in Chapter 1). The ringing of Lesser Crested Terns in this study can be considered the first major ringing activity for the country as there was no bird ringing scheme in the past, due principally to the lack of bird study organisations and limited number of ornithologists. The initial phase of ringing of this species started in 2006 and continued till 2008, with an initiative by the Nature Conservation Department at the Environment General Authority (EGA), with the support of the Regional Activity Centre for Specially Protected Areas (UNEP-MAP-RAC/SPA) and both the Maltese and Italian ringing schemes. It aimed at the monitoring of breeding sites and ringing the young in order to identify both staging and wintering areas (Fig. 4.1 and 4.2) of this Mediterranean seabird breeding population (Azafzaf et al., 2006, Hamza et al., 2007).

This chapter presents the results of the ringing operations conducted between 2009 and 2012 by the author as well as the results of ringing activities in the previous three years prior to the start of the present study.

#### **4.1.1 Aims and Objectives**

This chapter aims to:

1. Synthesize the information on post-natal dispersal and movements of the species based on ring sightings and field observations, and
2. To identify some wintering sites based on sightings provided from West Africa,
3. Monitor and report the recruitment of previously ringed birds at the breeding colonies.

#### **4.1.2 Hypotheses**

The Hypotheses tested through this work are:

- Fledged terns migrate in small groups to winter generally in West Africa.

- Terns bred at the Elba Island colony may have a different wintering area, as no previous sightings or recoveries are available to prove that they also winter in West Africa.
- Breeding ringed birds are philopatric to their natal sites.
- The age of first reproductive maturity is similar to what reported in other populations of the species.

## 4.2 Materials and Methods

Three breeding sites along the Libyan coast (for site descriptions and population size see Chapter 2) host 99% of the Mediterranean breeding population of Lesser Crested Tern *Thalasseus bengalensis emigrata* (Meininger et al., 1994, UNEP-MAP-RAC/SPA, 2003, Bricchetti and Foschi, 1987).

About 10 days after hatching, ringing was initiated by ensuring the young gathered in a crèche near the colony site, using a protective soft collar made of plastic mesh of 5 mm, about 50cm high (Figure 4.1) approximately 20-30m from the colony edges (Gara and Elba Island colonies). In smaller colonies where the site was too small to handle the young (Jeliana islet), to avoid disturbance to incubating birds, the chicks were collected by hand and placed in carrying carton boxes, then moved to the bank of Jeliana Lake for ringing, before being returned using the same method.

Metal rings were donated by Birdlife Malta and colour rings from the Italian Institute for Environmental Protection and Research (ISPRA). Only chicks of 10 days and older were targeted, as their tarsus are then almost similar in size to those of the adult bird, permitting ringing without causing injury. Ringing was conducted either during the early hours of the morning or in late afternoons, to avoid heat stress on both the chicks and incubated clutches. Ringing was carried out by teams composed of three persons, two ringers for each of the metal and colour rings, in addition to one reporter to write details on each ringed bird including site name, date, metal ring code, colour ring code, colour and code writing direction (up or down), in addition to the estimated age of each of the ringed chicks. In some years more than one team per site was present (e.g. in 2007 and 2008 at Gara); in order to ring large numbers of young in the shortest possible time (Figure 4.2).

Colour rings in 2007-2008 were made of white plastic rings of PMMA®, engraved with two digit alphabetic codes, while rings from 2009 onwards were made from PVC produced by INTERREX®. Both types of rings have the following dimensions: inner diameter 5.5mm, length 15mm, with 2-3 digits (e.g AA or A01). The alphabet letters used were: ABCDFHJKLNPSTVZ. All data on ringing were stored in excel sheets and uploaded regularly to the European colour ringing database (details at <http://www.cr-birding.org/node/1042>).

During the fledging period (mid-August to early September), field visits were made to coastal wetlands, small rocks/islets and Islands located to the west and the east of the breeding sites to monitor the migration pattern in terms of bird numbers and flock structure at some of the staging sites within Libya. One visit was also made in October 2010 to Djerba Island, southeast Tunisia (Figure 4.3) where the species is known to roost (staging site) during both the pre-breeding and post-breeding migrations from the breeding colonies (Isenmann et al., 2005). Ringed tern observations in staging and wintering areas were provided by birdwatchers to a central database, where life histories for each individual were produced and sent to the data provider.



Figure 4.1 Part of the Lesser Crested Tern crèche at Gara Island, Libya during ringing operation, August 2010



Figure 4.2 Ringing of Lesser Crested Tern young at the Elba Island.

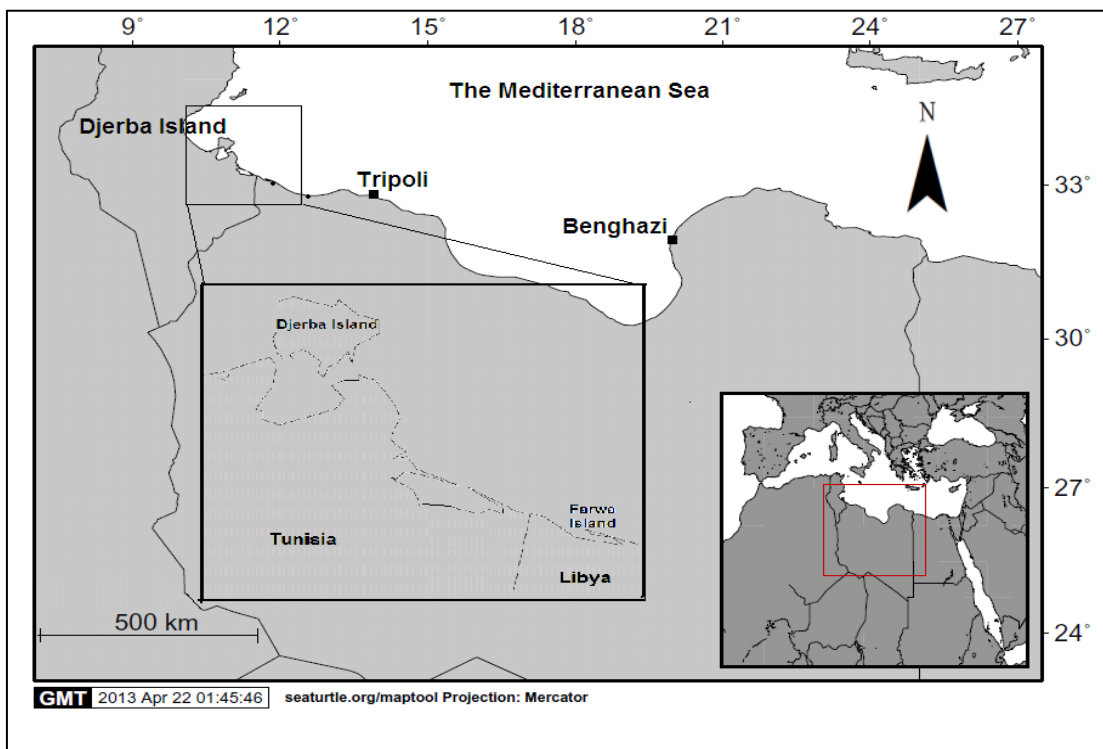


Figure 4.3 Map of Libya showing staging sites of Farwa and Djerba Islands.

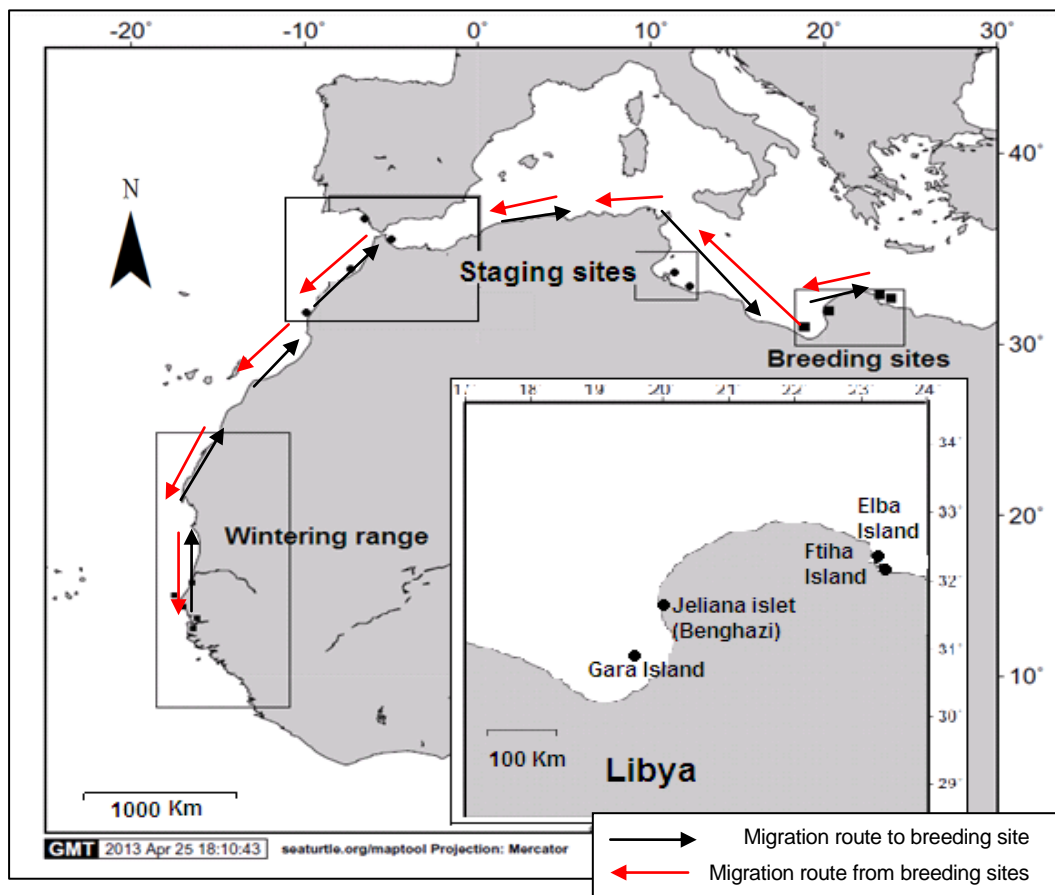


Figure 4.4 Map of the breeding, staging and wintering sites for Mediterranean Lesser Crested Terns.

### 4.3 Results

#### 4.3.1 Ringing operations

During six seasons, a total of 1,352 young plus two adult terns were ringed, the operations being conducted from 2006 to 2012 (Table 4.1). During the period of this study (2009, 2010 and 2012 seasons) 544 young (40.23%) were ringed using both metal and colour rings. Most of the study ringing effort was made at Gara (72.9%) being the largest colony with a population size of more than 2,000 breeding pairs, while only 19.9% and 7.25% of study rings were used at the Jeliana and Elba colonies, respectively. Ringed birds represented 40-60% of crèche size present in each season at both Gara and Jeliana colonies, while at Elba all young were ringed, except those which died before reaching the age of 10 days. In 2006 and 2007 metal rings only were used at Gara, due to unavailability of colour rings. In 2006 metal rings were used at Elba, but following a delivery, colour rings were used as from 2007.

Table 4.1 Numbers of ringed (young and adults) Lesser Crested Tern at breeding sites in Libya 2006-2012

	Previous studies			This study			Total
	2006	2007	2008	2009	2010	2012	
<b>Gara</b>	61*	425*	204	48	247	0	985
<b>Jeliana</b>	0	0	66	4	0	199	269
<b>Elba</b>	9*	25	18	25	8	13	98
<b>Total</b>	70	450	288	77	255	212	1,352

\* Metal rings only

Following the ringing programme A total of 28 ringed terns have been observed (Table 4.2) representing 2.07% of the total ringed 1,352 Lesser Crested Terns in Libya, during inward or outward migration via staging sites along the north-western coast of Africa and in southern Spain (7), and in wintering areas (7) of West African countries, in Senegal and The Gambia, or rings recovered from dead birds in Morocco (1) and Sierra Leone (1). The total includes the record of the first recruited breeding individuals, which have returned to the Libyan



breeding sites in 2011 and 2012 (Table 4.3 & Table 4.4). Details on ringing activity conducted during the present study are presented at Annex II.

Table 4.2 Numbers of observed and recovered ringed individuals on migration and at wintering sites in West Africa

Breeding site	On migration at staging sites	Wintering in West Africa	Dead recovery	Recruited breeder	Total
Gara	5	5	1	1 (at Jeliana)	12
Jeliana	1	2	1	9	13
Elba	1	0	0	2 (1at Jeliana)	3
Total	7	7	2	12	28

#### 4.3.2 Post-natal movements (staging and wintering areas)

Lesser Crested Tern young fledge in late July (Jeliana) and mid-August to early September (Elba and Gara). Adult birds with juveniles migrate along the coast of North Africa in groups of 5-15 birds. The juveniles continuing to depend on their parents for feeding during their first months, similar to Sandwich Terns (Smith, 1975) and Royal terns (Buckley and Buckley, 2002). They roost on small islets, estuaries and coastal wetlands during migration to and from wintering areas in West Africa between Senegal and Sierra Leone (Fig. 4.4 and 4.5). Some juveniles may spend their first winter with their parents, at scattered sites along the north and northwest African coast (Meininger et al., 1994) in what is known as a Nomadism period of the juvenile tern first year (Barlow, 1998). For example, during this study one bird with a colour ring code (C44) was reported by a fisherman in Tripoli harbour on December 27<sup>th</sup>, 2010 after being ringed that year in August 4<sup>th</sup> at Gara Island (Table 4.4).

Such movements have been monitored by the author at several sites on the Libyan coastline. For example, a small group of 3-5 birds were seen in late August 2009 for two days at Al-Ghbeba beach, west of Sirte. Another 18 birds were seen earlier on August 15<sup>th</sup> 2009 roosting with Little Terns *Sterna albifrons*, feeding their young at small rocky islets off the coast of Sabratah town (80 km west of Tripoli). Further to the west on the same day another group of 16 Adults

with 5 juveniles were observed, roosting on mudflats at the southern beach of Farwa Island (180 west of Tripoli), three of the adults had one metal ring each only (2006/2007 ringed birds). These observations reinforce the predicted migration routes and pattern in North Africa. However, no data was obtained on the movements of the species to the east of the Libyan breeding sites, tending to confirm that most breeding individuals in Libya winter in West Africa. There is at present no data available to indicate population dispersal/migration to the Red Sea area, despite some limited observations of the other subspecies on the Egyptian Mediterranean coastline (Goodman and Meininger, 1989) which may belong to foraging or non-breeding nominate subspecies *T. b. bengalensis*.

Post-natal migration continues in the following months, e.g. in early October 2010 about 80 birds (adults and juveniles) were counted within 25 minutes of observations off the north-western coast of Djerba Island, Tunisia, moving in small groups of 5-20 birds. This pattern of movement in small groups up to two months from the ringing date does explain the sparse information available on the species post-natal movements along the North African coast. It may also be the result of limited seabird monitoring effort during the summer months in both Tunisia and Algeria. However there is notably more information on the species from Morocco (e.g. Ceuta) as individuals may spend the winter at some Moroccan sites, where a higher monitoring effort on bird migration may contribute more to understanding the movement pattern and timing for the species. The African side of the straits of Gibraltar seems to represent a migratory bottleneck for this species, based on frequent ring sightings. This area is one of important bird areas (IBAs) for several migratory bird species between Europe and Africa, as well as within-Africa's migratory species (Paracuellos and Jerez, 2003).

Lesser Crested Terns are generally rare in southern Spain but a few individuals are detected every year around the area of Cadiz, mainly at Doñana National Park, Montijo and La Jara beaches (at the mouth of the Guadalquivir River), Salinas de la Tapa (El Puerto de Santa María) and Odiel marshes (Huelva). They usually associate with other tern species, mainly Sandwich tern *Thalasseus sandvicensis* and Common Tern *Sterna hirundo* (Carlos Gutiérrez, pers. com).

No Lesser Crested Terns have been reported to the east of the Elba Island breeding site, at least as far as the frontier with Egypt. However although all fledged young of this colony were ringed, over six seasons, only one dead recovery was reported from northwest Morocco, in April 2013 (Table 4.2) and 4 re-sighted as breeders in Elba and one at Jeliana in 2012 (Table 4.3). Further monitoring is needed to confirm migration routes and staging sites used by this subpopulation (Figure 4.4).

The post-natal dispersal pattern is unsynchronised, with some birds leaving the breeding sites much earlier than others. For example, three ringed juveniles with their parents were sighted in Ceuta (Spanish Morocco) within 48-59 days after ringing (Table 4.4). Taking into account that the young were 10-15 days old when ringed, this means that they fledged and left the breeding site some 15-20 days after ringing and were subsequently located at Ceuta after travelling for  $32.34 \pm 10.7$  days, hence an average daily distance travelled of 86 km.

#### **4.3.3 Post-natal movements (wintering range)**

The information obtained through ring sightings and recoveries indicate that the most distant dispersal of a newly fledged Lesser Crested Tern (colour ring VB, metal ring W1015) was reported from Turtle Islands, Sierra Leone, having travelled some 6,700 km in 149 days after ringing (Table 4.4), thus moving at an average of 45 km/day. This area may be the most southern wintering distribution as the species is not known to occur in countries south of Sierra Leone (Dowsett et al., 2013), although some resources mention the observation of small number of wintering individuals in Ghana (Grimes, 1987) and Nigeria (Meininger, 1988).

Guinea-Bissau is believed to host important numbers of wintering Lesser Crested Tern, between December 1986-January 1987, total of 400 individuals were counted along the coast, the total number of wintering individuals was estimated at 600-1000 (Meininger, 1988), no recent update on the status of the species is present, and no rings were reported so far from this country during the study period.

More monitoring is needed to better delineate the southern limit of the species' winter distribution. The species is known to occupy all available habitats to fulfil their energy requirements in most of the West African countries where they occur in winter, using both marine and estuarine habitats (Clive Barlow, *pers. com*). One of the more bird watching sites in Sierra Leone is River No.2 at the beaches of the Western Area National Park (Figure 4.5). In December 2008, several Lesser Crested Terns were found associated with other tern species at this site (Valentine, 2008).

Six of the sighted ringed individuals were wintering in Senegal and The Gambia (two in Dakar, two at N'Gor Island, Senegal and two at the Tanji Bird Reserve, The Gambia). Senegal hosts large concentrations of Lesser Crested Terns, e.g. over 825 birds were counted between 3-16 October 2005 at N'Gor, with a daily average of 59 birds (Holmström et al., 2005). Daily counts ranged between 9-143 individuals, these birds being described as migrants. The Tanji Bird Reserve and Barra Ferry Terminal on the Gambia River are also sites where the species has been frequently reported by several birdwatchers (Skov and Jensen, 2002). Senegal and The Gambia appeared to be located at the centre of the species wintering range and more monitoring is essential to understand where the species may be concentrated (Schricke et al., 2001).

Information on the species' occurrence in Mauritania indicates it is a passage migrant, one of the most important sites being the Banc d'Arguin National Park (Isenmann et al., 2010), the peninsula of Nouadhibou to the north of the country may represent the northern limit of the wintering range.

#### **4.3.4 Mortality at wintering range**

The deterioration of environmental conditions and human exploitation of birds at wintering areas can be considered as the most important limiting factor for Tern population survival, as most mortality occurs out of breeding areas (Nisbet et al., 2011, Bächler et al., 2010), food scarcity in wintering areas can also increase mortality levels (Stienen and Brenninkmeijer, 2002).

Ring recoveries of European terns from West African countries were attributed to the large-scale trapping of terns in these countries, especially in Ghana and Senegal (Meininger, 1988)



Figure 4.5 Lesser Crested Terns roosting at the mouth of River no.2, Sierra Leone.  
December 2011

Source (<http://www.flickr.com/photos/neumeyer/6824539889/in/photostream/>)

The Tern trapping practiced for leisure, food or acquire an income for poor villagers was suggested a major cause of several species mortality in West Africa (Figure 4.6), some 20% of caught birds released after removal of the rings from their tarsus (Wendeln and Becker, 1999). Different methods are used, including traps, snares and 'fishing' for terns using baited hooks on nylon lines (Meininger, 1988). Diving terns in shallow waters are being caught using baited line and hooks. More first year birds than adults are taken - scavenging may be normal behaviour for young birds, or a result of food shortage. Significant numbers of terns were believed to be trapped every year, it was estimated that almost 10,000 terns may have been trapped during the 1990s, mostly Sandwich Terns but including perhaps 500 terns of other species (Wendeln and Becker, 1999) .

Most surveys on tern trapping were conducted in Ghana during early 1980's and mid 1990's, the project Save the Seashore Birds –Ghana was launched after alarming declines of Roseate Tern *Sterna paradisea* breeding in the UK, in a response to trapping and ring removal by locals along the coast of Ghana (Avery et al., 1995). Senegal is also an important area where tern trapping is practiced by boys of 7-15 years old; terns were found to be trapped for leisure, food and in one locality tern meat were sold to European tourists, as a local delicacy. Species frequently caught in Senegal includes Caspian, Royal, Sandwich, Common, Black and white-winged terns (Meininger, 1988).



Figure 4.6 Trapped Terns by village boys in Senegal  
(Provided by P. Meininger, 2013)

Later between November 1995 and December 1997 large numbers of rings from terns and other seabirds, total of 210 rings was found, mainly in bracelets and necklaces, 80% belongs to Terns ringed at breeding colonies in the British Isles, Belgium and the Netherlands (Stienen et al., 1998).

Due to lack of recent updates, it is not known yet the present scale of tern trapping in West Africa. The potential impact of these practices on survival of wintering population of Lesser Crested Tern ringed in Libya needs to be updated and quantified with other wintering tern species in that region.

#### **4.3.5 Site fidelity and recruitment**

During the 2011 season one ringed Lesser Crested Tern (colour ring BA, Blue) was seen in flight and also bred at Elba Island after being ringed there in August 2007 (Figure 4.4). In 2012 three additional ringed terns were observed breeding at Elba but the flat landscape of the Island and the elevated vegetation near the colony, prevented a clear field view for reading the ring codes. One adult bird with a blue ring (ringed in 2009), the other two with white rings (ringed in 2010). The latter two were the youngest recruits to return to a breeding site during this study, however it was not possible to determine whether they were breeding or not.

At Jeliana, eleven ringed adults were seen to be breeding in the 2012 season, eight of which were ringed at the same site in August 2008 as young, in addition to one ringed in the 2009 season at the same site. Two ringed individuals from other sites chose Jeliana for breeding in 2012. One with ring code (AA) ringed at Gara Island in early August 2008, the other with ring code (BN) ringed at Elba in August 2007 (Table 4.3), the same bird being also observed at the Souss Massa National Park, in southern Morocco, on 23 April 2013 (Table 4.4).

Table 4.3 Recruited Lesser Crested Terns observed at breeding sites in Libya.

No.	Ringing date	Ringing site	Colour ring	Metal ring	observed/recovery date	Duration after ringing	Observation site
1	08/08/2007	Elba	BA	W0299	19/07/2011	3 years 11 months	Elba Island
2	20/07/2010	Elba	White – no code visible	-	26/07/2012	2 years	Elba Island
3	20/07/2010	Elba	White – no code visible	-	26/07/2012	2 years	Elba Island
4	20/07/2009	Elba	Blue – no code visible	-	26/07/2012	3 years	Elba Island
5	08/08/2007	Elba	BN	W0448	13/07/2012	4 years 11 months	Jeliana Islet
6	05/08/2008	Gara	AA	W0499	17/07/2012	3 years 11 months	Jeliana Islet
7	04/08/2008	Jeliana	DT	W0475	13/07/2012	3 years 11 months	Jeliana Islet
8	04/08/2008	Jeliana	LD	W0481	13/07/2012	3 years 11 months	Jeliana Islet
9	04/08/2008	Jeliana	LH	W0483	13/07/2012	3 years 11 months	Jeliana Islet
10	04/08/2008	Jeliana	LP	W0488	22/07/2012	3 years 11 months	Jeliana Islet
11	04/08/2008	Jeliana	VA	W1014	22/07/2012	3 years 11 months	Jeliana Islet
12	04/08/2008	Jeliana	ZC	W1030	13/07/2012	3 years 11 months	Jeliana Islet
12	04/08/2008	Jeliana	ZF	W1032	13/07/2012	3 years 11 months	Jeliana Islet
14	04/08/2008	Jeliana	LH	W0483	13/07/2012	3 years 11 months	Jeliana Islet
15	05/07/2009	Jeliana	BH	W1199	17/07/2012	3 years	Jeliana Islet



Table 4.4 Live observations and recoveries of ringed Lesser Crested Terns at staging and wintering sites

No.	Ringin g date	Ringin g site	Colour ring	Metal ring	observation/ recovery date	Duration after ringing (days)	observation / recovery site	Approximate Distance travelled (Km)
1	05/08/2008	Gara	FH	W1145	02/10/2008	58	Ceuta, Bahia Sur, Spanish Morocco	2,785
2	05/08/2008	Gara	PF	W1067	01/02/2010	545	Dakar, Senegal	5,750
3	05/08/2008	Gara	PJ	W1069	31/01/2010	544	Dakar, Senegal	5,750
4	18/07/2009	Gara	CF	-	18/07/2011	730	Donana N.P., Punta de Malandar, Spain	2,820
5	04/08/2010	Gara	B65	W0515	02/10/2010	59	Ceuta, Spanish Morocco	2,785
6	04/08/2010	Gara	C44	W0594	27/12/2010	145	Tripoli harbour	680
7	04/08/2010	Gara	D04	W0604	21/09/2010	48	Almadraba Bay, Ceuta, Spanish Morocco	2,785
8	04/08/2010	Gara	D21	W0621	06/03/2011	214	Baie de Ngor, Dakar, Senegal	5,773
9	04/08/2010	Gara	D24	W0624	08/07/2012	734	Montijo Beach, Chipiona, Cadiz Spain	2,911
10	04/08/2010	Gara	D17	W0617	13-10-2010 (dead recovery)	70	Tan Tan, Morocco	3,985
11	04/08/2010	Gara	C11	W0563	04/05/2013	1,004	Baie de Ngor, Dakar, Senegal	2,773
12	04/08/2008	Jeliana	ZB	W1029	22/01/2013	1,632	Tanji Bird Reserve Western Division Gambia	5,925
12	10/07/2012	Jeliana	A53	W1435	26/01/2013	200	Kartong, fishing village Western Division Gambia	5,950
14	24/07/2012	Jeliana	D66	W1481	02/09/2012	40	Los Lances beach, Tarifa Spain	2,816
15	04/08/2008	Jeliana	VB	W1015	31/12/2008 (dead recovery)	149	Turtle Island , Sierra Leone	6,700
16	08/08/2007	Elba	BN	W0448	23/04/2013	2085	National Park of Souss Massa, Agadir, Morocco	3,700

#### 4.4 Discussion

Monitoring post-natal movements of breeding Lesser Crested Terns and identification of migration routes, including determination of the relative importance of staging sites, are essential requirements for understanding the factors affecting populations during migration periods (Wernham et al., 2002). In the present study, fledging occur from late July to early September (Chapter 2), groups of adults and juveniles then moving westward from the breeding sites to wintering areas in West Africa (see also Cramp, 1985), utilising several staging areas. Some of the latter are known (Table 4.4) while others need more monitoring effort to be identified, particularly in northern Tunisia and Algeria. Migration is not synchronous, reflecting differences in fledging times at the breeding sites due to differences in breeding initiation dates. Ring sightings and recoveries of terns in the present study show that several sites along the North and Northwest African coast are being used as staging sites during migration to and from the breeding sites (Table 4.4).

Small numbers of Lesser Crested Terns may spend their first winter at certain sites along the flyway to the wintering areas of the species (Meininger et al., 1994, Brehme, 2003). For instance, in January 2011 at Farwa Island, fifteen individuals (adults and juveniles) were recorded in one day at this site in north-west Libya (Bourass et al., 2013) in addition to the usual observations of the species in the Farwa Island during both inward and outward migration (Etayeb and Essghaier, 2007). Within-Libya winter records ranged from three to fifteen individuals, reported during the wintering water-birds census between 2007 and 2011 (EGA-RAC/SPA-WCT, 2012, Bourass et al., 2013). It is not well known whether these individuals either continue their post-breeding migration to wintering sites in the following spring months or would rather join inward migrating terns and return to the Libyan breeding areas as non-breeders. Further monitoring is needed to clarify this phenomenon.

In Tunisia, the southern site of Ras Err'mal (Sand head) at the northwest side of Djerba Island, is known to host aggregations of several roosting tern species including Lesser Crested Terns (Isenmann et al., 2005). Some pre-breeding behaviour has been observed several times in the past years during early June at

this site without any actual breeding detected at this site (Hichem Azafzaf, *pers. com*).

The migration of Lesser Crested Terns from breeding sites in Libya to their wintering range in West Africa includes flying for considerable distances, but not far from the coast, migrating in small groups of 5-20 individuals (adults and recently fledged juveniles). The latter continue to depend on their parents for feeding during the first few months (del Hoyo et al., 1996), similar to Sandwich Terns (Smith, 1975), and Royal Terns (Buckley and Buckley, 2002), reflecting the availability of roosting/feeding sites. However, possibly because of this migration pattern, the species appears to be under-reported along the Tunisian and Algerian coastlines, also due to low seabird monitoring effort in most North African countries (Isenmann et al., 2005, Isenmann and Moali, 2000). The author counted a total of 80 Lesser Crested Terns in 25 min. of observation on migration to the north of Djerba Island, Tunisia in early October 2010 (*pers. obs.*).

With recent advances in bio-loggers (using transmitters to track migration routes) several seabird species migration routes, including the Arctic Tern (*Sterna paradisaea*) have been recorded using some stop-over points along their migration routes, suggested to act as refuelling stations (Egevang et al., 2010).

The findings of the present study also indicate a level of site fidelity for Lesser Crested Terns with some inter-colony movement. Eleven out of fifteen individuals previously ringed at Jeliana had returned to their natal site to breed in 2012.

Site tenancy (fidelity) has been reported in several tern species, for example (Austin, 1949) found that Common Terns once being nested, tended to return to the same site during the subsequent breeding seasons. He also found that many birds moved to a different colony to re-nest if their first nest failed, because of habitat degradation or egg/nestling predation, these birds also tending to return to their original natal site in subsequent years. Some studies have found that Common Terns keep their conspecific breeding neighbours from year to year at the same site (group adherence) a common feature in several gull and tern species (see review by McNicholl, 1975). Returning to natal sites after some years is a strategy that provides individuals with time to meet suitable mates and stay together until moving to non-breeding areas (Coulson, 2001). Several

benefits of seabirds returning to their natal sites, include increased breeding success (Greenwood and Harvey, 1982) and reduced cost of prospecting a new breeding site (Naves et al., 2006). It also keep the birds informed on food types, nearby foraging areas and refuges when faced with predator (Coulson, 2001, see review by Wittenberger and Hunt, 1985).

Movement of breeding seabirds from one colony site to another has also been reported in several species, Roseate Tern *Sterna dougallii* bred for several years at a colony in Massachusetts, USA had moved to another colonies located 200-400 Km in Maine, two of them bred for eight years in Massachusetts before moving, indicating that even long-term philopatric breeders can move to another colony (Spendelov et al., 2010). Moving to another colony can be caused as a response to human disturbance. Visits to seabird colonies for tourism, or even to conduct research activities that may flush ground-breeding seabirds, can make them susceptible to heat/cold stress or attack by opportunistic predators (Avery et al., 1995, Medeiros et al., 2007, Szczys et al., 2005). This disturbance can increase the possibilities for moving to a different colony site, either during the same season or in coming seasons, according to disturbance type, intensity and frequency, see review by Carney and Sydeman (1999). During the present study, the breeding population at Elba Island in 2010 moved to nearby Fteha Island and initiated a new colony. The most possible cause of this shift in breeding site is human disturbance (Chapter 2). Breeding habitat degradation can also cause colony desertion or breeding termination. Jeliana site habitat quality has deteriorated during 2010 due to increased water levels (flooding) causing the terns to desert the area and suspend breeding (see Chapter 2 for details). Breeding seabirds can react promptly and shift their colony site in response to flooding of their usual colony site (see Scarton, 2008 for Little Tern breeding biology). Changes in food availability can also stimulate a colony to move to another site in years of food scarcity (e.g. Greater Crested Terns *Thalasseus bergii* breeding decreased to 67% (Crawford, 2003)).

Two Lesser Crested Terns observed at Jeliana in the 2012 season were ringed as young at the Elba and Gara colonies. Inter-colony movement has been reported in several tern species e.g. Caspian Terns *Hydroprogne caspia* (Cuthbert, 1985), Sooty Terns *Sterna fuscata* in Seychelles (Feare and

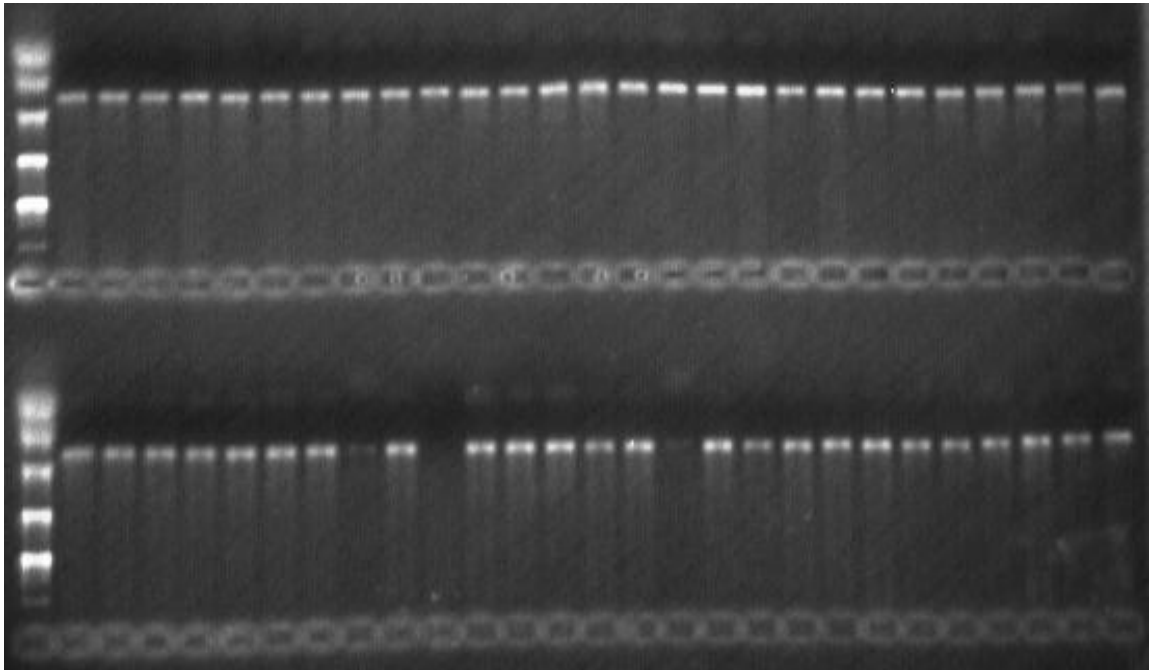
Lesperance, 2002) and nominate Crested Terns *T. bergii bergii* (Crawford et al., 2002). These movements between colonies may indicate a site preference of recently mated birds to breed at the other natal site, as a result of pairing at the same wintering or staging site(s) prior to arrival at the original breeding site. For example, some Caspian Terns may visit several nearby colony sites per day until they acquire a suitable mate before deciding on colony choice (Cuthbert, 1985). This behaviour may not exist for breeding Lesser Crested Terns in Libya due to the extended distances between breeding sites, considered to be beyond their daily movement range. Hence the presence of individuals originating from a different site may indicate that mate choice was made at either the wintering area or staging site(s) prior to arrival at the Jeliana colony site. However, In Common Terns, nest initiation started at one site and then a pair may move to more than 120 miles to re-nest at second breeding site (DiCostanzo, 1980) which poses the possibility of inter-colony movement between Libyan breeding sites. This topic needs further research using tracking technology to validate such a hypothesis.

Obtaining information on first age of reproductive maturity is an essential requirement of population modelling, in addition to mortality and survival parameters. This information needs to be collected in the coming seasons.

This study has shown that the age of first breeding (maturity) in Lesser Crested Terns breeding in Libya is two years. However, most of the recruited adults were four years old (n=10, 66.67%) and four birds (two each) returned after two and three years respectively (n=4, 26.6%) while one bird returned after almost five years in wintering range (n=1, 6.67%). It was not possible in the latter case to confirm whether this was a first breeding attempt. The age of first breeding of two years in the Libyan breeding population is in agreement with the presumptions of earlier data (del Hoyo et al., 1996). On the other hand some populations of Lesser Crested Terns may reach breeding age until three years, e.g. the Persian Gulf population (Ghasemi et al., 2011), which suggests a delay in sexual maturity for some populations. This may apply to part of the Libyan breeding population, as some individuals have returned to the colony sites to breed at the age of 3 to 5 years.

More monitoring is needed to refine the average daily distances travelled by the Libyan population between breeding and wintering sites, via the intermediate staging sites. It was not yet been possible to calculate the total duration of migration between breeding sites and wintering grounds for those birds that first bred in 2012 (ringed individuals), simply because to date there have been no sightings or recoveries beyond the breeding sites. The current ringing programme in Libya for the species is intended to continue as and when circumstances allow, followed by future monitoring and conservation activities, especially as the four Libyan breeding sites were shortlisted among other 24 coastal sites as proposed marine protected areas (Hamza et al., 2011).

**Chapter 5 Population Genetics of the  
Lesser Crested Terns  
*Thalasseus bengalensis***



## **Chapter.5 Population Genetics of the Lesser Crested Terns**

### ***Thalasseus bengalensis***

#### **5.1 Species taxonomy and the use of Genetic markers**

The identification, description and naming live organisms according to the Linnaean nomenclature are all encompassed within the branch of Biology called Taxonomy (Padial et al., 2010), Taxonomy recently became using phylogenetics to amend classifications proposed on morphological features alone. Phylogeny is the study of evolutionary relationships among groups of organisms (e.g. species, populations), which are discovered through molecular sequencing data and morphological data matrices. The result of phylogenetic studies is a hypothesis about the evolutionary history of taxonomic groups.

The determination of taxonomic status for a species as well as the systematic relationships among the different or closely related species are essential for accurate biodiversity assessment and implementing conservation measures (Avisé, 2000). The current species taxonomy and classification has started with the invention of the Linnaean nomenclature in 1758, composed of genus and species names. Some species further divided into subspecies (a geographically distinct species forms). Each year new species are either first discovered, named, renamed or its classification rearranged according to morphological or other biological characteristics (Agapow et al., 2004). The Earth today is estimated to host  $5 \pm 3$  million and the total number of species discovered so far is >1.3 million species (Costello et al., 2013).

Many avian species are divided into multiple subspecies. The subspecies is an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species and the debate between ornithologists on the validity of those subspecies divisions within avian taxonomy system has provided opportunities for discussions and rearrangements of that designation (James, 2010). This debate has increased with the recently emerged modern phylogenetic techniques, which led to some revisions in species and subspecies systematics (Avisé, 2000), Traditionally, subspecies have been recognized on the basis of discontinuities in the geographical distribution of phenotypic traits (Mayr



and Ashlock, 1991), a new global analysis of avian subspecies showed that 36% of avian subspecies are phylogenetically distinct (Phillimore and Owens, 2006), which poses the question of the subspecies category validity when tested by modern phylogenetic methods. One of these techniques is the use of genetic markers (mitochondrial and nuclear DNA segments used to measure the neutral genetic diversity at single loci) after the development of Polymerase Chain Reaction technique (PCR) in 1985, which allowed the production of millions of copies for a restricted zone of the DNA (Mullis et al., 1986). This technique with other phylogenetic methods have helped to rearrange several bird species taxonomy and nomenclature, for example, recently the New World Sandwich Tern *Thalasseus sandvicensis acufavidus/eurygnathus* complex has been recently split into two distinct species, Cabot's Tern *Thalasseus acufavidus* and the Old World Sandwich Tern should be *Thalasseus sandvicensis*, as a result a phylogeny that based on several mitochondrial and nuclear genetic markers (Efe et al., 2009). In some other cases these techniques have helped to review the phylogeny of a whole family, e.g. Tern family: Sternidae (Bridge et al., 2005) and Gulls family: Laridae (Pons et al., 2005) or reviewing a whole class of species, e.g. class Aves of the new world (John et al., 2013). On the other hand, some species may be combined into one species due to lack of significant phylogenetic variations among them, for example, the greenfinches (*Carduelis spp.*) found to represent a monophyletic group (Zuccon et al., 2012, Nguembock et al., 2009). Genetic markers can also show the evolutionary history of a species through time, e.g. the Kelp Gull *Larus dominicanus* in South America (Dantas et al., 2012).

Polymerase Chain Reaction technique (PCR), has allowed both the amplification of a selected segments or a whole gene from either nuclear or mitochondrial DNA, and the production of large quantities of that specific DNA from a complex DNA template in a simple *in vitro* enzymatic reaction, then by sequencing these specific parts of the DNA it is possible to identify the nucleotide sequence structure of that gene, and study the genetic variations between individuals from similar or different species or subspecies (Avice, 2000, Ball and Avice, 1992, Desjardins and Morais, 1990, Mullis et al., 1986).

### 5.1.1 Taxonomy and phylogeny of terns

The systematic status of the tern group was not stable in the classical evolutionary classification, in terms of the taxonomic position in relation to other seabird groups, the number of genera and within-group species relationships. Some taxonomists put terns within tribe Sternini, Family Sternidae, consisted of 7 genera and 45 species, with majority of terns (32 species) are listed under species *Sterna* (Sibley and Monroe, 1990), other classification schemes recognize the terns as a subfamily, Sterninae under Gull family, Laridae (Higgins et al., 1996). Moynihan (1959) has revised the Laridae family, including terns, and he divided terns according to morphological characteristics and behaviour into three groups: the noddies (*Anous*), the Inca tern (*Larosterna*), and the (*Sterna*) for black-capped terns (Bridge et al., 2005). Some more recent classifications rearranged terns in 10 genera (Gochfeld and and Burger, 1996), and separated the crested tern species from *Sterna* into a specific genus (*Thalasseus*).

Many of tern species is composed of more than one type species, i.e. two or more subspecies (as the case for Lesser Crested Tern), according to detailed morphological differences, extracted via systematic field observations (Gochfeld and and Burger, 1996). However division led to continuous debate on validity of such grouping, some supports using subspecies as being evolutionary important and deserve full taxonomic recognition, whilst others questioned the validity of such system, as subspecies were employed by the ornithological community, which includes scientists but also many amateur birdwatchers.

Bridge et al (2005) has used the mtDNA and some nuclear DNA genes (2008 nucleotide base pairs, later bp) to investigate the phylogeny of 33 tern species and additional two subspecies. They showed that head ornamentation patterns in alternate plumages were closely related to their phylogeny. The resulting phylogeny confirmed previous hypotheses of relationships within the group, and suggested a revision of the terns which recognizes 12 genera, and concluded the validity positioning of crested tern group under a separate genus *Thalasseus* sp, that have similar morphological features, particularly feather colouring and size. Similar results were obtained by using larger group of genetic markers on the genome level of tern group (Jackson et al., 2012), however it reassured that low

genetic variability in tern group makes perfect phylogeny a challenging task (see Faria et al., 2007).

### **5.1.2 Structure and importance of mitochondrial DNA in phylogeny**

Animal mitochondrial DNA (mtDNA) is a small circular, double-stranded molecule, found in mitochondria of all eukaryotic cells, with molecular size ranging from 16-20 kilobases, composed of 37 genes coding for 22 transfer RNAs, two ribosomal RNAs and 13 messenger RNAs, that lacks introns, repetitive DNA, pseudogenes and spacer sequences (Boonseub et al., 2012, Gissi et al., 2008). The two double strands of DNA are distinguished as a heavy strand (H) which is guanine rich and the light strand (L) which is cytosine rich (Avisé, 2000).

Several biological properties allowed mtDNA to become the most molecular marker used in molecular systematic of species, first the maternal inheritance of this whole molecule, which means that this molecule is behaving biochemically as a single, non-recombining locus, i.e. all sites on this molecule are sharing common genealogy, which simplifies the representation and analysis of intra-specific variation data (Galtier et al., 2009). Second the physiological function of mtDNA genes in respiration is considered more stable in evolving compared to other biological functions that require several adaptive processes to become evolved (Moritz et al., 1987) and finally the mutation-free evolution of this molecule, means that divergence levels of mtDNA reflects actual divergence times (Galtier et al., 2009, Avisé, 2000) and accordingly less generations are needed for any new divergence event in the evolutionary history of a particular species (Lau, 2008, Whittier et al., 2006).

The fast evolvement of mtDNA compared to single copy nuclear DNA, and the absence of differences in sequence lengths (except in the control region between species (see review by Moritz et al., 1987, Galtier et al., 2009), in addition the self-repairing mechanism to control the impacts of any mutations made it ideal for phylogeny studies.

## 5.2 Avian mitochondrial DNA

The first complete sequence of an avian mitochondrial genome was published from chicken tissues (Desjardins and Morais, 1990), and found to consist of and encode for the same set of 37 genes (13 proteins, 2 rRNAs and 22 tRNAs) found in mammals and reptiles (Kvist, 2000, Ball and Avise, 1992, Avise, 2000), with some differences in gene order, that caused because of avian mtDNA multiple independent origins being found in a divergent taxa, making them susceptible to parallel evolution (Mindell et al., 1998).

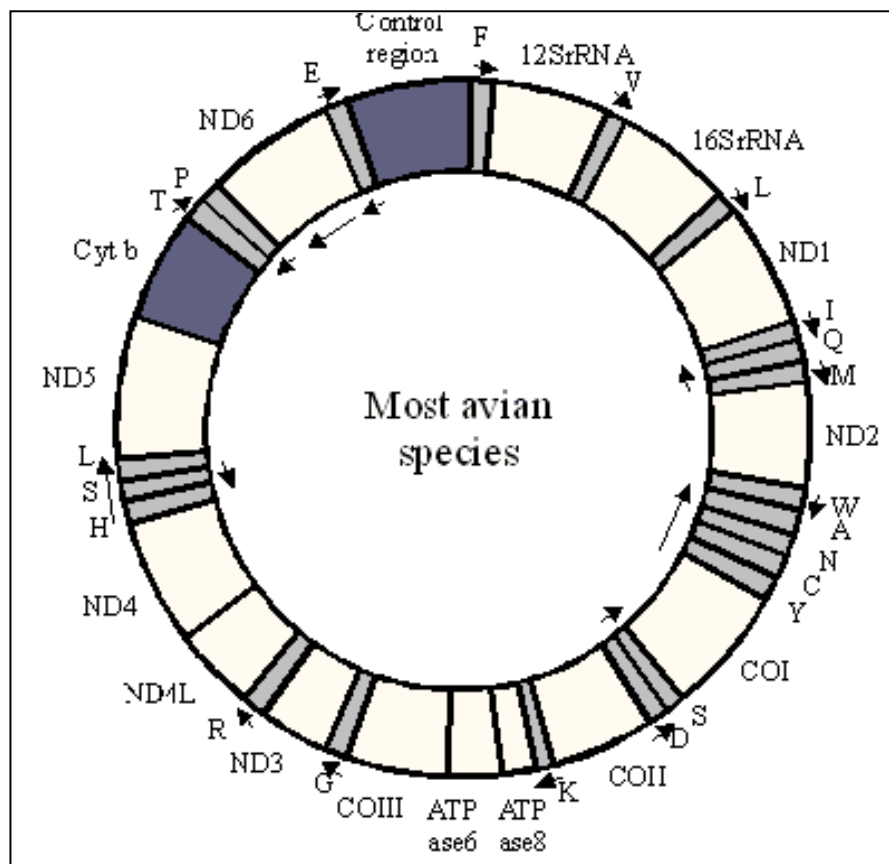


Figure 5.1 Structure of the mitochondrial DNA for most avian species  
Adapted from Kvist (2000)

### 5.2.1 The NADH Dehydrogenase subunit 2 gene (ND2)

The avian ND2 gene is located between the ND1 and COI genes, between 5250-6290 base pairs (bp) in the chicken (*Gallus gallus*) mtDNA while the ND2 gene in human mtDNA is between 4469-5510 bp. The ND2 gene coding sequence of avian species is about 1042 bp in length (Avise, 2000).

In terms of amino acid sequence, ND2 is the 3rd most variable gene after ATPase 8, which is very short (~165-168 bp) and therefore provides relatively little information, and ND6, which is also relatively short (~519-522 bp) and is generally more difficult to amplify and sequence, given its unusual base composition and location near the control region. In contrast, the complete ND2 gene can be amplified in one piece with primers these primer pair: L5216-H6313. (Sorenson et al., 1999). ND2 gene was recently considered a major species identification probe to protected bird species, where sampling is restricted by conservation regulations (Boonseub et al., 2012)

### **5.2.2 Cytochrome b (Cyt b)**

The mitochondrial gene, Cytochrome *b*, is the most widely used gene for phylogenetic work (Avice, 2000), mainly due to the fast rate of evolution in silent positions (Irwin *et al.* 1991). The wide use of this marker has become as a universal metric for phylogenetic and population genetic studies. Cyt *b* is thought to be variable enough for population level studies and conserved enough for clarifying deeper phylogenetic relationships. However, the Cytochrome *b* is under strong evolutionary constraints because some parts of the gene are more conserved than others due to functional restrictions (Meyer 1994). So far, it has been the most prevalent source of sequence data in avian studies. It could be considered as best choice for resolving relatively recent evolutionary history (Prychitko and Moore, 1997). The tendency of birds to have low sequence divergence rates at high taxonomic levels compared to other vertebrates makes cytochrome *b* a good choice as a marker (Kvist, 2000).

### **5.2.3 The chromo-helicase-DNA binding protein gene (CHD)**

The use of nuclear genes in addition to mitochondrial gene data is recently found to produce more robust results in constructing population structure, within and between species (Zink and Barrowclough, 2008). Nuclear gene data is important for quantitative estimates of the depths of haplotype trees, rates of population growth and values of gene flow, and combining both markers can yield finer

descriptions of phylogeographic pattern than can the single locus provided by mtDNA (Edwards and Bensch, 2009).

In birds The Z and W sex chromosomes evolved from different autosomal precursor chromosomes in birds compared to the mammalian X and Y chromosomes (Ellegren and Carmichael, 2001). Female birds are heterogametic and carry a copy of Z and W but males are homogametic and carry two copies of the Z sex chromosome (Handley et al., 2004).

The amplification of CHD-Z gene at the present study, aimed first at the estimation of sex ratio in samples collected from nestlings at the study sites in Libya and Egypt, and for samples collected from adult birds in the Gulf region (Bahrain). Then the sequences of female individuals were added to the phylogeny analysis to represent nuclear genetic data, due to the reasons mentioned above.

### **5.3 Sex ratio in Birds**

Sex ratios are produced approximately equal in bisexual species, Based on the Mendel laws of segregation of characters (Batellier et al., 2004) regardless of the sex determination mechanism, that might be determined by the ambient temperature in reptiles, e.g. marine turtles (Katselidis et al., 2012), where high nest temperature produces more females, and colder incubation temperature produces more males. In birds sex ratio is determined by genotypic mechanism, at fertilization, long before the incubation of eggs begins (Hardy, 2002). This phenomenon as avian females are the heterogametic sex, while males are homogenic (Fridolfsson and Ellegren, 1999). Sex determination might be under direct control of the adult female. The research on this aspect of animal biology has developed recently to wide range of applications, with insights of different sex ratio at hatching and fledgling phases of the bird breeding cycle, that have direct impacts on population viability and growth. Primary sex ratio can be altered using hormonal treatment of the adult female bird prior to egg laying, testosterone causes male biased sex ratio in pigeons for example (Goerlich-Jansson et al., 2013), while corticosteroids induced a female bias in first eggs. Habitat alteration also found to affect sex ratio in some bird species, the meadow pipet *Anthus pratensis* was found to respond to grazing regime by sheep, or a mixture of

sheep and cattle at their breeding range, male offspring was lowest in the ungrazed and intensively grazed treatments and highest in treatments grazed at low intensity (Prior et al., 2011). The Common Tern *Sterna hiurundo* on the other hand was found to invest more in sons, whenever the environmental and the partner conditions are good, while the proportion of daughters increases with clutch size and maternal breeding experience (Gonzalez-Solis et al., 2005).

In birds with three egg clutches, the last egg (c-egg) is smaller compared to the first two eggs, and hatch later, being laid third with less nutrients leading lower survival probability (Moore et al., 2000). The sex ratio of c-eggs was female biased in common terns, but not of earlier laid eggs, these female hatchlings had a significantly higher survival rates than male counterparts (Fletcher and Hamer, 2004), which presents a strong evidence that factors other than sexual size dimorphism are responsible for producing greater vulnerability of male offspring to adverse conditions during growth.

Therefore the study of sex ratio can be used to forecast the potential impacts of biased demographic population structure towards one sex, that resulted by several factors, which in turn can result in limitations for population growth and survival on the long term, especially when there are differences in philopatry between males and females (Sarzo et al., 2012). This study presents first insights on the nestlings' sex ratio at the three breeding colonies, using PCR protocol (Fridolfsson and Ellegren, 1999, Devlin et al., 2004).

#### **5.4 Evolutionary relationships within Tern species**

Several research efforts oriented to understand the evolutionary relationships between tern species, and the other closely related Laridae taxa. Most authors agree that terns in general are low variable group, with difficulties to amplify certain genetic markers. Most studies aimed at solving the evolutionary relationships among tern species were un-comprehensive (did not used data on all tern species), employing either the Cladistic approach (e.g.Chu, 1995) or the DNA-DNA hybridization approach (Sibley and Ahlquist, 1990). Furthermore within species phylogenetic studies used several mitochondrial, nuclear and microsatellite markers in attempt to draw the subspecies division within a single species taxon complex. For example the Eurasian and American Sandwich Terns

have found to form two separate species, based on both nuclear and mitochondrial markers (Efe et al., 2009). Table (5.1) summarises the available knowledge on tern genetic population studies.

Table 5.1 Summary of published tern population genetic studies and their main findings.

<b>Common Name</b>	<b>Papers</b>	<b>Loci</b>	<b>Results</b>
S. American Tern ( <i>Sterna hirundinacea</i> )	Faria et al. (2010)	microsatellites mt-ND2/CYTB	Low but significant variation between Brazilian and Patagonian population; homogeneity within populations
Angel Tern ( <i>Gigs alba</i> )	Yeung et al. (2009)	mt-ND2/CYTB	Extensive gene flow among all populations; merging of 2 species and 2/4 subspecies
Sandwich Tern ( <i>Thalasseus sandvicensis</i> )	Efe et al. (2009)	microsatellites mt-ND2/ND6/CYTB nuc-BFIB/MYO	Old World and New World terns are different species; low differentiation among North American and South American terns with complex genetic structure and gene flow
Least Tern ( <i>Sterna antillarum</i> )	Whittier et al. (2006)  Draheim (2010)	microsatellites mt-CR/CYTB nuc-G3PDH	Homogeneity within populations; extensive gene flow between eastern and western US populations
Roseate Tern ( <i>Sterna dougalli</i> )	Lashko (2004)  Szczyz et al. (2005)	microsatellites mt-ND2/ND6	Significant variation between Indo-pacific and Atlantic population; homogeneity within populations; recent expansion in both oceans; merging of 3/4 subspecies
Sooty Tern ( <i>Onychoprion fuscata</i> )	Avise et al. (2000), Peck and Congdon (2004)	mt-CR RFLP	Low but significant variation between Indo-pacific and Atlantic population; homogeneity within populations
Common Tern ( <i>Sterna hirundo</i> )	(Martin et al., 2000)	microsatellites isoelectric protein focusing	Extensive gene flow between adjacent states (MN, WI).



## 5.6 Aims and research questions and hypotheses

This chapter aims at:

1. Study the subspecies phylogenetics of the Lesser Crested Tern *Thalasseus bengalensis* breeding in Libya and to clarify their intra-specific phylogenetic relationships,
2. To quantify genetic structure and diversity patterns and determine if genetic data support the existence of three different subspecies taxonomy among the three breeding populations in Libya, the Red Sea (Egypt) and the Persian Gulf (Bahrain)
3. To investigate the sex ratio at each colony to forecast any impacts on demographic structure of the breeding population.

### The research questions:

1. How much genetic divergence present between the Libyan breeding *T. b. emigrata*, the Red Sea (*T. b. bengalensis*) and the Persian Gulf (*T. b. torresii*)?
2. Is the current three subspecies classification is valid after the using genetic markers and phylogeny?
3. What is the sex ratio of each breeding colony

### The research hypotheses:

- Terns are low variable group of birds at species level, thus, it is expected that genetic variability will be small if non among the sampled populations.
- The current subspecies division is expected to be valid, given the long isolation of the species breeding populations by vast Sahara (in Arabian Peninsula between *T.b. bengalensis* and *T. b. torresii*) and the absence of any documented connectivity between the Libyan breeding population *T.b. emigrata* and the Red Sea breeding population *T.b. bengalensis* due to distances between each of the groups at the wintering range (West vs East Africa respectively).

- The sex ratio is expected to be balanced within each sampled population, given the fact that this species populations on global level is stable, reflecting the production of equal ratios of males and females.

## 5.7 Methods

### 5.7.1 Study area

Primary wing feather samples were collected from 10 days old nestlings, during the banding operations in 2009 and 2010 (Table 5.2). Samples ranged from 2-3 feathers from each nestling, from Gara Island (n=27), Elba Island (n=25) and Jeliana islet (n= 2). In addition to tissue samples from broken eggs (embryos) from the Red Sea population at Ashrafi archipelago (n=7) and adult wing primaries from the Persian Gulf Island of Al Jarim, Bahrain (n= 28). For detailed description of sampling locations, see Chapter 2 (Table 2.1).

### 5.7.2 Sample collection and preservation

Two to three growing wing primers were sampled from 10 days chick, during ringing operations in 2009 and 2010 seasons. Each feather base contains some epithelial cells and blood, used as a source of DNA. Samples were preserved in absolute Alcohol, and stored in -20°C until further processing for genomic DNA isolation.

### 5.7.3 DNA extraction and amplification

Genomic DNA was isolated from the feathers using the Qiagen DNeasy® Tissue Kit- Animal Tissue, with modifications (Bush, 2005). Resulting DNA was re-suspended in 50 µl sterile water and stored at -20°C. DNA concentrations were quantified using a NanoDrop ND-1000 spectrophotometer. Genetic variation of *Thalasseus bengalensis* was assessed at two mitochondrial genes, full amplification of NADH Dehydrogenase subunit 2 gene (ND2; 1041bp) and partial amplification of Cytochrome *b* (Cyto *b*: 306 of 340), in addition to one nuclear gene, the CHD, the gene for chromo-helicase-DNA binding protein, which preserved within avian Z and W sex chromosomes.

Polymerase chain reactions (PCR) reactions were undertaken in 40 µl reaction volumes for both ND2 and Cyto *b* reactions contained ca. 2ng DNA, 0.25 µM

dNTPs, 2.5 mM MgCl<sub>2</sub>, 10x PCR reaction buffer (Bioline), 10 pM of each primer and 0.5 U *Taq* DNA polymerase (Bioline). Thermal cycling conditions consisted of an initial denaturation (5 min at 95°C) followed by 40 cycles with 30 s at 95°C, 30s at the optimal annealing temperature (58°C ) and 30s at 72°C, and a final extension step of 10 s at 72°C (Faria et al., 2007).

For CHDW reactions, the PCR reaction has contained ca. 2.5 ng DNA, 0.8 µM dNTPs, 2.5 mM MgCl<sub>2</sub>, 10x PCR reaction buffer (Bioline), 10 pM of each primer and 1 U *Taq* DNA polymerase (Bioline). Thermal cycling conditions consisted of an initial denaturation (2 min at 94°C) followed by 40 cycles with 45s at 94°C, 45s at the optimal annealing temperature (48°C ) and 45s at 72°C, and a final extension step of 5mins at 72°C (Griffiths et al., 1998, Fridolfsson and Ellegren, 1999).

PCR primers used in the multi-gene analysis are listed at (

Table 5.3). Following amplification, an aliquot of the PCR product (5 µl) was visualised on a 3% agarose gel stained with ethidium bromide and sized against a 1000 bp DNA size ladder (Bioline). For successful PCRs, the remaining PCR product volume (35 µl) has been purified and bi-directionally sequenced (Macrogen Inc., Republic of Korea) using standard conditions. In some cases sequencing conditions were fine tuned to obtain better chromatogram results. Trials were made to obtain the best results by amending the published cycling parameters, in terms of concentration of Magnesium Chloride (MgCl<sub>2</sub>) in the PCR mixture, as well as both the annealing time and temperature to amplification success (Faria et al., 2007, Whittier et al., 2006, Pons et al., 2005).

Table 5.2 Sampling locations of the *Thalasseus bengalensis* used in this study.

Country	Subspecies	Population	Latitude	longitud
Libya	<i>T.b. emigrata</i>	Libya: Gara island, Ajdabiyah	30°48' N	19°54' E
Libya	<i>T.b. emigrata</i>	Libya: Elba island, Derna	32°14' N	23°17' E
Libya	<i>T.b. emigrata</i>	Libya: Jeliana islet, Benghazi	32°05' N	20°03' E
Egypt	<i>T.b. bengalensis</i>	Egypt: Ashrafi archipelago, the Red Sea	27°46' N	33°41' E
Bahrain	<i>T.b. torresii</i>	Bahrain: Al Jarrim island	26°28' N	50°30' E

Table 5.3 PCR primer pairs and sequencing primers used in this study.

Gene	Primer name	Direction	$T_m^*$ (°C)	Primer (5' – 3')	DNA sequence source; Citation
Cyto b	L15008	Forward	58	AACTTCGGATCTCTACTAGG	Desjardin and Morais 1990
	H15326	Reverse		GAATAAGTTGGTGATGACTG	
ND2	L5216	Forward	58	GGCCCATACCCCGRAAATG	Desjardin and Morais 1990
	H6313	Reverse		ACTCTTRTTTAAGGCTTTGAAGGC	
CHDZ	P2	Forward	48	TCTGCA TCGCTA AATCCTTT	Griffiths et al. 1998
	P8	Reverse		CTCCCAAGGATGAGRAAYTG	

\* $T_m$  °C, annealing temperature; \* sequencing primers

#### 5.7.4 Sequence alignment and statistical analyses

DNA chromatograms (forward and reverse DNA sequences) were assembled and corrected in MEGA 5.0 (Tamura et al., 2011). Poor quality sequence data were excluded. Sequences were compared in a BLAST analysis against GenBank nucleotide collection to confirm gene and DNA origin. Reference DNA sequence data for the ND2, Cyto b genes was collated from GenBank (Accession: [AY631370.1](#) and [AY631298.1](#) respectively). There was no CHD-Z sequence data for the study species in the GenBank database, blast search found that the closest sequence for this gene is found in the Black-tailed Gull *Larus crassirostris* (Accession [AB252736.1](#)).

Multiple sequence alignments of *Thalasseus bengalensis* DNA sequences for each gene were carried out using ClustalW in MEGA 5.0 (Tamura et al., 2011).

ARLEQUIN 3.5 (Excoffier and Lischer, 2010) was used to estimate the amount of genetic variation within colonies through haplotype diversity (H) and nucleotide diversity ( $\pi$ ). Haplotype determination was conducted using DnaSP v5.10.1 (Librado and Rozas, 2009), For locations where multiple samples were of the same haplotype, a consensus sequence for each haplotype present was used in further analysis. Then Haplotype distribution networks were calculated using ARLEQUIN, and drawn manually according to the relative proportions of sampled within each haplotype and relative distances between haplotype unites in the network were based on ARLEQUIN estimates.

Genetic divergence among populations was examined by F-statistics and analyses of molecular variance AMOVA used to check the genetic variability significance between populations sampled and within each population.(Excoffier et al., 1992). Significance was estimated through 10,000 permutations tests as implemented in ARLEQUIN.

## 5.8 Results

### 5.8.1 DNA sequence analyses

DNA sequence alignments of the three markers: ND2 (n = 66; length 1041 bp), Cyt-b (n = 66; length 306 bp) and the CHDZ (n = 51; length 424 bp) were analysed. For CHDZ, samples obtained from male individuals (ZZ-single band) were sequenced successfully, however the quality of obtained sequences were not optimal, thus they were excluded, and only mitochondrial markers (ND2 and Cyt b) were used for further population analyses. Sex-linked gene amplification have been originally conducted in this study to estimate the sex ratio (male: female) at each of the breeding population sampled. Potential bias in sex ratio can have long-term impacts on population dynamics and survival. In common terns for example, sex specific mortality bias was detected towards male nestlings (Gonzalez-Solis et al., 2005).

The results sex ratio analysis showed that at both Elba and Gara male young proportions were more than females, while at Jeliana all four sampled young were females. In the Persian Gulf breeding population of Bahrain seems to be female biased (Figure 5.2), however because the trapping of terns has been conducted at the breeding colony on the Island of Jarim, Bahrain, probability of capturing flushed incubating females seems to be higher than trapping foraging returning males. Therefore the result of sex ratio analysis at this site was female biased. These results need further sampling from young birds to obtain comparable results with those obtained from Libyan breeding populations. Furthermore, the results displayed represents sex ratio in nestlings in early days of growth, the actual fledglings' sex ratio might be altered later, as a proportion of nestlings would not survive to fledging phase (See Table 2.10 on nest failure - Chapter 2).

Two samples of the four collected at Jeliana were successfully sequenced; the same happened for other sites, where sequencing quality was good for one marker and not for the other. Therefore sample size was reduced and limited to samples that have resulted both markers (ND2 and Cyt b).

### 5.8.2 Basic properties of study populations

Haplotype diversity using Cyt b locus, ranged from  $0.19 \pm 0.11$  in Gara population to  $0.81 \pm 0.07$  at the Persian Gulf population, with an overall average of  $0.40 \pm 0.2$  (Table 5.4). ND2 haplotype data showed greater diversity, it ranged from  $0.27 \pm 0.11$  at Gara population to  $0.64 \pm 0.10$  at Elba population, with an overall average of  $0.51 \pm 0.16$  (Table 5.5). The present haplotype diversity for the sampled populations may altered with the increase of sample size, for instance the small number of samples from The Red Sea population ( $n=6$ ) may underestimate the importance of haplotype diversity of this population.

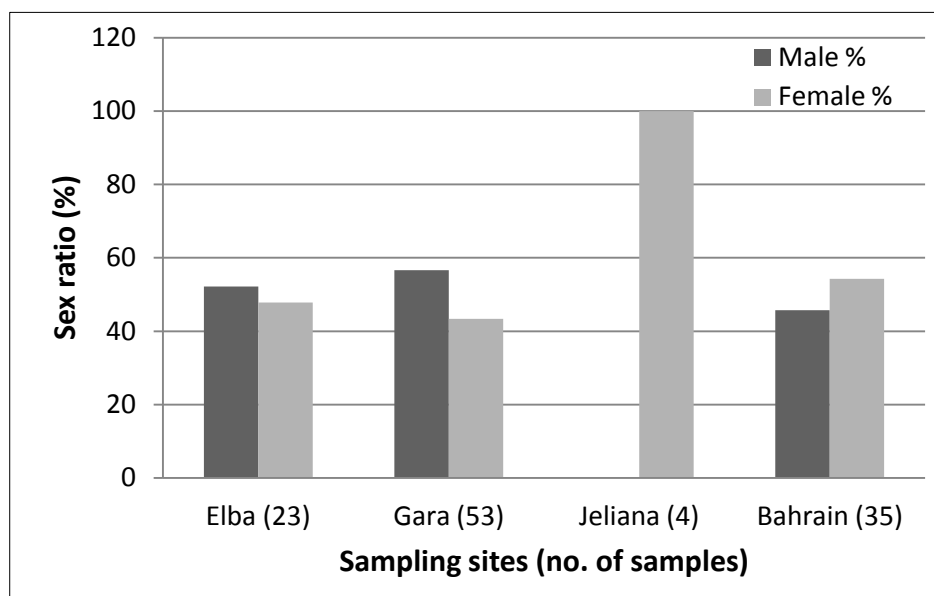


Figure 5.2 Sex ratio distribution of sampled breeding populations of Lesser Crested Terns.

It was not possible to collect more samples from this site, due to the timing of our visit to the site during mid-incubation period (See Chapter 2). Number of polymorphic loci for Cytochrome b has ranged from 4 at Gara and the Red Sea populations to 45 at the Persian Gulf population (Mean= $15 \pm 18.81$ ). Based on ND2 marker, number of polymorphic loci ranged from 4 at Gara to 48 at the Persian Gulf population (Mean =  $19.75 \pm 20.07$ ). In both loci, the Persian breeding population had more polymorphic sites than both of the Libyan and the Red Sea

populations. Mean number of alleles were almost identical among sampled populations (2.00-2.11) using Cytochrome b and was equal at 2.00 for the ND2 locus.

Table 5.4 Basic properties of study populations based on Cytochrome b marker.

Statistics	Gara	Elba	Red Sea	Persian Gulf
No. of gene copies	14	21	6	25
No. of loci	306	306	306	306
No. of polymorphic loci	4	9	4	45
Nucleotide diversity ( $\pi$ )	0.005 $\pm 0.004$	0.008 $\pm 0.006$	0.008 $\pm 0.007$	0.041 $\pm 0.022$
Haplotype diversity (H)	0.19 $\pm 0.11$	0.32 $\pm 0.12$	0.28 $\pm 0.19$	0.81 $\pm 0.07$
Mean Expected Heterozygosity ( $\pm$ s.d.)	0.16 $\pm 0.04$	0.11 $\pm 0.04$	0.28 $\pm 0.00$	0.12 $\pm 0.09$
Mean number of alleles ( $\pm$ s.d.)	2.00 $\pm 0.00$	2.11 $\pm 0.33$	2.00 $\pm 0.00$	2.07 $\pm 0.25$

Table 5.5 Basic properties of study populations based on ND2 marker.

Statistics	Gara	Elba	Red Sea	Persian Gulf
No. of gene copies	27	26	6	28
No. of loci	817	817	817	817
No. of polymorphic loci	4	20	7	48
Nucleotide diversity ( $\pi$ )	0.006 $\pm 0.0004$	0.002 $\pm 0.0015$	0.002 $\pm 0.002$	0.005 $\pm 0.003$
Haplotype diversity (H)	0.27 $\pm 0.10$	0.64 $\pm 0.10$	0.60 $\pm 0.21$	0.53 $\pm 0.11$
Mean Expected Heterozygosity ( $\pm$ s.d.)	0.09 $\pm 0.03$	0.098 $\pm 0.03$	0.33 $\pm 0.00$	0.09 $\pm 0.03$
Mean number of alleles ( $\pm$ s.d.)	2.00 $\pm 0.00$	2.00 $\pm 0.00$	2.00 $\pm 0.00$	2.00 $\pm 0.00$

### 5.8.3 Intra-specific sequence diversity

Sequences from 68 birds resulted in 20 haplotypes from each of Cyt b and ND2 markers (Table 5.6 and 5.7). Haplotype networks obtained by Alrequin have revealed multiple private Haplotypes at each breeding population and few primary haplotypes shared by all populations. The most common Cyt b haplotype (H1) was shared by 46 out of 69 individuals (66.67%) and 19 haplotypes were identified from the remaining 20 individuals. For ND2 the most common haplotype (H-b was shared by 44 out of 69 individuals (63.8%). Further 19 haplotypes were identified from the remaining 25 individuals.

All identified haplotypes (for either of Cyt b or ND2) were unique (private) as they differ from the sequences obtained from the Australian breeding population *T. b. bengalensis* (Accession: AY631298.1 GI: 55419843 and Accession: AY631370.1 GI: 55417569) respectively (Bridge et al., 2005). The Libyan breeding population *T.b. emigrata* at Gara (n=14) yielded three haplotypes (two private) for each of Cyt b and ND2, while at Elba (n=21) provided 5 haplotypes (four private) for Cyt b and 9 haplotypes (seven private) for ND2. At Jeliana (n=2) two haplotypes for each marker (one private for ND2). The Red Sea breeding subspecies *T. b. bengalensis* (n=6) provided two haplotypes for Cyt b (one private) and three haplotypes for ND2 (one private). The greatest private haplotype diversity were in the Persian breeding subspecies *T. b. torresii* (n=25), as it resulted 15 and 8 private haplotypes for Cyt b and ND2 respectively.

Table 5.6 Distribution of Cytochrome b haplotypes for the study populations

<b>Cyt b</b>	<b>Jeliana (2)</b>	<b>Elba (21)</b>	<b>Gara (14)</b>	<b>Red Sea (6)</b>	<b>Persian Gulf (25)</b>	<b>Total</b>
H-a	2	17	12	5	10	46
H-b				1		1
H-c		1				1
H-d		1				1
H-e		1				1
H-f		1				1
H-g			1			1
H-h			1			1
H-i					3	3
H-j					1	1
H-k					1	1
H-l					1	1
H-m					1	1
H-n					1	1
H-o					2	2
H-p					1	1
H-q					1	1
H-r					1	1
H-s					1	1
H-t					1	1
<b>Total</b>	<b>2</b>	<b>21</b>	<b>14</b>	<b>6</b>	<b>28</b>	<b>68</b>



Table 5.7 Distribution of ND2 haplotype for the study populations

<b>ND2</b>	<b>Jeliana (2)</b>	<b>Elba (21)</b>	<b>Gara (14)</b>	<b>Red Sea (6)</b>	<b>Persian Gulf (25)</b>	<b>Total</b>
H-1		2	1	1		4
H-2	1	12	11	4	17	45
H-3				1		1
H-4		1				1
H-5		1				1
H-6		1				1
H-7		1				1
H-8		1				1
H-9		1				1
H-10		1				1
H-11	1					1
H-12			1			1
H-13			1			1
H-14					1	1
H-15					2	2
H-16					1	1
H-17					1	1
H-18					1	1
H-19					1	1
H-20					1	1
<b>Total</b>	<b>2</b>	<b>21</b>	<b>14</b>	<b>6</b>	<b>28</b>	<b>68</b>

Haplotype distribution networks were calculated by Arlequin estimates of within haplotype connections using minimum spanning trees among haplotypes of each locus. The most shared haplotype among all populations was Haplotype a (H-a) in Cytochrome b data (Figure 5.3), while at ND2 locus the Haplotype 2 (H-2) were shared by all sampled populations, followed by H-1 (Figure 5.4). The network showed also the relative distribution of private haplotypes for each of the sampled population for both loci, and the spatial location within the network between each two haplotypes (nodes). Intermediate forms of haplotypes present between each two nodes.

These networks present only haplotype patterns found in the present investigation, however the shape of the network tree and relative proportions of each population contribution within the main shared haplotypes can change with the addition of more data, therefore these network trees represent the generic genetic structure of haplotype distribution of this study samples only, and

generally aimed at giving a first insights on intra-haplotype relationships resulted from the two mitochondrial loci.

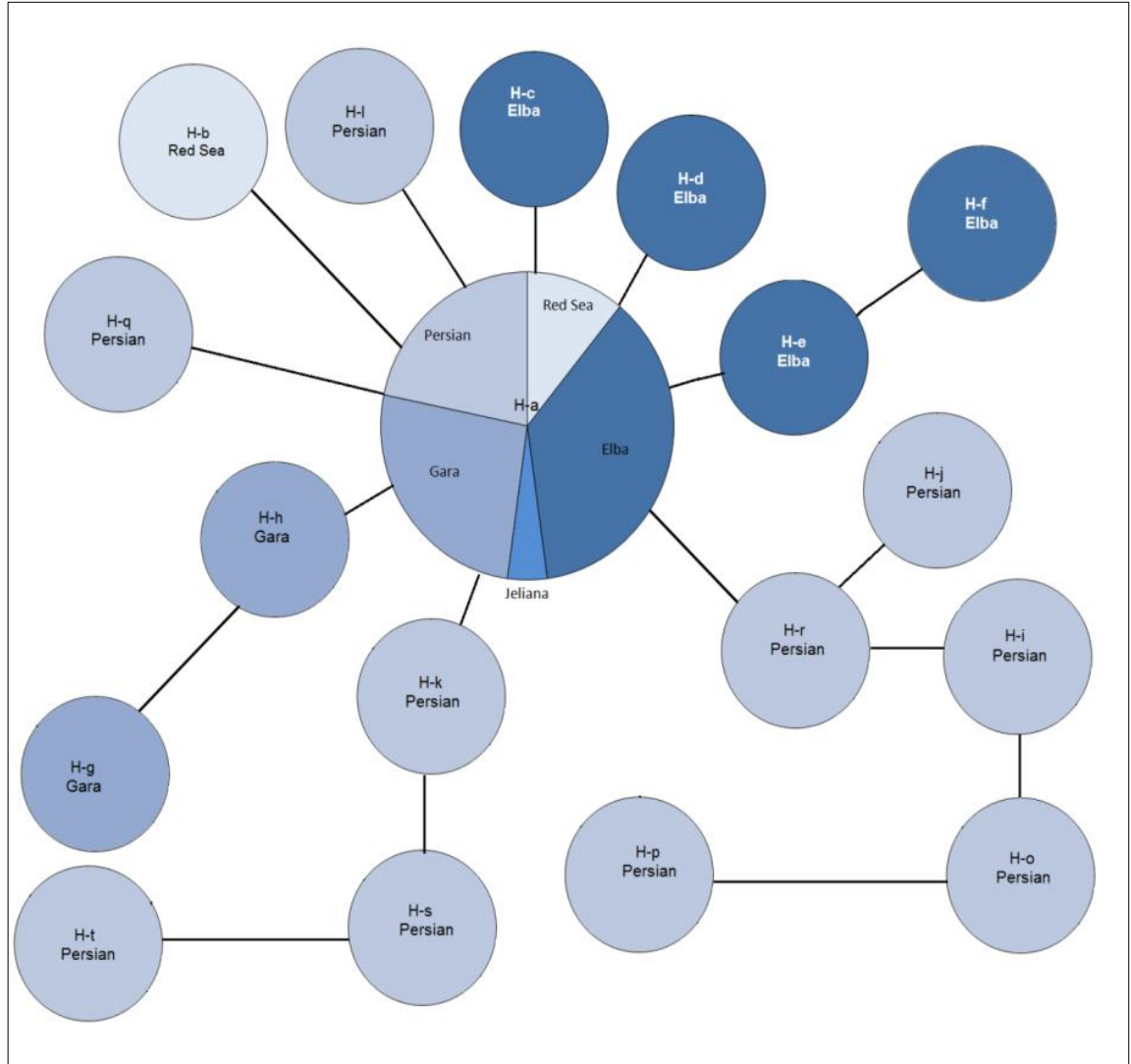


Figure 5.3 Haplotype distribution Network for four populations (3 subspecies) of Lesser Crested Tern *Thalasseus bengalensis* using Cyt b.

Haplotype designations correspond to those listed in Table 5.4. The diameter of the circles is proportional to the number of individuals represented by that haplotype.

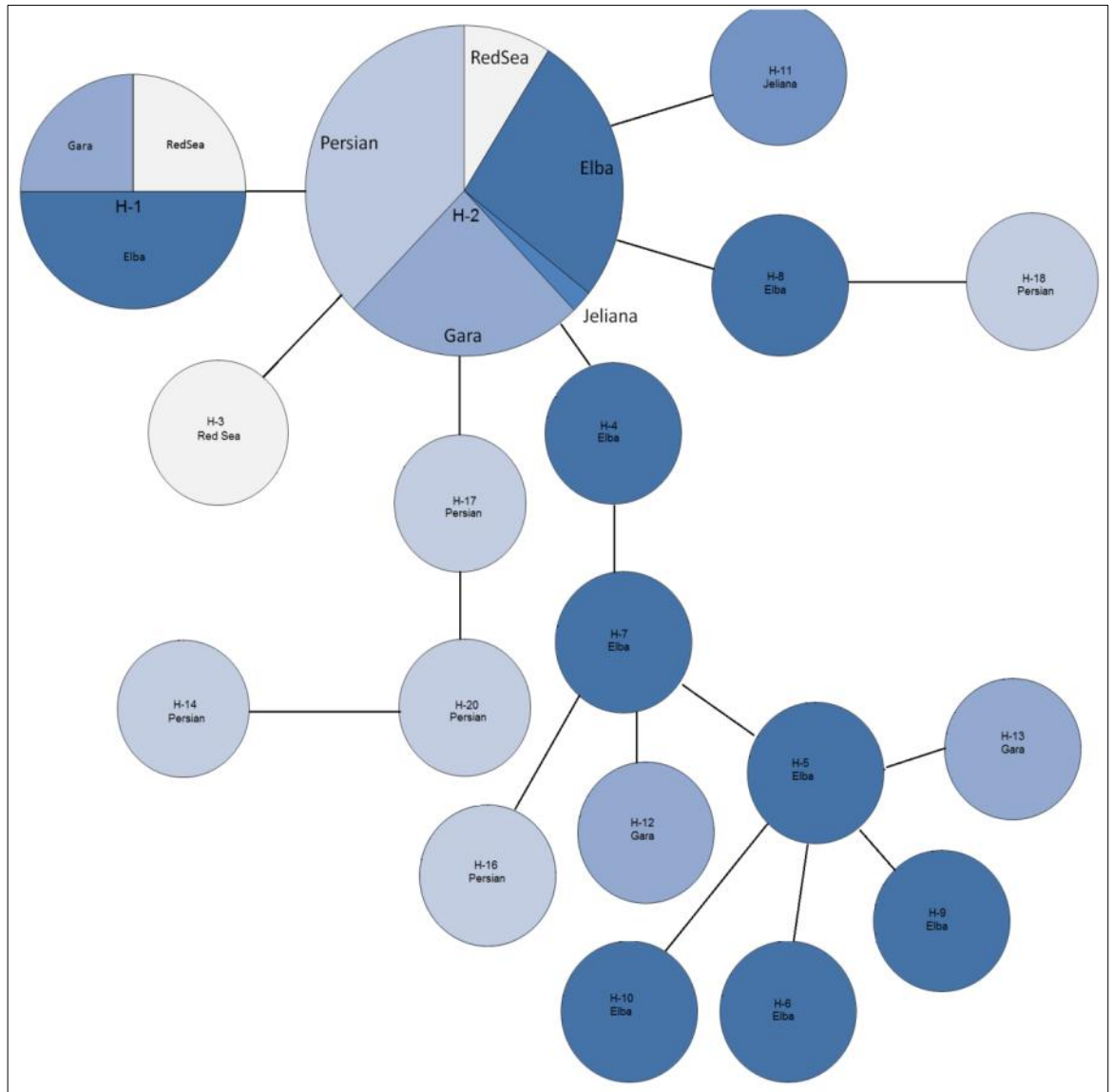


Figure 5.4 Haplotype distribution network for four populations (3 sub-species) of Lesser Crested Tern *Thalasseus bengalensis* using ND2.

Haplotype designations correspond to those listed in Table 5.5. The diameter of the circles is proportional to the number of individuals represented by that haplotype.

#### 5.8.4 Inter-population differentiations using mt-DNA loci

Results from AMOVA analysis of Molecular Variance using the  $F$ -Statistics of haplotype frequencies indicated significant genetic structure at the subspecies level for both Cyt b locus (Table 5.8;  $F_{ST}$ , Cyt b =0.103  $P<0.05$ ); but not for ND2 locus (Table 5.8;  $F_{ST}$ , ND2 = 0.017,  $P>0.05$ ). In Cyt b data, most significant pair-wise differences were found between Persian Gulf subspecies (*T. b. torresii*) and both populations at Gara and Elba (*T. b. emigrata*). Although the overall differentiation was not significant using ND2 locus using haplotype frequency data ( $F_{ST}$ ), pair-wise comparisons at population data differentiation have showed significant variations at the subspecies level (ND2  $\Phi_{ST}$  =0.011 ( $P<0.05$ )) particularly the Red Sea population (*T.b. bengalensis*) and Elba population (*T. b. emigrata*) as well as between Persian gulf population (*T. b. torresii*) and both of Elba and Gara populations (*T. b. emigrata*), which indicate validity of the current subspecies division taxonomy. The greatest variation was found between The Red Sea population and the Persian Gulf population using Cyt b locus (

Table 5.9;  $\Phi_{ST}$ , Cyt b =0.87  $P<0.05$ ), while it was between Elba population and the Red Sea using ND2 locus (Table 5.10;  $\Phi_{ST}$ , ND2 =0.76  $P<0.05$ ).

Table 5.8 Analysis of Molecular Variance AMOVA among and within populations using Cytochrome b and ND2 genetic markers.

	Source of variation	d.f.	Sum of squares	Variance components	Percentage of Variance
CYT b gene $\Phi_{ST}$ =0.037 ( $P<0.05$ )	Among populations	3	6.12	0.04Va	2.8
	Within populations	62	87.43	1.41 Vb	97.1
ND2 gene $\Phi_{ST}$ =0.011 ( $P<0.05$ )	Among populations	3	4.29	0.01 Va	1.13
	Within populations	62	95.42	1.16 Vb	98.8

Table 5.9 Genetic differentiation between tern populations (Pairwise comparison\*) based on Cytochrome b.

	Elba	Gara	Red Sea	Persian Gulf
Elba	0	0.10	<b>0.76</b>	0.01
Gara	-0.02	0	0.61	0.04
Red Sea	<b>0.03</b>	-0.043	0	<b>0.87</b>
Persian Gulf	0.06	0.05	<b>0.02</b>	0

Table 5.10 Genetic differentiation between tern populations (Pairwise comparison\*) based on ND2.

	<b>Elba</b>	<b>Gara</b>	<b>Red Sea</b>	<b>Persian Gulf</b>
<b>Elba</b>	0	0.05	<b>0.76</b>	0.02
<b>Gara</b>	-0.013	0	0.22	0.21
<b>Red Sea</b>	<b>0.006</b>	0.15	0	0
<b>Persian Gulf</b>	0.01	0.01	-0.060	0

\*Above the diagonal Exact test of population differentiation values, below the diagonal Fst values (in bold significant values ( $p < 0.05$ ))

## 5.9 Discussion

Recent advances in Molecular techniques used to infer taxonomic position of species present robust methods to discover inter/intra-specific evolutionary relationship, this approach became more used recently than the traditional taxonomy based on morphology or physiology of the taxon in question (Avice, 2000, Ball and Avice, 1992, John et al., 2013, Zuccon et al., 2012, Nguembock et al., 2009). The advances of using the phylogenetic approach on traditional approach are: DNA consists of four types of nucleotides (Adenine, Guanine, Thymine and Cytosine), which can be used to compare any groups of organisms via the pair-comparison matrices of data resulted from row nucleotides or the comparisons of relative proportions of those nucleotides in the gene pool section under investigation (Sibley and Ahlquist, 1990). Second, the evolutionary change of the DNA follows a regular pattern, therefore mathematical modelling is being used to formulate the expected change and compare DNA from both closely or distantly related organisms (Nei and Kumar, 2000).

The present study is an attempt to resolve the validity of subspecies taxonomy by investigating the genetic structure and evolutionary relationships among the Lesser Crested Tern *Thalasseus bengalensis* subspecies complex breeding in Libya, The Red Sea and the Persian Gulf. Previous studies mentioned this species with regards to its inter-specific positioning within the taxonomy of Family Sternidae, under subgroup of six species of crested terns (Bridge et al., 2005). In Bridge's study, the Lesser Crested Tern DNA samples were originated from the Western Australian population, that considered sedentary (Serventy et al., 1971), compared to the other migratory populations in the Persian Gulf, the Red Sea and North Africa (Cramp, 1985), yet the haplotypes resulted from the present

study were significantly different from those found in the Australian population, indicating a long term isolation between the two lineages.

The significant genetic variation between the Persian population (*T. b. torresii*) and the Libyan breeding population (*T. b. emigrata*) using the Cyt b locus, overall the insignificant differentiation using the ND2 locus poses the question on how strong is the variation between the species subpopulation, giving the fact that a very slight morphological variation is present among the three subpopulations. Some authors predict that they are a monophyletic species all together (Meininger, *Personal communications*) based on morphology and ecology of the species, but paleontological information indicated that the Mediterranean breeding population might be derived from the Red Sea (Zotier et al., 1999). However when haplotype frequency data were compared using the ND2 locus, there were some significant differentiations between the Red Sea and the Persian Gulf compared to Libyan population, supporting the hypothesis of long isolation between these populations due to differences in wintering and breeding ranges, making the current three subspecies layout. Despite strong natal philopatry and breeding site fidelity in many species, low genetic differentiation is reported in all tern genetic studies across subspecies and geographic range (Faria et al, 2007, 2010, Efe et al., 2009), suggesting long-term isolation and morphological differentiation due to selection, while experiencing ongoing gene flow.

The haplotype network tree showed that at least one common haplotype was shared by all subpopulations (H-1 and H-2 for ND2 locus, and H-a in Cyt-b), with the presence of an extended array of private haplotypes that characterises certain populations and not found at others. These results may reflect the variable sized samples used in the present investigation, where small data set were able to be collected from Jeliana for example, due to the 2009-2010 colony desertion because of water level rise and dredging works respectively (see Chapter 2 for details).

The results of nestlings' sex ratio at each breeding site showed variable results, both Elba and Gara were male biased, while the only four samples from Jeliana were females, contrasting female biased sex ratio at the Persian Gulf population. More sampling is needed to investigate any inter-annual variation in sex ratio at

each breeding site, linking the data on sex ration with foraging quality and recruitment can provide another insight into the dynamics of demographic structure of the breeding population in Libya and the other breeding populations of the species. Biased sex ratio may have long term effects on population structure, dispersal and growth (Becker et al., 2008, Sarzo et al., 2012), which may explain the relative stability in population size at the most important breeding colony of Lesser Crested Terns in Libya, giving the fact that no predation is evident there and the food resources seems to sustain larger breeding population than what is exist, unless a wintering range high mortality levels in juvenile birds, which affects the whole population growth, recruitment and survival rates .

Sex-specific mortality and dispersal are fundamental mechanisms of sex ratio adjustment at the population level, evidence of significant changes between nestlings and recruits sex ratio were found (Becker et al., 2008), indicating higher emigration to and immigration from other colonies in females, the less territorial and less philopatric sex compared to males.

Management of breeding population by introducing one sex to balance the deficiency in that sex for better population performance is an option of management used in many wild populations. However a precise knowledge of the sex ratio of the donor and/or recipient population is crucial, as such management decisions can significantly affect the success of reintroduction and reinforcement programs (Lambertucci et al., 2013), therefore the continuous monitoring of sex ratio both at hatching and of the recruits is essential prerequisite for better management of breeding populations.

Such studies when conducted in future alongside with historic population fluctuations extracted through the Bayesian analysis of genetic data can both allowing clear picture on the causes of population size apparent stability of the breeding lesser crested tern colonies in Libya, in the framework of available data since the recent start of population size monitoring (see Chapter 2).

Results obtained from other studies on tern phylogeny, were in general contradictory, as nuclear markers data or in combination with mitochondrial data provide varying results (Jackson et al., 2012), therefore, further sampling and the use of other methods targeting both mitochondrial and nuclear genome skimming

(e.g. Jackson et al., 2012) and the use of microsatellite markers (Faria et al., 2007, Szczys et al., 2005) can improve results on intra-specific evolutionary relationships among the three Lesser Crested Tern subspecies present in North Africa and the Middle East, as well as to further test the validity of the present study results.



## **Chapter 6**

# **General Discussion and conclusions**

## **Chapter.6 General Discussion and conclusions**

### **6.1 General discussion**

The present study has compiled a range of data on breeding chronology, ecology and breeding biology of the Mediterranean breeding population of Lesser Crested Tern in four breeding sites Libya, in addition to collecting limited data from a one-day visit to one of the species breeding sites at Ashrafi archipelago in the Red Sea.

The review of Libya's ornithological history with particular emphasis on seabirds shows the limited and incomplete research effort achieved, compared to the neighbouring countries. Libya is the less documented country in terms of bird species and studies.

The Lesser Crested Tern is the largest seabird breeding population in Libya, although this important Mediterranean population is yet not receiving the appropriate level of protection, due to lack of protection to the species and its breeding and feeding habitats. Hence there was the need for a study of the breeding biology, feeding ecology and migration aspects of this population to further understand the ecology to draft and implement future management plans for this population. The identification of the important stop-over of these birds in their route from and to the breeding sites, as well as the limited information on wintering sites, that needs further research to map these sites and identify threats to which they are exposed in West Africa. There is regional responsibility for conservation of those sites, as the protection of breeding sites only cannot guarantee the survival of the population, as they host the population only during the breeding months (June to August), while for the remaining nine months the species is either migrating or at wintering sites.

Three seasons of colony monitoring has particularly determined the breeding dates, breeding initiation at each site, as well as the dates of first hatching and fledging (Chapter 2). Breeding was started approximately three weeks earlier at Jeliana colony, in the last third of May; possibly due to different migration routes used by Jeliana terns. Other possible explanations discussed for this early breeding proposed were discussed at both Chapter 2 and 4, including differences

in migration initiation date from wintering sites, and the relative availability of prey species in terms of quality and quantity at Jeliana site compared to the other breeding sites. These explanations need further research using telemetry tracking techniques, to precisely identify why Jeliana birds arrive earlier to their breeding site. More monitoring efforts at potential staging sites in southern Italy and along the Tunisian-Libyan coastlines can provide insights on the validity of differences in arrival route assumptions. Hatching and fledging dates differed between Jeliana and the other sites, due to different breeding initiation dates. Identifying dates related to breeding phases is essential to plan for future conservation activities at each breeding site, as these dates were approximate being obtained from single or two visits /season/site in the past.

The present study added a new breeding site to the earlier discovered breeding sites of Gara (Moltoni, 1938), Elba (Meininger et al., 1994) and Jeliana colonies (Gaskell, 2005, Hamza et al., 2007). A small colony was found at Fteha Island to the northwest of Elba Island in 2010, where the breeding population at Elba was split between Elba and Fteha, possible causes were discussed, including human caused disturbance and its consequences on the future of the site at Elba (Chapter 2). The three seasons monitoring of population size at each colony has confirmed that Jeliana breeding site is the second most important breeding site of the Mediterranean species *T. b. emigrata* as it is hosting 200-400 breeding pairs. Anthropogenic activities, such as dredging, waste disposal, water pollution and mammalian (mostly dogs) predation of eggs and nestlings (at Jeliana), with poaching of nestlings and wildfowl unregulated hunting (at Elba and Fteha) were the main threats faced by this breeding population. At Gara, the largest colony (Azafzaf et al., 2006), these threats appear to be absent, except for the vulnerability to Oil spill pollution, potentially caused by Oil tankers traffic, from and to the Zuitina Oil terminal (Hamza et al., 2008), as they may discharge ballast water prior to reloading new oil from oil terminal, located less than ten nautical miles to the northeast of Gara Island. The other potential Oil spill came from the currently planned offshore Oil drilling and production, in an areas to the north of Gara island. The preparation of an emergency response plan and prepare national capacities to control Oil spills and other at seas oil shipping accidents, is an urgent priority for the Libyan authorities, in accordance to the Law no.15/2003 on Environmental Protection and Enhancement and the International Convention

for the Prevention of Pollution from Ships (MARPOL) from operational or accidental causes and its annexes, in addition to the Barcelona convention's prevention and Emergency Protocol, which ratified by Libya and they specify responsibilities on each stakeholder in case of emergencies. The National Oil Corporation and its affiliated oil companies are expected to coordinate with both of the National Safety Authority (NSA) and The Environment General Authority (EGA) in case of accidental or operational Oil spill case. However, previous incidents showed limited facilities to control offshore oil spills in the past (Pers. Obs.), and a great deal of coordination, training and communication is urgently needed to control such threat on the marine environment and its elements.

All breeding sites of Lesser Crested Tern in Libya were shortlisted and proposed, with other sites along the Libyan coast, to be marine protected areas. The proposal was prepared in late 2009 by the Environment General Authority and the Marine Biology Research Centre (MBRC) and submitted to the government in early 2010 (Hamza et al., 2011). The proposal was not approved; pending further information requested by the prime minister office at that time, nevertheless the government has approved Farwa Island and the peripheral waters as protected areas. The Environment General Authority (EGA) is currently preparing draft law on protected areas and national parks, including a section on marine protected areas (EGA, Pers. Comm.). This work is conducted in collaboration with both the United Nations Environment program (UNEP) and the United Nations Development Program (UNDP). The new legislation draft will be submitted for adoption at the National General Congress (NGC) by the end of this year (2013). The proposed law is expected to fill the gaps and establish a new nature conservation system for the country, including clear criteria for protected areas selection, management and finance. It will also benefit from past experiences in neighbouring countries in this field, as there will be a regional contribution in synthesis of the first draft by a consultant from the Regional Activity Centre for Specially Protected Areas.

In all studied breeding seasons, egg hatching and young fledging success were highest at the Gara breeding colony, being the largest, with no predation, poaching or development reported during the study period, while the other three sites showed lower breeding productivity levels, mainly due to habitat alteration

(Jeliana) or potential impact of predation/poaching (Elba and Fteha). Detailed information on the reproductive parameters of each site through the study seasons, Table (2.6) shows that mean breeding success (across study seasons) was highest at Gara, followed by Elba and Jeliana. Data on both Fteha and Ashrafi archipelago breeding sites were not obtained due to poaching of eggs at Fteha in 2010 and early visiting date for Ashrafi in 2009. No information is found in literature on either breeding performance at Ashrafi islands, most previous studies concerned the reporting of breeding only with no further details (Baha El Din, 2003, Goodman and Meininger, 1989, PERSGA/GEF, 2003). For Fteha Island, this study is the first to discover breeding at this site (Chapter 2).

Breeding sites are particularly vulnerable to disturbance, this disturbance can be anthropogenic or caused by other animal species. A variety of causes were found to affect the breeding productivity at each colony; Human-caused disturbance include flooding of the breeding site due to uncontrolled water levels (particularly at Jeliana being an inshore site), or poaching of both eggs and nestlings, flooding and predation by dogs at Jeliana and poaching with disturbance by local hunters/bathers at Elba and Fteha were the main concerns. Disturbance by visitors have also impacted breeding productivity at Gara in 2009, some chicks had fallen from the island cliff (where the colony is located), after being chased by local visitors (Personal Observations). Mortality of young nestlings in nest or during crèche development was also observed at all sites, some nestlings were lost at both the Elba and Fteha colonies during the 2010 season; Further inquiries with locals suggested that some poachers have used the nestlings to trap birds of prey in the nearby area, where falconry and falcon trapping is a common practice. Such violations to wildlife, if continued, may lead to long-scale consequences not only on the survival of the confined breeding Lesser Crested Tern population at these sites, it can affect other bird species targeted by poachers. Disturbance of breeding success by other animal species include the impact of other seabird fish-eating species (gulls, Mediterranean Shags and little terns) on the availability and quality of prey that consumed by those birds as part of their food.

At all sites strict measures should be taken to minimise the impact of visitation disturbance, examples of these regulations are applied on other tern colonies in

Australia (Claridge, 1997), whereas the Mediterranean seabird colonies are still lacking such measures.

The potential disturbance caused by predation of eggs and nestlings by the Yellow legged gulls (*Larus michahellis*) cannot be excluded, as their breeding coincide for the first two weeks or the Lesser Crested Tern breeding season. The gull young were observed at the beginning of the tern breeding season in mid-June at Gara and Elba. In a similar situation the Lesser Crested Tern in Australia, predation of eggs and chicks by the Silver Gull *Chroicocephalus novaehollandiae* resulted in deaths of 1.6-83.1% of the colony young, in addition to mortality caused by flooding and starvation due to adverse weather conditions (Hulsman, 1977).

Lesser Crested Tern at all studied colonies in this study were noted to respond to human approaching by flushing from their colony sites. Mobbing was the main behaviour exercised by terns against human disturbance. It was relatively more intense at Elba colony compared to other colonies (*Personal observations*). Disturbed birds emit alarm calls and fly at the intruder, diverting its attention and may make physical contact. Mobbing is common behaviour in Gulls and Common Terns (e.g. Clode et al., 2000, Burger and Gochfeld, 1991). In other tern species Moy (1995) found that, the Black Tern *Chlidonias niger* was able to differentiate between aerial and terrestrial predators, with different alarm call intensity and duration at each case.

The section on feeding ecology via regurgitated food from growing young has increased our knowledge of diet structure of the Mediterranean population of Lesser Crested Tern (Chapter 3), as the only data presently available is on the Australian subspecies population. Both the Gara and the Elba colonies depend on approximately 8-10 species of epipelagic fish, whilst the diet at the Jeliana colony was found to be composed of 18 fish species, including some common species found on both the Gara and Elba diet lists. This difference in taxon diversity of prey species at Jeliana might be caused by the larger sample size collected at this site, it also can be caused by the benthic habitat diversity in waters near the colony, in addition to the presence of many inshore water bodies, that serves as fish nurseries, such as Ain Ziana, El-lithama and Benghazi

lagoons where the species is frequently observed foraging. Inshore foraging is characterised this species at other breeding ranges, e.g. in Australia (Hulsman, 1988).

The annual variation in nestling diet structure based on the ANOSIM analysis showed that, there was more variation in diet structure between sites than within sites, indicating a degree of stable prey availability in the foraging areas around each breeding site. It is not clear whether this resulted from active selection by foraging terns for certain species or it just reflects what is available in the periphery of each colony. Future studies should compare diet structure using regurgitations and sampling the foraging areas to confirm either of these suggestions.

There was also an increase of the delivered fish length with the advancement of the breeding season, i.e. fish was significantly larger during late middle and provisioning period, responding to the increased demand for food by the fast growing chicks. Similar trend have been found in the Crested tern *Thalasseus bergii* breeding in Australia (McLeay et al., 2010, McLeay et al., 2009).

Ecological theory states that a species need to use a diverse food resources, when their preferred prey species decline (Bell and Ford, 1990). Thus diet breadth should expand when preferred resources are scarce, and a negative relationship between prey diversity and breeding productivity would be expected, especially when other environmental factors affects the preferred prey population dynamics (Pedersen et al., 2012). In the present study prey diversity was positively correlated with breeding productivity, where birds fed on more diverse food items are expected to have higher breeding performance compared to birds with limited dietary resources. This relationship may occur when there is marked spatial and temporal variation in the availability of preferred resources (Arroyo and Garcia, 2006). This trend was observed in many bird species including birds of prey (Arroyo and Garcia, 2006) and seabirds, e.g. (González-Solís et al., 1997, Jaquemet et al., 2008). The feeding niche breadth can provide the adult seabird with alternatives to switch between prey species, in response to decline in one or more of its prey population, due to either of prey population dynamics, overfishing or oceanographic climatic changes that may affect the availability and spatial

distribution of the prey species population (Barrett et al., 2007, Furness and Tasker, 1999, Furness and Tasker, 2000, Hamer et al., 1991, McLeay et al., 2008).

The dominant prey species and relative proportions of each prey species in the diet of the young of this tern species were identified at each breeding site, using the index of relative importance. At each site two or three prey species dominated the structure of the young food, with other species present in smaller proportions. The other species may represent less favourable prey types or merely reflect their relative abundance in the foraging areas used by the feeding terns. It was not possible to conduct sampling at these waters to estimate the relative density of each prey species. There was a significant increase of caught prey with the advancement of the provisioning periods of the nestlings, this increase in prey size may reflect an active selection by adult terns by targeting larger prey to save energy of catching small prey in several foraging journeys, it also indirectly indicates the growing need of the nestlings to more amounts of food to meet their energetic requirements. The colony food intake/energetic requirements for both adult and juvenile birds were not estimated during this study, however the literature indicates that requirements of a similar size species, the adult female Sandwich tern *Thalasseus sandvicensis* required 63g herring per day, equivalent of 361kJ/day (Brenninkmeijer et al., 1997), while the energy intake for juveniles ranged between 45kJ/day to 385kJ/day (Stienen et al., 2002). This difference in energy requirements indicates the need for chicks to reach fledging phase in shortest time possible, to fly and avoid terrestrial based threats.

Independent sampling of epipelagic fish and other fish species found in the diet of the Lesser Crested Tern was not possible due to logistical constraints, such as suitable sized boats and sampling tools. However if such sampling is conducted in the future and fish sizes compared with those delivered to chicks, it could support the prediction made in the present study that adult terns actively select specific fish sizes that suits the physical abilities of the young to swallow and to fulfil its dietary requirements. It can also support another hypothesis on Tern breeding phenology is largely driven by food quality and availability, therefore a natural synergy exists between prey size growth and the advancement of the young growth during the breeding season, which then show an excellent example



of synchronisation in tern breeding when biotic and a biotic conditions at the optimum, to allow the best possible breeding success.

The increase in sea surface temperature SST around the eastern region of the Libyan coast, during April and early May, in addition to both water depth and marine currents are all explain most of the variation regarding potential spawning sites for fish in the region (D'Elia et al., 2009, Giannoulaki et al., 2011, Palomera et al., 2007, Pawson and Giama, 1985). Pawson and Giama (1985) indicated that gonad growth in Round Sardine in Libya (main prey species) commences in April, when they feed on zooplanktone and there were temporal variations in spawning season, resulting in spatial and temporal differences in fish length within the population, during the summer and autumn seasons. This in turn provide the foraging tern with a mixed sized supply of high-energy food to nourish their young.

The availability of food resources combined with a sudden increase in marine productivity at this particular region of the Mediterranean, may represent the main drive behind the selection of these Libyan Islands as the main breeding sites for Lesser Crested Tern Mediterranean population, leaving many other potential breeding sites that can provide the physical (Site) requirements for breeding but cannot fulfil the dietary requirements for the colony to be established.

More information was collected on habitat requirements at the breeding range, in addition to a synthesis of published information on pre/post breeding movements of the Mediterranean Lesser Crested Tern population, along the North and Northwest African countries, Tunisia (Isenmann et al., 2010), Algeria (Isenmann and Moali, 2000), Morocco (Thévenot et al., 2003) and Mauritania (Isenmann et al., 2005) . The overall information collected on ecology and migration of the study population lead to synthesising a model presenting the temporal relationship between wintering, staging and breeding ranges was prepared (Figure 6.1).

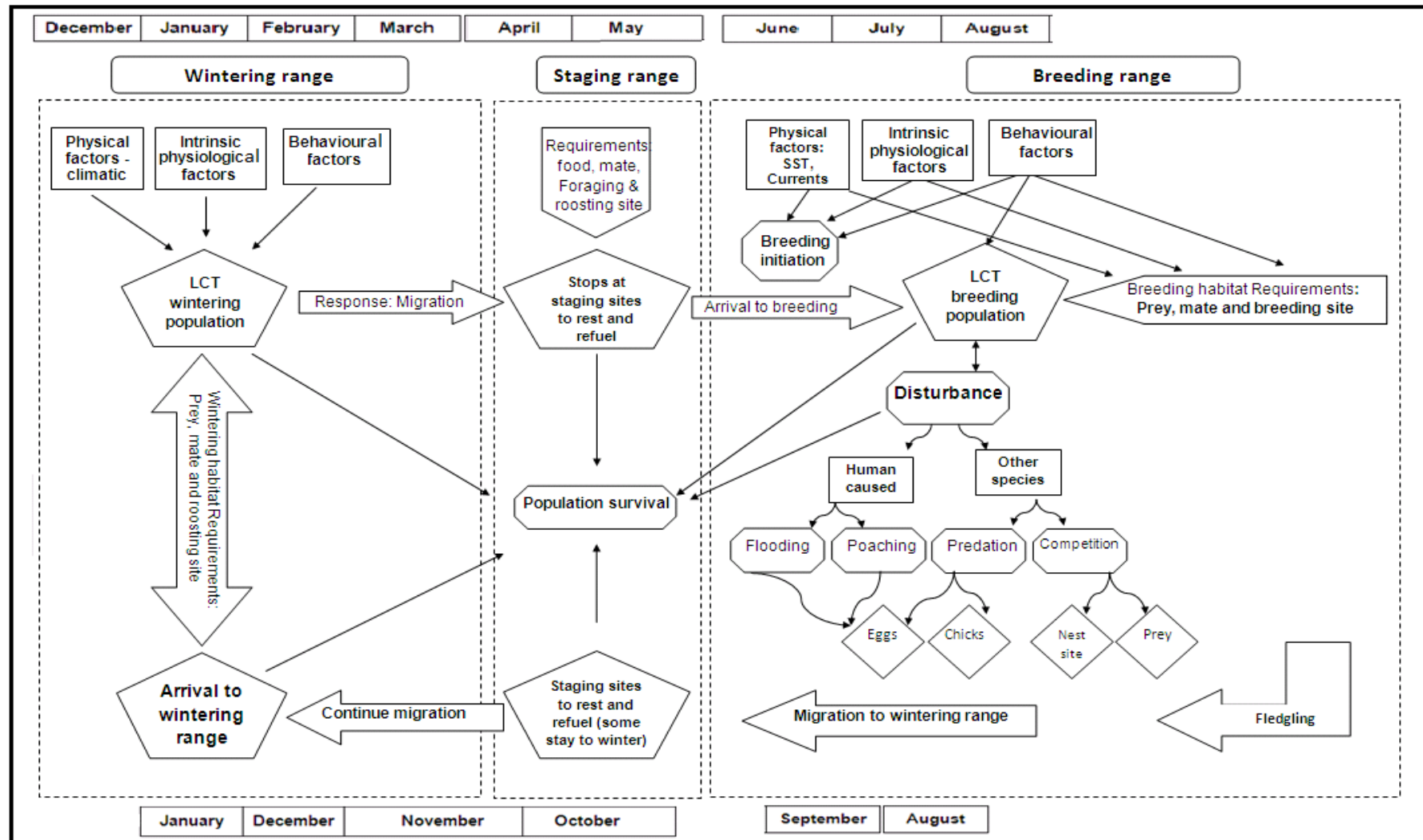


Figure 6.1 Simplified model of Lesser Crested Tern at the breeding, staging and wintering ranges.

At the wintering range the Lesser Crested Tern wintering population is mixed with several other terns and other seabird species (Clive Barlow, *Pers. com*). They spend winter months feeding at the coastal Atlantic Ocean waters, and within inshore waters and coastal wetlands. Then start migrating to the breeding sites in early spring. Several factors are known to trigger the initiation of migration journey, including physical and climatic factors, in addition to both intrinsic physiological changes of the birds, resulting in behavioural responses. The timing of migration in animals inhabiting environments with only slight seasonal fluctuations such as the tropics is poorly understood (Wingfield et al., 1992, Hau et al., 1998). It is of note that the migration of Lesser Crested Tern is characterised by the movement of small groups of birds along the coastal area between wintering and breeding range, through several staging sites. These numbers may range from a few individuals to hundreds of birds (based on ring sightings reports from West Africa, Morocco and Spain). The migrating birds then stop to roost, feed and possibly mate at the staging sites. One of these sites is Ras Ramel at the north coast of Djerba Island, Tunisia, which in fact situated >800 Km to the west of the closest breeding site in Libya (Gara Island). Field observations at this site confirmed that terns display mating and couple formation behaviour (Isenmann et al., 2005), indicating that some arrived birds to breeding sites mated already and immediately start egg laying after arrival. Other birds mate and lay their eggs within 5-10 days at the breeding site (reported for the common tern- (Ehrlich et al., 1988)). The time spent by terns at staging sites is estimated as two months (mid-March to mid-May), the first birds do occupy their nest site at Jeliana, followed by others breeding at both Gara and Elba by mid-June. The asynchrony of breeding initiation (Chapter 2), suggests several differences in breeding initiation, including potential use of shorter migration route between northern Tunisia and the Italian southern small Islands to arrive at Jeliana earlier than terns of the other two colonies. If this hypothesis was rejected by monitoring at these migration points, the asynchronic start of migration from wintering grounds can be responsible on this early breeding at Jeliana compared to the other sites. Asynchrony of arrival to breeding sites suggests some degree of population segregation during the migration period. Faria et al. (2010) found that birds depends on a high diversity prey range do arrive earlier to breeding sites than those having lower prey diversity food niche. The number of prey

species found at Jeliana birds is double the number of prey species used by Lesser Crested Terns breeding at Elba or Gara. The availability and diversity of food at Jeliana from both inshore and offshore waters may trigger the population to arrive earlier than those at other breeding sites.

Although the initiation and duration of the breeding season are ultimately controlled by food availability, on which the young depend, in addition to photoperiod, hormones and breeding density (see review by Dawson, 2008). Other factors can affect the breeding initiation, including climatic conditions and oceanographic factors (e.g. Marine currents and Sea Surface Temperature) which both affect the proliferation of phytoplankton and zooplankton. The latter are the primary food for the epipelagic fish which constitute the main food of Lesser Crested Tern near the breeding sites. Such an indirect relationship between climatic and oceanographic conditions were also shown for other seabird species using long term data, e.g. the rhinoceros auklet *Cerorhinca monocerata* and Japanese cormorant *Phalacrocorax filamentosus* (Watanuki and Ito, 2012).

Other factors affecting the breeding initiation date include the physiological changes within the body hormones of the adult bird (Hau, 2001), and behavioural factors that govern the mating and egg laying phase (Blanchard and Morris, 1998, Moore et al., 2000, Vedder, 2012, Votier et al., 2009). Several habitat requirements are crucial for a successful breeding season, including prey availability and quality (Crawford, 2009, Dänhardt and Becker, 2011, Enstip et al., 2006, McLeay et al., 2008, Weimerskirch, 2007) and mate availability for birds that did not mated during migration (Moore et al., 2000). In addition to habitat suitability for breeding is formed by factors such as soil type, plant cover, presence of other breeding/non-breeding species, site protection against predators and flooding (Fasola and Canova, 1996). The habitat requirements mentioned for wintering, staging and breeding phases are all contributing to population survival through each of those phases.

A detailed study of adult food of these species is needed to identify food guilds of each species near the breeding sites.

The predation of eggs and nestlings by stray dogs at Jeliana is a limiting factor for breeding success there; these dogs use the lowered water level at the end of breeding season to access the small islet where the colony is located, and feed on both eggs and nestlings. In 2010 the whole colony was destroyed by stray dogs.

Other species might also impact the breeding Lesser Crested Terns via the competition on nest sites, within each breeding site. The breeding of Black-winged Stilt and Little terns at Jeliana contributed to nest space competition, which may have adverse consequences on breeding success of each of those species.

Hatching and fledging dates and success rates were estimated for all colonies of the species in Libya, although this was not possible for the Red Sea colony of Ashrafi archipelago, as the visit was conducted during the early incubation period in August 2009 (difference in breeding start between Mediterranean and Red Sea breeding terns). It was not possible to revisit the Red Sea sites during the following seasons. Fledging starts in late July at Jeliana and in August and September at Elba, Fteha and Gara colonies. The parent birds continue to feed the newly fledged juvenile birds (Cramp, 1985) on migration route to wintering sites through the staging range in north and northwest Africa.

Migration pattern through Ring sightings and recoveries were studied by using the available data provided by birdwatchers and the author own observation on migration and at breeding sites respectively (Chapter 4). The speed of movement from breeding to wintering areas was estimated based on ringed bird sightings along the migration route. The first post-natal migratory birds arrived to Ceuta in Spanish Morocco (staging site) within 32 days, passing an average of 86 Km/day. This shows the importance of ringing technique and monitoring on staging sites to study the migration of this population. However, lack of monitoring and site protection at other potential staging sites are some of the difficulties preventing the collection of further information on migration pattern and existing threats at these sites. The conservation of a species does not only depend on breeding site protection, without similar measures at staging and wintering sites. Field monitoring and literature indicated that some individuals may prefer spending the

winter at the staging range (Brehme, 2003, Meininger et al., 1994, EGA-RAC/SPA-WCT, 2012). Adult terns and their growing juveniles then arrive to the wintering sites by late October/ early November in small groups and conclude the migration by December (Clive Barlow, *pers. com*).

## 6.2 Threats to breeding Lesser Crested Tern

The field study of the breeding ecology of Mediterranean Lesser Crested Tern in Libya for three seasons has revealed several threats that negatively affecting breeding success and the future of each of the colonies.

These threats were identified and ranked in severity using both information from the literature and the data collected during the present study. Threat severity values were assigned to each threat per site, according to the relative whole site threat ranking method (Margoluis and Salafsky, 1998). Threat severity ranking and description are given in (Table 6.1).

Table 6.1 Severity index used to quantify threat severity on Mediterranean breeding Lesser Crested Tern Colonies. Adapted from Margoluis and Salafsky (1998)

Severity ranking	Description
4 = Very High	Threat is likely to destroy or eliminate the population or reduce it by 71-100% within ten years or three generations.
3 = High	Threat is likely to seriously degrade/reduce the population by 31-70% within ten years or three generations.
2 = Medium	Threat is likely to moderately degrade/reduce the population by 11-30% within ten years or three generations.
1 = Low	Threat is likely to slightly degrade/reduce the population by 1-10% within ten years or three generations.

All direct threats were compared to one another at each colony site and then cumulative threat severity values of the four study sites has been calculated to present threat severity ranking on the whole breeding population level (Table 6.2). This finding provide the conservation authorities in Libya and the regional Action Plan on Seabirds listed in SPA protocol and Biodiversity in the Mediterranean with a weighted list of threats they need to deal with for better protection of the Lesser Crested Tern breeding population. This threat list can be the basis of future conservation projects aims at mitigating these threats and

provide an opportunity for pan-Mediterranean collaboration to conduct these projects. For example a project on habitat enhancement in Jeliana and Benghazi in General can be the base on which several other activities can be conducted, including awareness campaign on the importance of treating wastewater, importance of Benghazi as a hub for bird migration between Europe and Africa and within Africa migration as well. Guidelines on site visitation and ecotourism at seabird breeding sites can also be elaborated with support from other countries in the Mediterranean with more experience in this field. Blast fishing impact on local environment, on human health and safety also can be a core for a project targeting both blast fishers and fish consumers where these practices are exercised. Therefore the threat list is nothing but a start for rationally organise priorities in next phase of education, awareness, restoration and legal enforcement projects targeting the breeding Lesser Crested Tern and other species and habitats used including human welfare and safety.

Table 6.2 Rated list of threats to Mediterranean breeding Lesser Crested Tern, column width is scaled to colony size.

Threat type / Severity (1-4)	Mediterranean breeding sites-Libya *				Cumulative threat severity index (0-16)
	Gara	Jeliana	Elba	Fteha	
Lack of legal protection (A)	4	4	4		16
Oil pollution (A)	4	3	3	3	13
Disturbance by site visitors (A)	2	3	3	3	11
Blast Fishing (A)	3	3	2	2	10
Noise pollution (A)	3	4	1	1	9
Solid Waste pollution (A)	2	4	2	1	9
Egg/Check poaching (A)	1	2	3	4	9
Mammalian predation (N)	1	4	1	1	8
Predation by other birds (N)	1	2	2	2	7
Competition on breeding space (N)	1	4	1	1	7
Light pollution (A)	1	4	1	1	7
Municipal development/habitat degradation (A)	1	4	1	1	4
Wastewater pollution (A)	1	4	1	1	4

\* Column width in accordance to colony size. A= Anthropogenic threat. N= Natural threat.



### 6.2.1 Legal framework

The Lesser Crested Tern in particular and birds generally in Libya are not included at any special species or habitat protection act. In the present study this legal protection was the most important threat to both the Lesser Crested Tern breeding population and its breeding and feeding habitats, in addition to other breeding or migrating species. Absence of specific legal protection of both species and their fragile habitats can expose both to several threats (mainly anthropogenic), which can significantly reduce the population size, breeding success and the habitat availability. Poaching of eggs and chicks, hunting, site development by construction for tourism or industry without a proper Environment Impact Assessments are a few of the law violations, that without a clear updated legal mandate, the local and national law enforcement authorities cannot account offenders.

Most if not all present environmental legislations deal indirectly with nature conservation in general, without a clear identification of what species or habitats these laws are targeting, Table (6.3) presents details on the three main legal texts related to nature conservation.

There is an urgent need to revise and issue new legislative framework for nature and species conservation in the country, to follow the trend of adopting most regional and international multilateral treaties and conventions dealing with bird and nature conservation in General, as the past laws (mentioned in Table 6.3) were some laws are designed and implemented by one ministry, depending on its own duties, not on the basis of common goals for the conservation activity, that is necessary needs partnership between different governmental agencies and the civil society sector as well (NGO's). Single sided laws ignite disputes among governmental sectors and cannot lead to any actual achievements on the ground. The law no.14/1989 on marine wealth exploitation for instance, included a chapter on marine protected areas gave the Marine Biology Research Centre the responsibility of selecting and management of marine protected areas, without any details on finance, management or partnerships with other stakeholders. The result that after several decades no single marine protected areas is exists in Libya, although the field studies on marine birds, marine turtles

and marine vegetation species showed several sites that have a regional importance.

The lack of a comprehensive biodiversity strategy making the efforts spent by different stakeholders in the field of conservation either with short comes or with limited impact on the actual status of species and habitats in the country. The new Libyan authorities are facing a long term consequences of the work system left by the former regime, it will need to set clear goals and inventory the available financial and human resources available, to start a well-planned national programme on conservation of species and habitats.

Table 6.3 Present Libyan legislations on marine and coastal conservation

Legislation number/date	Executing Ministry /Agency	Related Articles
Executive regulations of the Law no.14/1989 on marine resources exploitation	The Ministry of Agriculture and Marine Resources/ Marine Biology Research Centre (MBRC)	Chapter 6: article 75-77 of this legislation concerning marine protected areas, definition, identification and scientific supervision.
Law no.15/2003 on Environment Protection and improvement	The Environment General Authority (EGA).	Chapter 9, of this law dealing with wildlife conservation, article 56 states: "All species of animals and birds should be protected from extinction by different hunting means; protected areas should be identified where hunting/fishing is completely banned". Article 57 specifies conditions for obtaining hunting licences and hunting seasons.
The General People's Committee Decision No. 37/ 2005	The Ministry of Agriculture and Marine Resources/ Animal Sciences Research Institute	Declares a national protected economic fishing zone along the Libyan coastline, prohibiting all methods of fishing in the declared permitted zones without advance permission issued by the official authority.

The threat of oil spill came as the second most important threat on breeding Lesser Crested Tern colonies after lack of legal protection. The Libyan economy depends largely on Oil exploration and production, generated about 95% of total fiscal revenue and 98% of export receipts in 2011-12 (World-Bank, 2013). The Libyan marine area is a passage for most oil tankers that leave Libyan oil terminals as well as tankers coming through the Suez channel to Western European countries and vice versa. Ballast water cleaning produces Tar and sludge suspended in water column, then form Tar balls drifted onto the coast and the shores of islands (Golik et al., 1988), although recent international regulations prohibit ballast water release into open waters, because of pollution and invasive species introductions, some tankers in absence of surveillance still practice this harmful procedure. This contributes significantly to the amount of Oil pollution drifting on to Island shores, presenting a threat on both breeding and feeding seabirds of the area, directly by trapping the birds or their nests within sticky tar, or indirectly with the oil impact on fish and other marine invertebrates that made the food for seabirds and their offspring (Symens and Evans, 1993, Burger, 1997). For example, the northern shore of Elba Island is almost covered with layers of tar drifted from the oil tanker cleaning.

The proximity of the Zouitina Oil terminal to the Gara Island puts this most important site for Mediterranean breeding Lesser Crested Tern in danger in case of any oil spill or oil tanker accident. The offshore exploration of oil and gas in the area is adding to the severity of this imminent potential threat unless safety procedures are put in place at each of these pollution sources. The National Oil Corporation (NOC) with the Environment General Authority (EGA) are liaising with national and foreign Oil companies operating in Libya, to ensure the adherence of environmental standards of exploration and production according to both national (Law no.15/2003) and international Oil exploration environmental standards.

Blast fishing using dynamite extracted from landmines of the World War II is a widespread fishing practice on most of the central and eastern coasts of Libya (Hamza et al., 2011). The use of such a method is not only kills more fish than required or can be harvested but also causes extreme damage to the sea floor and sedentary fauna and flora (Figure 6.2). The recent spread of explosives after

the war of 2011 has increased the usage of such a destructive fishing method, with the availability of different types of landmines remains and the weak law enforcement authorities (*Personal observations*).



Figure 6.2 Blast fishing in Benghazi, July 2011.  
(source: AP Photo/Sergey Ponomarev)

The law No.15/2003 on Environment protection and improvement prohibits the use of dynamite or chemicals in fishing. Enforcement actions are needed to control these practices and stop the trading networks in such dangerous materials throughout the country (Hamza et al., 2011). The Environmental Police force that mentioned in the above law have not formed, and it is by law the enforcement agency that deal with wildlife crime.

The effect of blast fishing near the Lesser Crested Tern breeding sites is indirect, as explosives can affect the prey availability for the terns, as it does not differentiate between fish sizes and fishermen, who usually harvest only the larger species because of their higher commercial value and leave the rest. The great reduction of several species has been attributed to blast fishing, particularly during seasonal fish spawning aggregations (Naughton, 1985).

The acoustic noise disturbance caused by blast fishing to incubating birds, when blast is detonated near the colony site is another effect on incubating tern behaviour. Underwater personal observations around the breeding sites at Gara

and Benghazi coast indicate an intensive use of blast fishing in shallow waters near the edges of these islands. Elba and Fteha areas suffer less intense blast fishing, however it was recently reported to be of concern there (Badalamenti et al., 2011).

It is important to formulate and train the Environmental Police to identify fish caught by blast fishing, to allow immediate persecution and control the leak of such goods to the consumers. Public awareness by local authorities and NGO's in areas where the blast fishing is widespread, can help to educate consumers on adverse impacts this destructive fishing on local marine/coastal habitats and on the expected deterioration of fish stocks in future. This is an example of results of a scientific study of Lesser Crested Tern breeding ecology can show the importance and the integration of marine and coastal ecosystem components, and how anthropogenic practices can affect both the bird and the human population. Publicizing results of this study to local and national media, in a simple way, can help to raise the awareness of this issue.

Disturbance by visitors to the breeding sites is a concern to the survival of those colonies, breeding season coincide with the summer season, when Libyans go to the sea more often. The artisanal fishing is also more active in summer with better weather conditions and an increased seasonal fishing for certain target species, such as the Common Dolphinfish *Coryphaena hippurus* and the Greater Amberjack *Seriola dumerili* using both Long-lines and Purse-seine. Some important proportion of the Amberjack catch is blast fished during June and July (*Personal observations*).

It is of note that, in addition to the predation threat by stray dogs and disturbance from development works, visitors and poachers to the lake area at Jeliana, other threats can further affect the tern survival at this second most important breeding site. The illegal disposal of solid wastes including house-hold organic waste on the Lake perimeter causing fires due to the fermentation produced Methane ignition, with high air temperature during the summer months. Such conditions can also encourage the spread of other mammalian predators such as Rats and Mice, which could become a limiting factor for the Tern and other breeding bird populations' survival. Rodents consume bird eggs and small checks, their fast

reproduction can threaten the whole subspecies population in a few years, especially at Jeliana. Linear models showed that rats have affected seabird species distributions more on the smaller islands and on islands than larger islands, due to their higher densities in small area(Martin et al., 2000). On the other hand, Rats represent a hazard for the public health, as they can spread diseases and parasites in such highly inhabited area. Immediate action is needed by the local authorities in Benghazi to prevent such consequences on both the nature and the public health.

After the 2011 war, decreased state control made guns, including hunting rifles, very common in Libya, this made the Wildlife to become under an increased poaching pressure. Evidence of bird shooting was noted at both Elba and Jeliana sites during the 2012 season (Figure 6.4), although poachers usually target migratory duck and larger waterbirds, and tendency was observed to target and consume seabirds, the disturbance caused by shooting could have negative impacts on the colony at both sites.



Figure 6.3 Household mixed waste dumped by people on the edges of Jeliana Lake.



Figure 6.4 Poaching evidence of birds at Jeliana (Left: seats used by local poachers, Right: Rifle bullets)

The dredging of Jeliana Lake and building of several towers on its banks has started in late 2009 then halted in early 2011, due to the uprising events in Benghazi. These works were conducted by a Canadian contractor with the Libyan government, without conducting any Environmental impact Assessment, that in fact required by the Law no.15/2003 on Environmental Improvement and Protection to conduct such activity. The works are expected to resume during the first half of 2014 (EGA Benghazi office, Pers. com) although there are some attempts to minimise the impact of this project on the local biodiversity at the site. The project plan includes the construction of a shopping mall, hotels and a water park, in addition to the deepening of the lake, which expected to have adverse consequences on avifauna of the site. The establishment of a man-made Island was discussed with the developer in 2010; however the expected noise and light pollution can make breeding Lesser Crested Tern at this site difficult.

Fortunately 7 kilometres to the north of Jeliana Lake is another Lake of El-Lithama, which was a former salt marsh wetland. Between 2006 and 2010 there

was a project to restore this site, and deepen the salt marsh to become a lake, connected to the sea, with two man-made channels (

Figure 6.5 and 6.6). At this site, because apparently of noticing thousands of wintering waterbirds, particularly European Cormorant *Phalacrocorax carbo* and several waterfowl species, the engineering works included the construction of four man-made Islands. Once the new lake at El-Lithama being connected to the sea, it could become an ideal new breeding and inshore foraging site for Lesser Crested Terns. The terns already use this area when crossing north to roost at Ain Ziana lagoon. During the 2012 season tens of Lesser Crested Terns were observed repeatedly foraging at this site, indicating the presence of fish, most likely introduced Cichlids. Further site enhancements at those small islands would be needed to encourage the terns to use this new site, such as clearing tall vegetation and prepare the substrate soil, to become similar to that of Jeliana islet.

Both the sites at Jeliana and El-Lithama offer a very good examples of interaction between water birds and the human environment, the potential of using these sited to raise the public awareness on issues related endangered species, wetland conservation, wetland flora and fauna, bird watching, bird migration, water pollution, water resources management, aquatic and terrestrial invasive species and other related environmental protection topics in general are of great importance, to Benghazi inhabitants in particular and to the wider audience in Libya in general.





Figure 6.5 Satellite image of El-Lithama Lake (larger scale right). Note the recently built four Islands with connecting channels to the Mediterranean (Google Earth).



Figure 6.6 Aerial view of El-Lithama Lake with Jeliana lake (top left) and the Mediterranean Sea (top right) at the horizon of the view (© F. Bujwary).

### 6.2.3 Implications of the study results

#### i. Status of breeding sites

There is an urgent need to protect both the breeding sites and the species, based not only on the importance of Lesser Crested Tern breeding as the largest seabird breeding population in the country, but on the regional importance of this relatively small Mediterranean population *T.b. emigrata* compared with the total population of the species globally. The data collected in this thesis can be used by Libyan conservation authorities as a basis to setup a management plan, aims at the protection of Lesser Crested Tern breeding sites, to increase population size and to educate the general public near the breeding sites on threats facing such special sites, in addition to the importance of terns in general as an indicators of the ecosystem health.

Threats to breeding sites are listed above in Table (6.2), are more intense at Jeliana breeding site, compared to the remaining three sites, as it is the site affected largely with terrestrial anthropogenic activities (Dredging, water pollution, mammalian predation, noise and light pollution...etc). Continuous monitoring of these threats can provide insights on appropriate mitigation measures. The overall aim is to manage breeding sites in a way to acquire an acceptable level of protection to maintain the species and its breeding and feeding habitats.

Threats to the species at staging and wintering sites are poorly known, some data on tern trapping in West Africa was discussed in Chapter 4 based on data collected in Senegal (Meininger, 1988). The present situation of this threat is unknown, as no recent updates were available from that region. The other documented threat for terns in West Africa is Food scarcity, which can increase mortality levels for both Juveniles, who are starting to become independent feeders, as well as on adults (Stienen and Brenninkmeijer, 2002). Collaboration with West African ornithologists in the future can enlighten the current threats facing Lesser Crested Tern and other wintering seabird populations.

The Libyan authorities and the other North and West Africa countries, where Mediterranean breeding Lesser Crested Terns are passing through or spend the Winter, all have moral responsibilities to implement the regional and international

conventions, that listing both the breeding habitats (Islands and islets) and the population itself as an endangered population, including the Mediterranean Action Plan on Seabirds listed under the Protocol on marine and coastal Biodiversity in the Mediterranean- Barcelona Convention (UNEP-MAP-RAC/SPA, 2003), the African Eurasian Waterbird Agreement (AEWA) under the Convention of migratory species (CMS), the Convention on Biodiversity (CDB) and the Ramsar Convention on Wetlands. However the optimum implementation of those conventions is constrained by several factors, among them the limited human and financial resources and the weak legal framework of nature conservation, where the member state obligations towards regional and international conventions does not yet reflected on the national laws related to conservation. Although the commitment of implementing these conventions' guidelines and regulations are not compulsory, and done on the basis of moral commitment towards the environment, some conventions such as RAMSAR convention on Wetlands, have a special record (Montreux Record) of Ramsar sites where changes in ecological character have occurred, are occurring or are likely to occur. Such sites are kept in this record till substantial steps conducted by that country to restore the site to the quality it was designated up on as a Ramsar site. Technical and financial assistance from these conventions might be halted if significant violations are being conducted in a certain member state.

Bilateral negotiations between Libya and the European Union were held in 2003, to sign a joint trade treaty including fisheries investment, no agreement was reached at that time. Negotiations resumed in 2009, one of the outcomes of this negotiations is liberalisation of economic activities to open the door for European investment in several sectors. The Libyan fishing production for export to the EU is expected to increase significantly, aggravating existing and severe overfishing (George et al., 2010).

Future cooperation with the EU in conservation sector, on the other hand, can lead to the adaptation of some European regulations related to the habitat and species and wild bird directives in countries of the southern Mediterranean Sea. This can put further responsibility on national authorities to monitor and implement wider conservation measures on development and other human land uses at or near fragile natural sites.

The following (Table 6.4) is an analysis of the present situation of Libya in relation to the adoption of a sound conservation policy in Libya.

Table 6.4 The strengths and weaknesses for Libya to adopt a sound nature conservation policy

Strengths	Weakness	Proposed future actions
Small population in Large area	Most of the country is aired environment, and the coastline is under increasing development pressure.	Establish a national project on biodiversity mapping for both important habitats and current species distributions.
Significant sections of the coastline still in pristine condition.	23 out of 29 important coastal sites are targeted by tourist development (Hamza et al., 2011).	Liaison between EGA, Ministry of Tourism and the Libyan Spatial Planning Agency (LSPA) to select sites for development and protect fragile habitats.
Availability of recent updated data on several endangered bird species and habitats (e.g. this thesis, the Atlas of Wintering waterbirds of Libya).	Lack of legal protection for species and habitats, and lack of enforcement of the existed regulations.	Urgent effort should be made by EGA to revise existing regulations and propose a new legal framework for protecting species and habitats. Consultation with other governmental agencies and Civil Society partners NGO's is needed prior to submitting the proposed law to the National General Congress for adoption.
Legal framework of species and habitat is present within the law no.15-2003 on Environment Protection, in addition to the law no.14-1989 on marine resources.	Legal conflicts in legal texts gave different agencies similar duties related to conservation, making conflicts between Environment and Agricultural ministries a continuous problem.	Holding periodic meetings of stakeholders and NGO's to find ways of amending existing regulations and solve intra-governmental managerial conflicts.
Libya is a member in most conservation conventions on regional and international levels.	The actual benefit from these conventions support is limited because of bureaucracy and limited number of qualified staff at their national focal point authorities. In addition to the absence of NGOs role in such activities.	Revising the focal point appointments and encourage more Libyans to take part in internships at those international organisation working in conservation sector to acquire practical experiences that can help the government to effectively run this issue.
Availability of funds that can be used in conservation programmes, from national, regional and international donors.	Lack of active partners (NGO's) that have sufficient experience in managing conservation projects. Bureaucracy and limited qualified conservationists prevent the best use of the national financial resources in conservation.	Promote, train and support nature conservation NGO's to take share in nature conservation works of the country.

## ii. Diet study

Number of prey species at Jeliana is approximately double what have been found at each of Gara and Elba. However, the dependence on limited number of prey species, can put the whole population at risk of decline, if some or all of these prey population decreased under the critical threshold, as a result of overfishing, climate change affecting spawning habitats physical or biological conditions. Changes in prey population dynamics can also lead to niche competition with alien species from the Red Sea. (See Chapter 3 discussion).

More data are needed to assess the extent and impacts of illegal blast fishing in Libya on fish population at affected sites, and its effects on prey population abundance and on spawning habitats. The destruction caused by blast fishing around the colonies, if continued in such trend, can lead to a sudden drop in availability of some prey species.

The increasing trend of established invasive fish populations in the Libyan waters needs further field studies to map the current distribution and investigate the immediate and long-term impacts of these species on local fish fauna and habitats.

## iii. Ringing and Migration

Ring monitoring of adult tern returning to breeding sites can be used to estimate adult bird survival from season to another, which in turn reflects the population survival rates at both staging and wintering sites. Our knowledge of staging and wintering sites is still limited, although this study revealed some hotspots of the Lesser Crested Tern distribution out of breeding season. Recently a North African Ornithological Network was established, aiming at exchange of data and collaborate in joint projects.

More information is still needed to estimate the risk of tern catching in West Africa for both consumption and collection of rings. Previous surveys in the late 1970's and mid 1990's indicated that the practice of wintering tern catching is wide spread along coastal villages in Ghana (Avery et al., 1995) and Senegal (Stienen et al., 1998, Meininger, 1988). Terns were caught for food and to collect rings to

be sold as souvenirs to foreign tourists. It is not clear whether there is any preference for a particular tern species, as the mentioned surveys indicated the impact of tern catching by village children on both roseate tern and sandwich terns.

#### iv. **Population Genetics**

The low genetic variability is a common feature of all Sternidae family, although the results of population genetics showed some degree of genetic variability, it also showed that more sampling is needed to identify all haplotype structures found at each population, some of these haplotypes are already shared by all populations sampled, while others are specific to one subpopulation. The use of other phylogenetic methods such as microsatellites and whole genome skimming, can further identify the potential divergence between the three subspecies and can further predict population dynamics at each of them, in terms of population increase or bottle-nose inbreeding for smaller populations, as in the case of Mediterranean breeding population. The limited financial resources didn't allowed the use of these techniques during the present study, as the aim of this study was to investigate the general genetic structure of these three subspecies and attempt to quantify the validity of the present taxonomy.

#### **6.2.4 Conservation measures**

Given the above information, it is possible to make the following recommendations and conservation measures:

- Local authorities in Benghazi should take the necessary steps to control stray dogs at Jeliana area, not only due to their impact on breeding terns but as they represent a hazard on public health.
- Habitat enhancement and restoration can provide more nest spacing and mitigate the inter-specific competition on nest space; in 2012 the breeding area was positively correlated to breeding population size and breeding success (Chapter 2).

- The habitat requirements of the breeding, staging and wintering ranges affect population survival, hence the identification and quantification of these requirements at staging and wintering can provide data on conservation of this Mediterranean breeding population of Lesser Crested Tern.

### **6.3 Proposed Action Plan**

The following is a summarised Action Plan for the conservation of the Mediterranean breeding Lesser Crested Tern population at National level (Breeding range) and on regional level (staging and wintering ranges).

#### **6.3.1 Actions required on National Level**

##### **Thematic issue 1: Protection of the species**

The Lesser Crested Tern is the largest breeding population of any seabird in Libya. The country holds a moral responsibility to maintain the breeding population of the Mediterranean subspecies, and so far (except the adoption of the Mediterranean Action Plan for Seabirds and conducting annual monitoring) no actual protection measure was in place.

##### **Actions proposed:**

1. Develop national species protection legislation, and emphasise on protection of breeding seabirds and their habitats in particular.
2. Setup a national Action Plan to protect marine birds (Migrant and residents), with emphasis to the Mediterranean Guidelines available and the Regional Action Plan for seabirds of SPA protocol.
3. Declare and reinforce the protection of both Gara and Elba islands as Marine Protected Areas as soon as possible, and revise the legal texts regarding marine traffic, Oil spill and fishing, by taking the breeding and feeding habitats of seabirds in consideration.
4. Regulate wildlife hunting activities and control poaching and illegal hunting.
5. Utilize the available data collected since 2006, to setup a national programme to monitor and protect Lesser Crested Tern colonies and their sites.

**Thematic issue 2: Education and public awareness****Actions proposed:**

1. Organising of awareness campaigns towards the general public and governmental civil servants prior to and throughout the breeding season, on the importance of Libyan small islands to the Mediterranean population of Lesser Crested Tern.
2. Use the Lesser Crested Tern as a flagship species for Seabird conservation activities of the country.

**Thematic issue 3: Cooperation****Actions proposed:**

1. Strengthening the existing collaboration with Zueitina Oil Company and other local authorities near the breeding sites, to help in protecting and monitoring the known breeding sites for the species in Libya.
2. Continue the ringing programme, to enhance our understanding of migration routes, staging and wintering sites.
3. Training of Libyan ornithologists to acquire more experiences in seabird monitoring and conservation fields.

**6.3.2 Action Plan/ Regional level****Thematic issue 1: Cooperation****Actions proposed:**

1. Work with other Mediterranean states to implement the Action Plan on marine birds listed in SPA protocol, with particular attention to regional actions regarding the Mediterranean breeding population of Lesser Crested Tern.
2. Strengthening regional monitoring activities for sighting ringed Lesser Crested Terns migrating from and to wintering areas.
3. Information and data exchange regarding movements and identifying both staging and wintering areas along North and West African coastline (e.g. via the recently launched North African Ornithological Network).
4. Organize workshops and scientific symposia on Mediterranean Seabirds regularly.



## **Thematic issue 2: Knowledge Improvement**

### **Actions proposed:**

1. Monitoring the Med population: undertaking field surveys of LCT in wintering areas in west Africa and strengthening for the next 3 years the integrated monitoring program currently implemented on the species in Libya.
2. Apply protection status of the species and breeding habitats in Libya: Help the new Libyan authorities to setup conservation policy to protect Lesser Crested Tern as a national flagship species with regional Mediterranean importance.

### **6.3.3 Action Plan coordination and time frame**

This Action plan shall be coordinated by the Environment General Authority EGA, with support from the Regional Activity Centre for Specially Protected Areas (UNEP-MAP-RAC/SPA). Local non-governmental Organisation actively involved in Bird Conservation would be given an Action Plan partner status.

The time span of this proposed Action Plan is 5 years, the whole actions need to be reviewed and updated afterwards.

### **6.4 Further study**

The present study showed several opportunities and fields of research that can enhance the knowledge regarding this special population of terns in Libya and the Mediterranean. These future study themes are:

1. Studying the migration using light-weight data loggers, such as Geolocators and/or miniature satellites telemetry, to follow the population journey between breeding and wintering sites. This will help to identify migration routes, staging areas and wintering areas, in addition to estimating the duration of both inward and outward migrations.
2. Setup a regional surveillance network to monitor ringed individuals and build a database of signings and recoveries, which will help in estimating population dynamics for the population.

3. Study migration using stable isotope analysis of feathers, egg membranes and other tissues and compare the stable isotopes with those of expected food species in staging and wintering sites, to allocate individuals to their wintering sites. Stable isotopes can be further used to determine whether breeding individuals in Libya are Income (utilising local food resources prior to egg laying) or Capital breeders (depending on food resources outside of breeding area).
4. Study the sympatric seabird species foraging habitats and guilds to investigate any niche overlap that may affect the Lesser Crested Tern breeding success in future.
5. Conduct a study on the possible education and awareness resources and alternatives for raising public awareness with the importance of birds generally and the breeding seabirds in particular in communities near breeding sites in Libya.
6. Continue the monitoring of the population size, diet structure and breeding success at each of the colonies to collate a dataset that be able in future to examine population trends categorise threats and suggest management mitigations.
7. Monitor the spread and potential impacts of invasive fish and other marine organisms on the marine and coastal ecosystems near breeding sites.

#### **6.4 Critique of the study**

The present study was conducted in non-conventional circumstances, the following constrains contributed to the quality and quantity of the collected data:

1. The weather conditions during the breeding season, air temperature can reach 46°C, with high humidity, which limits the duration of fieldwork during the day and most work was done either during early morning hours or in the evening. Weather conditions at sea also contributed to delays in visiting the Gara Island in 2010 and Fteha Island in 2012, due to the Etesian (annual)

winds locally known as Meltem combined with stormy conditions for periods up to 10 days.

2. It was not possible, because of health and safety concerns, to visit the colonies in 2011 due to the war condition and to some colonies in 2012 due to security concerns after the war, which caused some gaps in the collected data.
3. Long distances among breeding sites contributed to the limitations in data collection, some the distance between Gara and Elba for instance is more than 600Km.
4. All breeding sites were neither protected nor managed, and suffer from visitors that may vandalise the colonies. In 2010 part of the nestling monitoring planned was to weigh nestlings to monitor weekly growth rates at Gara, to compare it with other colonies growth rate. A visitor entered the colony the same week and removed sections of the fence erected to enclosure selected nestlings for this experiment.
5. Absence of supporting infrastructure at or near the breeding sites (except at Jeliana) to support the accommodation and monitoring of sites for longer periods, as most field visits lasted for few hours each week.
6. The researcher experience contributed to the data quality, after the first data collection in 2009, the author has modified many aspects in the fieldwork planning to utilize most time in collecting data within the shortest time frame.
7. Limited availability of appropriate boats, most of the transportation to the Islands were conducted using small artisanal fishing boats, which are not suitable to conduct surface fish sampling for instance, as it lacks speed meter or other navigational instruments to follow transects at sea. Therefore sampling of surface fish to compare the fish diversity and morphometrics with what in the diet of nestlings was not possible.
8. Limited number of field assistants, to help with collecting fish samples and ring larger number of nestlings in both 2009 and 2012 seasons.

9. The cost of fieldwork itself, including accommodation, transportation among sites located hundreds of kilometres apart, in addition to purchasing field equipment. For example, it was planned to have a chapter on estimating foraging radius and frequency of foraging trips, using GPS tracking. A miniGPS logger was needed, but the cost of the high quality units was high (Euros 500-800 per unit), thus a cheaper version was bought, but the size of harness on tern back was not appropriate after trying it in the field, which led to the cancelling of the experiment due to limited financial resources.
  
10. The cost of extracting, amplifying and sequencing DNA samples was also high; therefore a limited number of samples and genetic markers were used.

## **Annexes**

**Annex I Distribution, Ecology and status of fish species found in the diet of Lesser Crested Tern young.**

**Annex II: Data on ringing of Lesser Crested Tern young during the present study (2009-2012)**

**Annex I Distribution, Ecology and status of fish species found in the diet of Lesser Crested Tern young.**

	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
1.	<i>Spicara smaris</i>	Picarel	Zrega	Eastern Atlantic and whole Mediterranean and Black sea	Schooling species, depth range 15 – 328,. Feeds on zooplanktons. Max. Length (TL) 20cm	Marine; pelagic-oceanic; inhabit <i>Posidonia</i> beds and muddy bottom	Not Evaluated	Native	Gara/Jeliana
2.	<i>Atherina boyeri</i>	Big-scale sand smelt	n/a	Eastern Atlantic: Portugal and Spain to Mauritania and Madeira, and throughout the Mediterranean and Black Sea. Some isolated populations in England and the Netherlands	Schooling species, depth range 1–? . Carnivorous, feeds on small crustaceans, worms, mollusc. Juveniles feed mainly on zooplankton. Max. Length (TL) 20cm	Marine; freshwater; brackish; demersal;	Least Concern (LC)	Native	Jeliana only
3.	<i>Belone belone</i>	Garfish	Yebrahim	Eastern Atlantic and Mediterranean Sea, with other two subspecies in north sea.	Lives close to the surface and has a migratory pattern. Feeds on small fishes, particularly clupeids and <i>Engraulis</i> . max length : 93.0 cm TL.	Marine; brackish; pelagic-oceanic;	Not Evaluated	Native	Elba only

	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
4.	<i>Lipophrys trigloides</i>	Grey Blenny	n/a	Eastern Atlantic and Mediterranean Sea,	Very inactive except during spawning when become diurnally active. Feeds on mussels, other benthic invertebrates and algae.  Max length : 13.0 cm TL	Intertidal, occurs in wave-battered rocky shores of coastal waters under rocks or seaweed.	Not Evaluated	Native	Jeliana only in 2012
5.	<i>Salaria pavo</i>	Peacock blenny	n/a	Eastern Atlantic: Atlantic coast from France to Morocco; also in the Mediterranean and Black seas and in the Suez Canal.	Intertidal, shallow bottoms, on rocks or sand between pebbles and vegetation. Feeds on benthic invertebrates, mainly molluscs, also algae. Max. Length 13cm,	Marine; brackish; demersal,	Not Evaluated	Native	Jeliana only in 2012
6.	<i>Seriola dumerili</i>	Greater amberjack	Barema	Circumglobal. Distribution including pacific, Atlantic oceans and Mediterranean sea.	Found in deep seaward reefs; occasionally entering coastal bays, Small juveniles associate with floating plants or debris in oceanic and offshore waters. Juveniles form small schools or solitary. Max length : 190 cm TL.	Marine; reef-associated;	Not Evaluated	Native	Elba only

	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
7.	<i>Tilapia zilli</i>	Redbelly tilapia	Telabia / Bolty	Africa and Eurasia: introduced to many countries by aquaculture.	Occasionally form schools; mainly diurnal. Prefer shallow, vegetated areas, juveniles are found in the seasonal floodplain. Herbivorous, feed on water plants and epiphyton, and some invertebrates.	Freshwater; brackish; benthopelagic	Not Evaluated	Introduced	Jeliana only in 2012 (may originate from Ain Ziyana lagoon).
8.	<i>Sardinella aurita</i>	Round Sardine	Sardina	Eastern Atlantic, the Mediterranean and Black Sea. Western Atlantic: Cape Cod, USA to Argentina. Bahamas, Antilles, Gulf of Mexico and Caribbean coast	Schools in coastal waters from inshore to edge of shelf, Juveniles tend to stay in nursery areas. Feeds mainly on zooplankton, especially copepods. Juveniles on phytoplankton. Max length : 31.0 cm TL	Marine; brackish; reef-associated; oceanodromous	Not Evaluated	Native	At the three sites.
9.	<i>Coryphaena hippurus</i>	Common dolphinfish	Lambuka	Atlantic, Indian and Pacific: in tropical and subtropical waters. Highly migratory species, present during summer and autumn months in the Mediterranean for spawning.	Schooling species, feeds on several fish species, crustaceans and squid. Max length : 210 cm TL	Marine; brackish; pelagic-neritic; oceanodromous	Least Concern (LC)	Native	At Elba



	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
10.	<i>Lichia amia</i>	Leerfish	Strelia	Eastern Atlantic: southern Bay of Biscay to South Africa, including the Mediterranean. Western Indian Ocean: South Africa to Delagoa Bay, Lourenço Marques.	Found in coastal waters including estuaries. Feed mainly on fish; juveniles prefer crustaceans. Max length : 200 cm	Marine; brackish; pelagic-neritic; oceanodromous	Not Evaluated	Native	Jeliana only in 2012
11.	<i>Engraulis encrasicolus</i>	European Anchovy	Inshoga	Eastern Atlantic: from Norway South Africa, The Mediterranean Black and Azov seas	A coastal schooling marine species, in some areas enters lagoons, estuaries and lakes, especially during spawning (April-November), tends to occupy surface waters in summer, retreating and descending in winter. Feeds on planktonic organisms. Max length : 20.0 cm SL.	Marine; brackish; pelagic-neritic; oceanodromous	Not Evaluated	Native	At Gara and Jeliana
12.	<i>Cheilopogon heterurus</i>	Mediterranean flyingfish	Hamama	Western Mediterranean (probably seasonally migrating, as never recorded in winter), north-eastern subtropical Atlantic near Gibraltar	Pelagic, coastal species which can fly over long distances escaping predators. Spawns in summer. Max length : 40.0 cm TL	Marine; pelagic-neritic; oceanodromous	Not Evaluated	Native	Gara and Elba

	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
13.	<i>Hemiramphus far</i>	Black-barred halfbeak	Bumishfa	Indo-West Pacific: Red Sea and East Africa, south to northern Australia and New Caledonia. Migrated to the Mediterranean Sea via the Suez Canal.	Schooling species, occur in coastal waters; generally in areas rich in vegetation and sand flats. Adults feed mainly on sea grasses, to a lesser extent on green algae and diatoms. Max length : 45.0 cm TL.	Marine; brackish; reef-associated;	Not Evaluated	Invasive/ alien	Gara and Elba
14.	<i>Coris julis</i>	Mediterranean Rainbow wrasse	Araisa	Eastern Atlantic: Sweden to Gabon. Also known from the coastal waters of the Mediterranean Sea.	Occurs in the littoral zone, near rocks and eelgrass beds. Usually found between 1-60m. solitary, among rocks, often with numerous specimens in its immediate vicinity. Max length : 30.0 cm SL.	Marine; reef-associated; depth range ? - 120 m	Least Concern (LC)	Native	Jeliana only in 2012
15.	<i>Chromis chromis</i>	Damselfish		Eastern Atlantic and Mediterranean Sea	Adults form small shoals in midwater above or near rocky reefs or above seagrass meadows (Posidonia). They feed on small planktonic or benthic animals. Spawn in summer. Max length : 25.0 cm TL.	Marine; reef-associated; non-migratory; depth range 2 - 40 m	Not Evaluated	Native	From the three sites.
16.	<i>Sparisoma cretense</i>	Parrotfish	Ghazla	Eastern Atlantic and Mediterranean Sea: more common in the eastern and southern Mediterranean coasts.	Shallow water along rocky shores. Feed on algae and small invertebrates, spawns from July to September. Max length : 50.0 cm TL	Marine; reef-associated; depth range 20 - 50 m	Least Concern (LC)	Native	From the three sites.

	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
17.	<i>Scomber scombrus</i>	Atlantic mackerel	Kawalli	North Atlantic and the Mediterranean	Forms large schools near the surface. Mainly diurnal, it feeds on zooplankton and small fish. Max length : 60.0 cm FL.	Marine; brackish; pelagic-neritic; oceanodromous; depth range ? - 1000 m	Least Concern (LC)	Native	From Gara and Jeliana
18.	<i>Boops boops</i>	Bogue	Bougah	Eastern Atlantic, Mediterranean and Black Sea.	Found on the shelf or coastal pelagic on various bottoms (sand, mud, rocks and seaweeds). Gregarious, ascending to the surface mainly at night. Omnivorous, feeding mainly on crustaceans and planktons. Max length : 36.0 cm	Marine; demersal; oceanodromous ; depth range ? - 350 m	Not Evaluated	Native	From the three sites.
19.	<i>Diplodus vulgaris</i>	Common two-banded seabream	Garagous mwashim	Eastern Atlantic, the Mediterranean and Black Sea	Euryhaline species inhabiting rocky and sometimes sandy bottoms, more commonly in less than 50 m. Juveniles may found in seagrass beds. Adults feed on crustaceans, worms and molluscs. Max length : 45.0 cm TL	Marine; benthopelagic; oceanodromous	Not Evaluated	Native	Elba and Jeliana
20.	<i>Diplodus sargus</i>	White seabream	Garagous	Eastern Atlantic: Mediterranean and Black Sea. Eastern Central Atlantic: Madeira Island	Inhabits coastal rocky reef areas and <i>Posidonia oceanica</i> beds. very active and frequents the surf zone, primarily at dawn. Feeds on shellfish and other benthic invertebrates. Max length : 45.0 cm TL	Marine; brackish; demersal; oceanodromous; depth range: ? - 50 m	Not Evaluated	Native	Elba only in 2012

	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
21.	<i>Oblada melanura</i>	Saddled seabream	Kahla	Eastern Atlantic: Bay of Biscay, the Mediterranean, and from the Strait of Gibraltar to Angola. Also known from Madeira, Cape Verde and the Canary Islands.	Gregarious, forms aggregations over rocky bottoms or seagrass beds ( <i>Zostera</i> and seaweeds). Omnivorous but feeds mainly on small invertebrates Max length : 34.0 cm TL	Marine; benthopelagic; oceanodromous; depth range? - 30 m	Not Evaluated	Native	Elba only
22.	<i>Pagellus erythrinus</i>	Common Pandora	Morjan	Eastern Atlantic: Norway and the Mediterranean to Guinea-Bissau	on inshore waters, at various bottom (rock, gravel, sand and mud). Omnivorous, but feed mainly on benthic invertebrates and small fishes Max length : 60.0 cm SL	Marine; benthopelagic; depth range ? - 300 m usually 20 - 100 m	Not Evaluated	Native	Elba only
23.	<i>Lithognathus mormyrus</i>	Sand steenbras	Mankus	Eastern Atlantic and Mediterranean, Black, and Azov seas to Cape of Good Hope, South Africa and the Mozambique Channel. Also found around the Canary Islands, Cape Verde and Madeira. Western Indian Ocean: Red Sea; southern	Found on the shelf, over sandy and muddy bottoms as well as seagrass-beds and estuaries. Gregarious, sometimes forming sizeable schools. Feeds on worms, mollusks and small crustaceans. Max	Marine; brackish; demersal; depth range ? - 150 m	Not Evaluated	Native	Jeliana only

	Species	Common name	Libyan name	Distribution	Biology	Habitat	IUCN Red list status	Status in Libya	Found at:
24.	<i>Sarpa salpa</i>	Salema	Shelba	Eastern Atlantic and the Mediterranean Sea.	Over rocky substrates and sandy area with algal growth. Gregarious, sometimes forming sizeable schools. Young mainly carnivorous on crustaceans, adults almost exclusively herbivorous, feed on seaweeds. Max length : 51.0 cm.	Marine; brackish; benthopelagic; oceanodromous; depth range 5 - 70 m	Not Evaluated	Native	Jeliana only
25.	<i>Siganus luridus</i>	Dusky spinefoot	Batata	Western Indian Ocean: Red Sea and East Africa to Islands in the western Indian Ocean. Immigrant to Mediterranean via the Suez Canal.	Small schools in very shallow water close to the bottom. Solitary adults and groups of 3 or 4 adults have also been observed. Feed on a wide range of benthic algae. Max length : 30.0 cm TL	Marine; reef-associated; depth range 2 - 40 m	Not Evaluated	Invasive/ alien	Jeliana only
26.	<i>Siganus rivulatus</i>	Marbled spinefoot	Shefshah	Western Indian Ocean: several localities in East Africa, and from the Red Sea to the eastern Mediterranean via the Suez Canal.	Inhabits shallow waters and generally in schools of 50 to several hundred individuals. Feeds by grazing on algae. Max length : 27.0 cm TL	Marine; brackish; reef-associated; depth range? - 30 m	Least concern (LC)	Invasive/ alien	Jeliana only

**Annex II: Data on ringing of Lesser Crested Tern young during the present study (2009-2012)**

<b>Jeliana Islet Colony 2009</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (days)</b>
05/07/2009	W 1196	<b>BA</b>	UP	Blue	10-15
05/07/2009	W 1197	<b>BB</b>	UP	Blue	10-15
05/07/2009	W 1198	<b>BC</b>	UP	Blue	07-10
05/07/2009	W 1199	<b>BH</b>	UP	Blue	07-10
<b>Gara island Colony 2009</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
18/07/2009	W 1200	<b>BD</b>	UP	Blue	Adult bird
18/07/2009	W 1242	<b>BJ</b>	UP	Blue	5-10
18/07/2009	W 1243	<b>BK</b>	UP	Blue	5-10
18/07/2009	W 1244	<b>BL</b>	UP	Blue	5-10
18/07/2009	W 1245	<b>BN</b>	UP	Blue	5-10
18/07/2009	W 1246	<b>BP</b>	DOWN	Blue	5-10
18/07/2009	W 1247	<b>BT</b>	UP	Blue	5-10
18/07/2009	W 1248	<b>BV</b>	UP	Blue	5-10
18/07/2009	W 1249	<b>BZ</b>	UP	Blue	5-10
18/07/2009	W 1250	<b>CA</b>	DOWN	Blue	5-10
18/07/2009	n/a	<b>CB</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CC</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CD</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CF</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CH</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CJ</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CK</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CL</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CP</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CS</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CT</b>	UP	Blue	5-10
18/07/2009	n/a	<b>CV</b>	UP	Blue	5-10
27/07/2009	W 1266	<b>CZ</b>	UP	Blue	10-15
27/07/2009	W 1267	<b>DA</b>	UP	Blue	10-15
27/07/2009	W 1269-left	<b>DC-Right</b>	UP	Blue	10-15
27/07/2009	W 1270	<b>DD</b>	UP	Blue	10-15
27/07/2009	W 1271	<b>DF</b>	UP	Blue	10-15

<b>Gara island Colony 2009</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
27/07/2009	W 1272	DH	UP	Blue	10-15
27/07/2009	W 1273	DJ	UP	Blue	10-15
27/07/2009	W 1274	DK	UP	Blue	10-15
27/07/2009	W 1275	DN	UP	Blue	10-15
27/07/2009	W 1276	DP	UP	Blue	10-15
27/07/2009	W 1277	DS	UP	Blue	10-15
27/07/2009	W 1278	DT	UP	Blue	10-15
27/07/2009	W 1279	DV	UP	Blue	10-15
27/07/2009	W 1280	DZ	UP	Blue	10-15
27/07/2009	W 1281	FA	UP	Blue	10-15
27/07/2009	W 1282	FB	UP	Blue	10-15
27/07/2009	W 1283	FC	UP	Blue	10-15
27/07/2009	W 1284	FD	UP	Blue	10-15
27/07/2009	W 1285	FF	UP	Blue	10-15
27/07/2009	W 1286	FH	UP	Blue	10-15
27/07/2009	W 1287	FJ	UP	Blue	10-15
27/07/2009	W 1288	FK	UP	Blue	10-15
27/07/2009	W 1289	FL	UP	Blue	10-15
27/07/2009	W 1290	FN	UP	Blue	10-15
27/07/2009	W 1291-Left	FP-right	UP	Blue	10-15
<b>Elba island Colony 2009</b>					
<b>Date</b>	<b>Ref Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
20/07/2009	W 1251	AA	UP	Blue	7-10
20/07/2009	W 1252	AB	UP	Blue	7-10
20/07/2009	W 1253	AC	UP	Blue	7-10
20/07/2009	W 1254	AD	UP	Blue	7-10
20/07/2009	W 1255	AF	UP	Blue	7-10
20/07/2009	W 1256	AH	UP	Blue	7-10
20/07/2009	W 1257	AJ	UP	Blue	7-10
20/07/2009	W 1258	AK	UP	Blue	7-10
20/07/2009	W 1259	AL	UP	Blue	7-10
20/07/2009	W 1260	AN	UP	Blue	7-10
20/07/2009	W 1261	AP	UP	Blue	7-10
20/07/2009	W 1262	AS	UP	Blue	7-10
20/07/2009	W 1263	AT	UP	Blue	7-10
20/07/2009	W 1264	AV	UP	Blue	7-10
20/07/2009	W 1265	AZ	UP	Blue	7-10
07/08/2009	W 1292	FV	UP	Blue	7-10
07/08/2009	W 1293	BS	UP	Blue	7-10
07/08/2009	W 1294	FZ	DOWN	Blue	7-10

Elba island Colony 2009					
Date	Ref Metal ring	Colour ring	Direction	Colour	Age (Days)
07/08/2009	W 1295	FS	UP	Blue	7-10
07/08/2009	W 1296	CC	UP	Black	7-10
07/08/2009	W 1297	KT	UP	Black	7-10
07/08/2009	W 1298	KV	UP	Black	7-10
07/08/2009	W 1299	KS	UP	Black	7-10
07/08/2009	W 1300	KK	UP	Black	7-10
07/08/2009	W 1301	KL	UP	Black	7-10
Gara island Colony 2010					
Date	Ref Metal ring	Colour ring	Direction	Colour	Age (Days)
18/07/2010	W1302	n/a	n/a	n/a	1-3
18/07/2010	W1303	n/a	n/a	n/a	1-3
18/07/2010	W1304	n/a	n/a	n/a	1-3
18/07/2010	W1305	n/a	n/a	n/a	1-3
18/07/2010	W1306	B46	UP	White	1-3
18/07/2010	W1307	n/a	n/a	n/a	1-3
18/07/2010	W1308	n/a	n/a	n/a	1-3
18/07/2010	W1309	n/a	n/a	n/a	1-3
18/07/2010	W1310	n/a	n/a	n/a	1-3
18/07/2010	W1311	n/a	n/a	n/a	1-3
18/07/2010	W1312	n/a	n/a	n/a	1-3
18/07/2010	W1313	A00	UP	White	1-3
18/07/2010	W1350	n/a	n/a	n/a	1-3
25/07/2010	W1322	n/a	n/a	n/a	4-8
25/07/2010	W1323	n/a	n/a	n/a	4-8
25/07/2010	W1324	n/a	n/a	n/a	4-8
25/07/2010	W1325	D37	DOWN	White	4-8
25/07/2010	W1326	n/a	n/a	n/a	4-8
25/07/2010	W1327	n/a	n/a	n/a	4-8
25/07/2010	W1328	C38	DOWN	White	4-8
25/07/2010	W1329	B21	UP	White	4-8
25/07/2010	W1330	C13	DOWN	White	4-8
25/07/2010	W1331	n/a	n/a	n/a	4-8
25/07/2010	W1332	B45	DOWN	White	4-8
25/07/2010	W1333	n/a	n/a	n/a	4-8
25/07/2010	W1334	n/a	n/a	n/a	4-8
25/07/2010	W1335	n/a	n/a	n/a	4-8
25/07/2010	W1336	n/a	n/a	n/a	4-8
25/07/2010	W1337	n/a	n/a	n/a	4-8
25/07/2010	W1338	n/a	n/a	n/a	4-8
25/07/2010	W1339	n/a	n/a	n/a	4-8
25/07/2010	W1340	D42	UP	White	4-8



Gara island Colony 2010					
Date	Ref Metal ring	Colour ring	Direction	Colour	Age (Days)
25/07/2010	W1341	n/a	n/a	n/a	4-8
25/07/2010	W1342	n/a	n/a	n/a	4-8
25/07/2010	W1343	n/a	n/a	n/a	4-8
25/07/2010	W1344	n/a	n/a	n/a	4-8
25/07/2010	W1345	n/a	n/a	n/a	4-8
25/07/2010	W1346	n/a	n/a	n/a	4-8
25/07/2010	W1347	n/a	n/a	n/a	4-8
04/08/2010	W0551	<b>A01</b>	UP	White	4-8
04/08/2010	W0552	<b>A02</b>	DOWN	White	4-8
04/08/2010	W0553	<b>A03</b>	UP	White	4-8
04/08/2010	W0554	<b>A04</b>	DOWN	White	4-8
04/08/2010	W0555	<b>A05</b>	UP	White	4-8
04/08/2010	W0556	<b>A06</b>	DOWN	White	4-8
04/08/2010	W0557	<b>A07</b>	UP	White	4-8
04/08/2010	W1349	<b>B01</b>	DOWN	White	4-8
04/08/2010	W1348	<b>B02</b>	DOWN	White	4-8
04/08/2010	W1351	<b>B03</b>	UP	White	4-8
04/08/2010	W1352	<b>B04</b>	DOWN	White	4-8
04/08/2010	W1353	<b>B05</b>	UP	White	4-8
04/08/2010	W1354	<b>B06</b>	DOWN	White	4-8
04/08/2010	W1355	<b>B07</b>	UP	White	4-8
04/08/2010	W1356	<b>B08</b>	DOWN	White	4-8
04/08/2010	W1357	<b>B09</b>	UP	White	4-8
04/08/2010	W1358	<b>B10</b>	UP	White	4-8
04/08/2010	W1359	<b>B11</b>	DOWN	White	4-8
04/08/2010	W1360	<b>B12</b>	UP	White	4-8
04/08/2010	W1361	<b>B13</b>	UP	White	4-8
04/08/2010	W1362	<b>B14</b>	DOWN	White	4-8
04/08/2010	W1363	<b>B15</b>	UP	White	4-8
04/08/2010	W1364	<b>B16</b>	DOWN	White	4-8
04/08/2010	W1365	<b>B17</b>	UP	White	4-8
04/08/2010	W1366	<b>B18</b>	DOWN	White	4-8
04/08/2010	W1367	<b>B19</b>	UP	White	4-8
04/08/2010	W1368	<b>B20</b>	DOWN	White	4-8
04/08/2010	W1369	<b>B22</b>	DOWN	White	4-8
04/08/2010	W1370	<b>B23</b>	UP	White	4-8
04/08/2010	W1371	<b>B24</b>	UP	White	4-8
04/08/2010	W1372	<b>B25</b>	DOWN	White	4-8
04/08/2010	W1373	<b>B26</b>	UP	White	4-8
04/08/2010	W1374	<b>B27</b>	DOWN	White	4-8
04/08/2010	W1375	<b>B28</b>	UP	White	4-8
04/08/2010	W1376	<b>B29</b>	DOWN	White	4-8

<b>Gara island Colony 2010</b>					
<b>Date</b>	<b>Ref Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
04/08/2010	W1377	<b>B30</b>	UP	White	4-8
04/08/2010	W1378	<b>B31</b>	DOWN	White	4-8
04/08/2010	W1379	<b>B32</b>	UP	White	4-8
04/08/2010	W1380	<b>B33</b>	DOWN	White	4-8
04/08/2010	W1381	<b>B34</b>	UP	White	4-8
04/08/2010	W1382	<b>B35</b>	DOWN	White	4-8
04/08/2010	W1383	<b>B36</b>	UP	White	4-8
04/08/2010	W1384	<b>B37</b>	UP	White	4-8
04/08/2010	W1385	<b>B38</b>	DOWN	White	4-8
04/08/2010	W1386	<b>B39</b>	UP	White	4-8
04/08/2010	W1387	<b>B40</b>	DOWN	White	4-8
04/08/2010	W1388	<b>B41</b>	UP	White	4-8
04/08/2010	W1389	<b>B42</b>	DOWN	White	4-8
04/08/2010	W1390	<b>B43</b>	UP	White	4-8
04/08/2010	W1391	<b>B44</b>	DOWN	White	4-8
04/08/2010	W1392	<b>B47</b>	DOWN	White	4-8
04/08/2010	W1393	<b>B48</b>	UP	White	4-8
04/08/2010	W1394	<b>B49</b>	DOWN	White	4-8
04/08/2010	W1395	<b>B50</b>	UP	White	4-8
04/08/2010	W1396	<b>C01</b>	DOWN	White	4-8
04/08/2010	W1397	<b>C02</b>	UP	White	4-8
04/08/2010	W1398	<b>C03</b>	DOWN	White	4-8
04/08/2010	W1399	<b>C04</b>	UP	White	4-8
04/08/2010	W1400	<b>C05</b>	DOWN	White	4-8
04/08/2010	W0558	<b>C06</b>	UP	White	4-8
04/08/2010	W0559	<b>C07</b>	DOWN	White	4-8
04/08/2010	W0560	<b>C08</b>	UP	White	4-8
04/08/2010	W0561	<b>C09</b>	DOWN	White	4-8
04/08/2010	W0562	<b>C10</b>	UP	White	4-8
04/08/2010	W0563	<b>C11</b>	DOWN	White	4-8
04/08/2010	W0564	<b>C12</b>	UP	White	4-8
04/08/2010	W0565	<b>C14</b>	DOWN	White	4-8
04/08/2010	W0566	<b>C15</b>	UP	White	4-8
04/08/2010	W0567	<b>C16</b>	DOWN	White	4-8
04/08/2010	W0568	<b>C17</b>	UP	White	4-8
04/08/2010	W0569	<b>C18</b>	DOWN	White	4-8
04/08/2010	W0570	<b>C19</b>	UP	White	4-8
04/08/2010	W0571	<b>C20</b>	DOWN	White	4-8
04/08/2010	W0572	<b>C21</b>	UP	White	4-8
04/08/2010	W0573	<b>C22</b>	UP	White	4-8
04/08/2010	W0574	<b>C23</b>	DOWN	White	4-8
04/08/2010	W0575	<b>C24</b>	UP	White	4-8
04/08/2010	W0576	<b>C25</b>	DOWN	White	4-8

<b>Gara island Colony 2010</b>					
<b>Date</b>	<b>Ref Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
04/08/2010	W0577	<b>C26</b>	UP	White	4-8
04/08/2010	W0578	<b>C27</b>	DOWN	White	4-8
04/08/2010	W0579	<b>C28</b>	UP	White	4-8
04/08/2010	W0580	<b>C29</b>	DOWN	White	4-8
04/08/2010	W0581	<b>C30</b>	DOWN	White	4-8
04/08/2010	W0582	<b>C31</b>	UP	White	4-8
04/08/2010	W0583	<b>C32</b>	DOWN	White	4-8
04/08/2010	W0584	<b>C33</b>	UP	White	4-8
04/08/2010	W0585	<b>C34</b>	DOWN	White	4-8
04/08/2010	W0586	<b>C35</b>	DOWN	White	4-8
04/08/2010	W0587	<b>C36</b>	UP	White	4-8
04/08/2010	W0588	<b>C37</b>	UP	White	4-8
04/08/2010	W0589	<b>C39</b>	UP	White	4-8
04/08/2010	W0590	<b>C40</b>	DOWN	White	4-8
04/08/2010	W0591	<b>C41</b>	UP	White	4-8
04/08/2010	W0592	<b>C42</b>	DOWN	White	4-8
04/08/2010	W0593	<b>C43</b>	UP	White	4-8
04/08/2010	W0594	<b>C44</b>	DOWN	White	4-8
04/08/2010	W0595	<b>C45</b>	UP	White	4-8
04/08/2010	W0596	<b>C46</b>	DOWN	White	4-8
04/08/2010	W0597	<b>C47</b>	UP	White	4-8
04/08/2010	W0598	<b>C48</b>	DOWN	White	4-8
04/08/2010	W0599	<b>C49</b>	UP	White	4-8
04/08/2010	W0600	<b>C50</b>	DOWN	White	4-8
04/08/2010	W0651	<b>C51</b>	DOWN	White	4-8
04/08/2010	W0652	<b>C52</b>	DOWN	White	4-8
04/08/2010	W0653	<b>C53</b>	UP	White	4-8
04/08/2010	W0654	<b>C54</b>	DOWN	White	4-8
04/08/2010	W0655	<b>C55</b>	DOWN	White	4-8
04/08/2010	W0501	<b>B51</b>	DOWN	White	4-8
04/08/2010	W0502	<b>B52</b>	UP	White	4-8
04/08/2010	W0503	<b>B53</b>	DOWN	White	4-8
04/08/2010	W0504	<b>B54</b>	UP	White	4-8
04/08/2010	W0505	<b>B55</b>	DOWN	White	4-8
04/08/2010	W0506	<b>B56</b>	UP	White	4-8
04/08/2010	W0507	<b>B57</b>	DOWN	White	4-8
04/08/2010	W0508	<b>B58</b>	UP	White	4-8
04/08/2010	W0509	<b>B59</b>	DOWN	White	4-8
04/08/2010	W0510	<b>B60</b>	UP	White	4-8
04/08/2010	W0511	<b>B61</b>	DOWN	White	4-8
04/08/2010	W0512	<b>B62</b>	UP	White	4-8
04/08/2010	W0513	<b>B63</b>	DOWN	White	4-8
04/08/2010	W0514	<b>B64</b>	UP	White	4-8

Gara island Colony 2010					
Date	Ref Metal ring	Colour ring	Direction	Colour	Age (Days)
04/08/2010	W0515	<b>B65</b>	DOWN	White	4-8
04/08/2010	W0516	<b>B66</b>	UP	White	4-8
04/08/2010	W0517	<b>B67</b>	DOWN	White	4-8
04/08/2010	W0518	<b>B68</b>	UP	White	4-8
04/08/2010	W0519	<b>B69</b>	DOWN	White	4-8
04/08/2010	W0520	<b>B70</b>	UP	White	4-8
04/08/2010	W0521	<b>B71</b>	DOWN	White	4-8
04/08/2010	W0522	<b>B72</b>	UP	White	4-8
04/08/2010	W0523	<b>B73</b>	DOWN	White	4-8
04/08/2010	W0524	<b>B74</b>	UP	White	4-8
04/08/2010	W0525	<b>B75</b>	DOWN	White	4-8
04/08/2010	W0526	<b>B76</b>	UP	White	4-8
04/08/2010	W0527	<b>B77</b>	DOWN	White	4-8
04/08/2010	W0528	<b>B78</b>	UP	White	4-8
04/08/2010	W0529	<b>B79</b>	DOWN	White	4-8
04/08/2010	W0530	<b>B80</b>	UP	White	4-8
04/08/2010	W0531	<b>B81</b>	DOWN	White	4-8
04/08/2010	W0532	<b>B82</b>	UP	White	4-8
04/08/2010	W0533	<b>B83</b>	DOWN	White	4-8
04/08/2010	W0534	<b>B84</b>	UP	White	4-8
04/08/2010	W0535	<b>B85</b>	DOWN	White	4-8
04/08/2010	W0536	<b>B86</b>	UP	White	4-8
04/08/2010	W0537	<b>B87</b>	DOWN	White	4-8
04/08/2010	W0538	<b>B88</b>	UP	White	4-8
04/08/2010	W0539	<b>B89</b>	UP	White	4-8
04/08/2010	W0540	<b>B90</b>	DOWN	White	4-8
04/08/2010	W0541	<b>B91</b>	DOWN	White	4-8
04/08/2010	W0542	<b>B92</b>	UP	White	4-8
04/08/2010	W0543	<b>B93</b>	DOWN	White	4-8
04/08/2010	W0544	<b>B94</b>	UP	White	4-8
04/08/2010	W0545	<b>B95</b>	DOWN	White	4-8
04/08/2010	W0546	<b>B96</b>	UP	White	4-8
04/08/2010	W0547	<b>B97</b>	DOWN	White	4-8
04/08/2010	W0548	<b>B98</b>	UP	White	4-8
04/08/2010	W0549	<b>B99</b>	DOWN	White	4-8
04/08/2010	W0550	<b>B00</b>	UP	White	4-8
04/08/2010	W0601	<b>D01</b>	DOWN	White	4-8
04/08/2010	W0602	<b>D02</b>	UP	White	4-8
04/08/2010	W0603	<b>D03</b>	DOWN	White	4-8
04/08/2010	W0604	<b>D04</b>	UP	White	4-8
04/08/2010	W0605	<b>D05</b>	DOWN	White	4-8
04/08/2010	W0606	<b>D06</b>	UP	White	4-8
04/08/2010	W0607	<b>D07</b>	DOWN	White	4-8

<b>Gara island Colony 2010</b>					
<b>Date</b>	<b>Ref Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
04/08/2010	W0608	<b>D08</b>	DOWN	White	4-8
04/08/2010	W0609	<b>D09</b>	UP	White	4-8
04/08/2010	W0610	<b>D10</b>	DOWN	White	4-8
04/08/2010	W0611	<b>D11</b>	UP	White	4-8
04/08/2010	W0612	<b>D12</b>	DOWN	White	4-8
04/08/2010	W0613	<b>D13</b>	UP	White	4-8
04/08/2010	W0614	<b>D14</b>	DOWN	White	4-8
04/08/2010	W0615	<b>D15</b>	UP	White	4-8
04/08/2010	W0616	<b>D16</b>	DOWN	White	4-8
04/08/2010	W0617	<b>D17</b>	UP	White	4-8
04/08/2010	W0618	<b>D18</b>	DOWN	White	4-8
04/08/2010	W0619	<b>D19</b>	UP	White	4-8
04/08/2010	W0620	<b>D20</b>	DOWN	White	4-8
04/08/2010	W0621	<b>D21</b>	UP	White	4-8
04/08/2010	W0622	<b>D22</b>	DOWN	White	4-8
04/08/2010	W0623	<b>D23</b>	UP	White	4-8
04/08/2010	W0624	<b>D24</b>	DOWN	White	4-8
04/08/2010	W0625	<b>D25</b>	UP	White	4-8
04/08/2010	W0626	<b>D26</b>	DOWN	White	4-8
04/08/2010	W0627	<b>D27</b>	UP	White	4-8
04/08/2010	W0628	<b>D28</b>	DOWN	White	4-8
04/08/2010	W0629	<b>D29</b>	UP	White	4-8
04/08/2010	W0630	<b>D30</b>	DOWN	White	4-8
04/08/2010	W0631	<b>D31</b>	UP	White	4-8
04/08/2010	W0632	<b>D32</b>	DOWN	White	4-8
04/08/2010	W0633	<b>D33</b>	DOWN	White	4-8
04/08/2010	W0634	<b>D34</b>	UP	White	4-8
04/08/2010	W0635	<b>D35</b>	DOWN	White	4-8
04/08/2010	W0636	<b>D36</b>	UP	White	4-8
04/08/2010	W0637	<b>D38</b>	UP	White	4-8
04/08/2010	W0638	<b>D39</b>	DOWN	White	4-8
04/08/2010	W0639	<b>D40</b>	UP	White	4-8
04/08/2010	W0640	<b>D41</b>	DOWN	White	4-8
04/08/2010	W0641	<b>D43</b>	DOWN	White	4-8
04/08/2010	W0642	<b>D44</b>	UP	White	4-8
04/08/2010	W0643	<b>D45</b>	UP	White	4-8
04/08/2010	W0644	<b>D46</b>	DOWN	White	4-8
04/08/2010	W0645	<b>D47</b>	UP	White	4-8
04/08/2010	W0646	<b>D48</b>	DOWN	White	4-8
04/08/2010	W0647	<b>D49</b>	UP	White	4-8
04/08/2010	W0648	<b>D50</b>	DOWN	White	4-8
04/08/2010	W0649	<b>A08</b>	UP	White	4-8
04/08/2010	W0650	<b>A09</b>	DOWN	White	4-8

<b>Gara island Colony 2010</b>					
<b>Date</b>	<b>Ref Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
04/08/2010	W0651	A10	UP	White	4-8

<b>Elba island Colony 2010</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
20/07/2010	W1314	A14	UP	White	5-7
20/07/2010	W1315	A15	UP	White	5-7
20/07/2010	W1316	A16	UP	White	5-7
20/07/2010	W1317	A17	DOWN	White	5-7
20/07/2010	W1318	A18	UP	White	5-7
20/07/2010	W1319	A19	UP	White	5-7
20/07/2010	W1320	A21	DOWN	White	5-7
20/07/2010	W1321	n/a	n/a	n/a	5-7

<b>Elba island Colony 2012</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
16/07/2012	W1557	C73	UP	White	5-7
16/07/2012	W1559	C74	UP	White	5-7
16/07/2012	W1558	C75	UP	White	5-7
16/07/2012	W1566	C76	UP	White	5-7
16/07/2012	W1652	C77	UP	White	5-7
16/07/2012	W1565	C78	DOWN	White	5-7
16/07/2012	W1555	C79	DOWN	White	5-7
16/07/2012	W1564	C80	UP	White	5-7
16/07/2012	W1560	C81	UP	White	5-7
16/07/2012	W1563	C82	UP	White	5-7
16/07/2012	W1561	C83	UP	White	5-7
16/07/2012	W1556	C84	UP	White	5-7
16/07/2012	non	C85	UP	White	5-7

<b>Jeliana islet Colony 2012</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
10/07/2012	W1401	A11	UP	White	8-10
10/07/2012	W1402	A12	UP	White	8-10
10/07/2012	W1403	A13	UP	White	8-10
10/07/2012	W1404	A22	UP	White	8-10
10/07/2012	W1405	A23	UP	White	8-10
10/07/2012	W1406	A24	UP	White	8-10
10/07/2012	W1407	A25	UP	White	8-10
10/07/2012	W1408	A26	UP	White	8-10
10/07/2012	W1409	A27	UP	White	8-10
10/07/2012	W1410	A28	UP	White	8-10
10/07/2012	W1411	A29	UP	White	8-10
10/07/2012	W1412	A30	UP	White	8-10
10/07/2012	W1413	A31	UP	White	8-10
10/07/2012	W1414	A32	UP	White	8-10
10/07/2012	W1415	A33	UP	White	8-10
10/07/2012	W1416	A34	UP	White	8-10
10/07/2012	W1417	A35	UP	White	8-10
10/07/2012	W1418	A36	UP	White	8-10
10/07/2012	W1419	A37	UP	White	8-10
10/07/2012	W1420	A38	UP	White	8-10
10/07/2012	W1421	A39	UP	White	8-10
10/07/2012	W1422	A40	UP	White	8-10
10/07/2012	W1423	A41	UP	White	8-10
10/07/2012	W1424	A42	UP	White	8-10
10/07/2012	W1425	A43	UP	White	8-10
10/07/2012	W1426	A44	UP	White	8-10
10/07/2012	W1427	A45	UP	White	8-10
10/07/2012	W1428	A46	UP	White	8-10
10/07/2012	W1429	A47	UP	White	8-10
10/07/2012	W1430	A48	UP	White	8-10
10/07/2012	W1431	A49	UP	White	8-10
10/07/2012	W1432	A50	UP	White	8-10
10/07/2012	W1433	A51	UP	White	8-10
10/07/2012	W1434	A52	UP	White	8-10
10/07/2012	W1435	A53	UP	White	8-10
10/07/2012	W1436	A54	UP	White	8-10
10/07/2012	W1437	A55	UP	White	8-10
10/07/2012	W1438	A56	UP	White	8-10
10/07/2012	W1439	A57	UP	White	8-10
10/07/2012	W1440	A58	UP	White	8-10
10/07/2012	W1441	A59	UP	White	8-10
10/07/2012	W1442	A60	UP	White	8-10

<b>Jeliana islet Colony 2012</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
10/07/2012	W1443	A61	UP	White	8-10
10/07/2012	W1444	A62	UP	White	8-10
10/07/2012	W1445	A63	UP	White	8-10
10/07/2012	W1446	A64	UP	White	8-10
10/07/2012	W1447	A65	UP	White	8-10
10/07/2012	W1448	A66	UP	White	8-10
10/07/2012	W1449	A67	UP	White	8-10
10/07/2012	W1450	A68	UP	White	8-10
10/07/2012	W0657	A69	UP	White	8-10
10/07/2012	W0658	A70	UP	White	8-10
10/07/2012	W0659	A71	UP	White	8-10
10/07/2012	W0660	A72	UP	White	8-10
10/07/2012	W0661	A73	DOWN	White	8-10
10/07/2012	W0662	A74	DOWN	White	8-10
10/07/2012	W0663	A75	DOWN	White	8-10
10/07/2012	W0664	A76	DOWN	White	8-10
10/07/2012	W0665	A77	DOWN	White	8-10
10/07/2012	W0666	A78	DOWN	White	8-10
10/07/2012	W0667	A79	DOWN	White	8-10
10/07/2012	W0668	A80	DOWN	White	8-10
10/07/2012	W0669	A81	DOWN	White	8-10
10/07/2012	W0670	A82	UP	White	8-10
10/07/2012	W0671	A83	UP	White	8-10
10/07/2012	W0672	A84	UP	White	8-10
10/07/2012	W0673	A85	UP	White	8-10
10/07/2012	W0674	A86	UP	White	8-10
10/07/2012	W0675	A87	UP	White	8-10
10/07/2012	W0676	A88	UP	White	8-10
10/07/2012	W0677	A89	UP	White	8-10
10/07/2012	W0678	A90	UP	White	8-10
10/07/2012	W0679	A91	UP	White	8-10
10/07/2012	W0680	A92	UP	White	8-10
10/07/2012	W0681	A93	UP	White	8-10
10/07/2012	W0682	A94	UP	White	8-10
10/07/2012	W0683	A95	UP	White	8-10
10/07/2012	W0684	A96	UP	White	8-10
10/07/2012	W0685	A97	UP	White	8-10
10/07/2012	W0686	A98	UP	White	8-10
10/07/2012	W0687	A99	UP	White	8-10
10/07/2012	W0688	C56	UP	White	8-10
10/07/2012	W0689	C57	UP	White	8-10
10/07/2012	W0690	C58	UP	White	8-10
10/07/2012	W0691	C59	UP	White	8-10



<b>Jeliana islet Colony 2012</b>					
<b>Date</b>	<b>Metal ring</b>	<b>Colour ring</b>	<b>Direction</b>	<b>Colour</b>	<b>Age (Days)</b>
10/07/2012	W0692	<b>C60</b>	UP	White	8-10
10/07/2012	W0693	<b>C61</b>	UP	White	8-10
10/07/2012	W0694	<b>C62</b>	DOWN	White	8-10
10/07/2012	W0695	<b>C63</b>	UP	White	8-10
10/07/2012	W0696	<b>C64</b>	UP	White	8-10
10/07/2012	W0697	<b>C65</b>	UP	White	8-10
10/07/2012	W0698	<b>C66</b>	DOWN	White	8-10
10/07/2012	W0699	<b>C67</b>	DOWN	White	8-10
10/07/2012	W0700	<b>C68</b>	DOWN	White	8-10

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