Estuarine, Coastal and Shelf Science 92 (2011) 188–194

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta

Javier Cotin [a,*], Manuel García-Tarrasón [a], Carolina Sanpera [a], Lluis Jover [b], Xavier Ruiz [1]

[a] Department Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain
[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain

A R T I C L E I N F O

Article history:
Received 9 June 2010
Accepted 26 December 2010
Available online 11 January 2011

Keywords:
aquatic birds
trophic ecology
stable isotopes
biomonitoring
Mediterranean

A B S T R A C T

The Ebro catchment, the largest river basin in Spain, includes various heavily industrialized areas. Among these is the Flix site, where a chemical plant has been operating since the beginning of the 20th century. This extended operational period, together with the construction of a dam next to the factory around 1960, resulted in the accumulation of large amounts of heavily polluted sediments in the adjacent riverbed, many of which are contaminated by mercury. Pollutants from Flix are carried downstream by the Ebro River to its delta. In order to assess the transfer of mercury to the complex river estuary ecosystem, we studied the ecology of the tern community living there as these birds segregate into a range of habitats. For this purpose, first we used stable isotope analysis (SIA) ($\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$) of eggs to determine the trophic ecology and habitat partitioning of several tern species (Common, Sandwich, Little, Gull-Billed and Whiskered Tern) breeding sympatrically, in order to link their foraging ecology with habitat types. Next we measured mercury concentrations in eggs to monitor the input of this metal into the diverse habitats. With the exception of the Little Tern, the other terns used a restricted habitat range in the Ebro Delta, as shown by C and S isotopes; the Gull-Billed and Whiskered Tern foraged in freshwater habitats, while the Common and Sandwich Tern used marine habitats. This restricted feeding behavior of the Gull-Billed and Common Tern contrasts with previous reports in other breeding sites. The Little Tern, which showed a wide range of isotopic values, was found to be an opportunistic forager but fed mainly in saltpans, a feeding habitat not reported previously for this species in this area. We found that mercury concentrations are related to foraging habitat and diet, and are unexpectedly higher in species feeding on demersal prey in marine habitats and also higher in birds feeding in saltpans than in those feeding in freshwater habitats. The mercury concentrations found in the Little and Common Tern eggs sampled in "Punta de la Banya" may be sufficiently high to endanger breeding success.

1. Introduction

The Ebro catchment is the largest river basin in Spain. Covering an area of 85,362 km^2, this catchment receives the potential influence of 3 million people and also contains some heavily industrialized areas. One of these areas is the Flix site, where a chemical industry has been in operation since the beginning of the 20th century. This long operational period, together with the construction of a dam next to the plant around 1960, resulted in the accumulation of high amounts of heavily polluted sediments in the adjacent riverbed (Fernandez et al., 1999). Recently, a project to remove the polluted sediments has started; however, this initiative implies a high risk of dispersal. Pollutants originated at the Flix site are carried downstream by the Ebro River to its delta 90 km away (Llorente et al., 1987; Pastor et al., 2004), especially during floods.

Previous studies in the Flix reservoir have reported the effects of pollutants on birds at this site and also in the Ebro Delta (Quiros et al., 2008; Barata et al., 2010) an area also affected by intensive agricultural activity, which has a significant impact on wildlife (Mañosa et al., 2001). Mercury is a special source of concern, as it mainly enters organisms through diet (Burger et al., 1992). The Ebro Delta (NE Spain: 40°37′N, 00°35′E) is one of the largest wetlands in the Western Mediterranean region and is home to extensive bird colonies. Occupying an area of 320 km^2, 75% of the delta surface is devoted to rice fields (c.a. 20,000 ha) and farmland, 20% to natural preserved areas and the remaining 5% to urban uses. This wetland presents a wide variety of habitats. The inner part of the delta is dominated by rice fields and farmland, which are connected to the river through channels, while the coastline is characterized by abandoned fields, lagoons, salt marshes and beaches. On each side

* Corresponding author.
E-mail address: jcotin@ub.edu (J. Cotin).
[1] Deceased 27th April 2008.

0272-7714/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ecss.2010.12.024

of the Ebro Delta and surrounded by sea there are two arrow-shaped sandy areas, known as the 'Punta del Fangar' and 'Punta de la Banya' (with an extension of 500 and 2500 ha respectively), the latter hosting a saltpan industry, which occupies over 1000 ha. Finally, the open sea and the northern and southern bays, molded by the two 'Puntas', conform the patchwork of habitats. The coastal lagoons, although connected to the sea and thus expected to hold brackish water, receive considerable freshwater input from the rice fields from spring to autumn, thereby lowering their salinity, which almost reaches that of freshwater during those seasons.

In order to study input of mercury from the Flix reservoir into the diverse habitats of the Ebro Delta, it is necessary to examine suitable target bioindicator species that forage in all the habitats present, as species used by Quiros et al. (2008) and Barata et al. (2010) (family Ardeidae) have a narrower range of foraging habitats.

Terns are gull-related seabirds. They comprise 45 species grouped in 12 genera (Bridge et al., 2005) which are found worldwide; most of these are migratory birds and they have been widely used as bioindicators of pollution (Becker et al., 1993b; Nisbet et al., 2002; Guitart et al., 2003; Tavares et al., 2009). The following five tern species breed in the Ebro Delta: Common Tern (*Sterna hirundo*, Linnaeus, 1758), Sandwich Tern (*Sterna sandvicensis*, Latham, 1787), Little Tern (*Sternula albifrons*, Pallas, 1764), Gull-Billed Tern (*Gelochelidon nilotica*, Gmelin, 1789) and Whiskered Tern (*Chlydonias hybrida,* Pallas, 1811). A wide variety of foraging habitats has been reported for these species: Freshwater habitats for the Whiskered Tern (Latraube et al., 2005; Paillisson et al., 2007); marine for the Sandwich Tern (Stienen et al., 2000) and both marine and freshwater for the Common Tern (Becker et al., 1997; Neubauer, 1998). The last two species have been described as generalist foragers. The Little Tern uses several foraging habitats in Portugal (Paiva et al., 2008a), and in the Ebro Delta it has been observed foraging in channels, lagoons and lagoon mouths (Bertolero et al., 2005). The Gull-Billed Tern feeds in terrestrial (Cabo and Sanchez, 1986), freshwater (Dies et al., 2005), and even marine habitats (Stienen et al., 2008). Given their feeding strategies, these five tern species have the capacity to forage over the whole of the Ebro Delta. This trait thus makes them suitable candidates as bioindicators of mercury for the whole area.

These five Tern species have large colonies in the Ebro Delta (c.a. 350 pairs of Little Tern, 5000 of Common Tern, 500 of Gull-Billed Tern, 2000 of Sandwich Tern and 1200 of Whiskered Tern), thus making this wetland one of the tern hotspots in Spain. These species have recently increased their breeding populations in the delta, except the Little Tern, which has decreased about 50% (Oro et al., 2004). Although all these species are classified as of "Least Concern" by Birdlife, terns face several threats, such as habitat loss, human disturbance, contamination, and predation by introduced species (feral cats, rats). Furthermore, in the Ebro Delta, tern populations have suffered fluctuations (Ferrer and Martinez-Vilalta, 1986), these normally related to human disturbance of their breeding sites and foraging areas.

Mercury biomagnifies along trophic webs, but its bioavailability can vary among habitats. Therefore to assess mercury input to terns, a full understanding of their foraging ecology in the area is needed (Nisbet et al., 2002; Sanpera et al., 2007b; Tavares et al., 2007; Catry et al., 2008). Stable isotope signatures of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) have been extensively used in studies of seabird communities, focusing on their trophic ecology and relationships, to evaluate the relative importance of dietary sources to consumers (Hobson et al., 1994; Forero and Hobson, 2003; Catry et al., 2008; Cherel et al., 2008; Koiadinovic et al., 2008). $\delta^{15}N$ reflects the trophic level, with consumer signatures being higher than in their prey (Forero et al., 2005). This parameter therefore allows interpretation of trophic positions and food webs. Information about the source of carbon entering a food web can be obtained from $\delta^{13}C$ (Hobson, 1999), as there is a negative gradient between terrestrial – freshwater – marine habitats, and inshore/benthic-linked food webs are more enriched in ^{13}C than pelagic/offshore ones (Michener and Schell, 1994). Isotopic sulphur signature ($\delta^{34}S$) is useful for determining the food sources of consumers (marine vs. terrestrial), because the essential sulphur-bearing compounds are incorporated into tissues without significant fractionation (Connolly et al., 2004).

The present study was performed on eggs. Terns are income breeders (Hobson, 2006); under this assumption most materials used in clutch formation are obtained directly from diet (Ruiz et al., 1998; Hobson et al., 2000), thus egg signatures reflect mainly the diet obtained in the neighboring area during this period of time. Moreover, tern eggs also reflect male foraging habitat as males provide females with prey during clutch formation (Nisbet, 1973; Wiggins and Morris, 1986; Gonzalez-Solis et al., 2001).

Here we assessed the potential exposure of an aquatic bird community that inhabits the Ebro Delta to mercury from the Flix reservoir. For this purpose, five tern species were used as bioindicators and the relationship between habitat use and mercury concentrations in clutches was examined. The first objective was to determine the trophic ecology and habitat partitioning of these species, in order to link their foraging ecology with certain habitats and compare them with the literature. The second objective was to measure mercury concentrations in tern eggs in order to establish whether values merit conservational concern. We tested the following main hypotheses; mercury concentrations are higher in terns feeding in freshwater habitats (which receive input from the Flix reservoir) than those feeding in marine and especially terrestrial habitats (as mercury bioavailability is higher in marine than terrestrial habitats). The results may be useful to define conservation policies to be applied in this area or other wetlands with similar tern communities (Fasola and Bogliani, 1990; Goutner, 1990; Peste et al., 2004; Paiva et al., 2008a). This is of particular relevance in the Ebro Delta as the project that is being undertaken to remove polluted sediments may alter the rate of sediments carried downstream.

2. Material and methods

2.1. Sampling

Egg sampling was conducted during the breeding seasons of 2006 and 2008 at several tern colonies located in the Ebro Delta (see Fig. 1). The Whiskered Tern ($n = 11$) colony sampled was located in 'La Tancada' lagoon. Little Terns ($n = 24$), Sandwich Terns ($n = 20$) and Common Terns ($n = 10$) samples were collected at 'Punta de la Banya' while Gull-Billed Terns ($n = 20$) and the other Common Tern colonies ($n = 19$) were sampled at 'Punta del Fangar'. The two Common Tern colonies were 23 km apart (36 km following the coastal line) (Fig. 1). To avoid pseudo-replication, only one egg was collected per nest. To avoid population impacts, only nests with 3 eggs were sampled for the Gull-Billed, Common, Whiskered and Little Tern, and with 2 eggs in the case of the Sandwich Tern. Eggs were collected following the protocol approved by the Ebro Delta Natural Park and with the permission of the *Serveis de Fauna i Pesca*, *Generalitat de Catalunya* (Spain).

Eggs were labeled and kept refrigerated until reaching the laboratory where they were kept frozen (−20 °C) until analysis. Egg content was then separated from the egg shell, weighed and placed into a glass container for freeze-drying. Freeze-dried samples were homogenized and a sub-sample was lipid-extracted for stable isotope analysis using methanol and chloroform, following Folch's method (Folch et al., 1957).

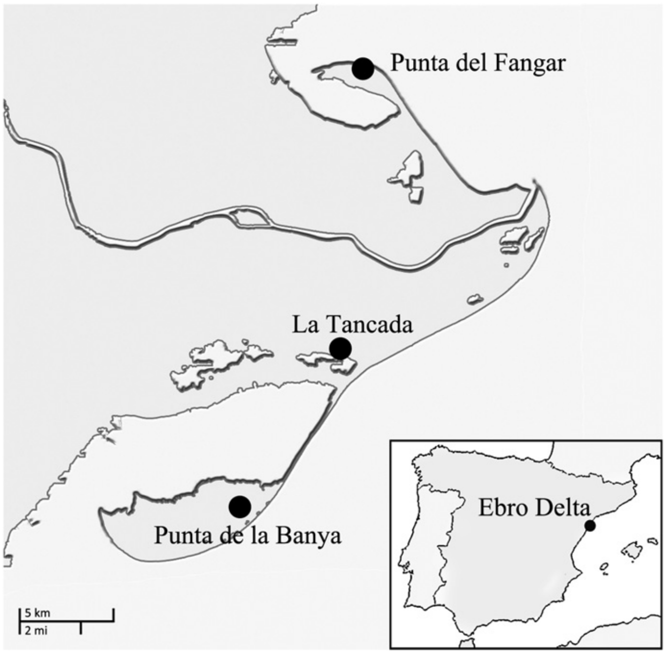

Fig. 1. Map showing the sampling sites.

2.2. Stable isotope analysis (SIA)

Lean sub-samples (ca. 0.36 mg for $\delta^{13}C$ and $\delta^{15}N$; 3.6 mg for $\delta^{34}S$) of the homogenized eggs were placed in tin buckets and crimped for combustion. Isotopic analyses were carried out by EA-IRMS (elemental analysis-isotope ratio mass spectrometry) by means of a Thermo Finnigan Flash 1112 (for C and N)/1108 (for S) elemental analyzer coupled to a Delta isotope ratio mass spectrometer via a CONFLO III interface.

Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) following the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000,$$

where X is ^{15}N or ^{13}C or ^{34}S and R is the corresponding $^{15}N/^{14}N$, $^{13}C/^{12}C$ or $^{34}S/^{32}S$ ratio. The standards for ^{15}N, ^{13}C and ^{34}S were atmospheric nitrogen (AIR), Pee Dee Belemnite (PBD) and Canyon Diablo Troilite (CDT), respectively. Precision and accuracy for $\delta^{13}C$ measurements was ≤0.1‰, ≤0–0.3‰ for $\delta^{15}N$ and ≤0.4 for $\delta^{34}S$. The laboratory applies international standards, which are generally run for every 12 samples: IAEA CH_7 (87% of C), IAEA CH_6 (42% of C) and USGS 24 (100% of C) for ^{13}C; IAEA N1 and IAEA N2 (with 21% of N) and IAEA NO_3 (13.8% of N) for ^{15}N; and IAEA-S1, IAEA-S2 and IAEA-S3 for ^{34}S.

2.3. Mercury

Mercury concentrations were measured on an ICP-MS Perkin Elmer ELAN 6000. Before the measurement, freeze-dried samples were digested in H_2NO_3 (3 ml) and H_2O_2 (2 ml) in Savilles Teflon digestion vessels for 12 h at 100 °C. Accuracy of analysis was checked by measuring certified reference material (Lobster hepatopancreas Tort-2 and Dogfish liver Dolt-3; National Research Council Canada). Mean recoveries ranged 98–100% for total mercury and no corrections were made. Mercury concentrations were expressed on a dry weight basis (ng g^{-1} dw).

Mercury and SIAs were performed at the *Serveis Científico-Tècnics (Universitat de Barcelona)*

2.4. Statistical methods

Mercury concentration and stable isotope ratio data were routinely checked for normal distribution. The former showed clear skewed distributions, which were normalized by applying a logarithmic transformation. The isotopic values of one Common Tern egg from 'Punta del Fangar' were considered extreme ($\delta^{15}N = 13.8$, $\delta^{13}C = -23.54$ and $\delta^{34}S = 4.38$) and were excluded from further statistical analyses and tables.

Species were compared using a one-way analysis of variance and applying the Levene test to check for homoscedasticity. The Welch correction was used accordingly. To test for "a posteriori" pairwise differences, we used SNK or Tamhane tests. Descriptive statistics and mean difference between groups and their 95% confidence intervals were used to show the results. Pearson's correlation coefficient was used to explore relationships between stable isotopes and mercury, applying the Bonferroni correction only for the whole group of species in order to maintain overall $\alpha = 0.05$. Statistical analysis was carried out using SPSS 15.0.

3. Results

Little and Whiskered Terns presented the highest variability in stable isotopes and mercury concentrations (Table 1). The comparison of the six tern colonies showed significant differences in all the stable isotopes ($\delta^{13}C$: $F_{Welch\ 5,37.2} = 793$, $p < 0.001$; $\delta^{34}S$: $F_{Welch\ 5,36.7} = 2613$, $p < 0.001$; $\delta^{15}N$: $F_{Welch\ 5,37.2} = 117$, $p < 0.001$). With regard to carbon signatures, the pairwise test showed four groups. Higher $\delta^{13}C$ values corresponded to the Little Tern, followed by the Common Tern "Fangar". The Common Tern "Banya" and the Sandwich Tern formed a third group, with significantly

Table 1
Descriptive statistics of stable isotope signatures and mercury in eggs from the five tern species included in the study (*Sternula albifrons, Gelochelidon nilotica, Chlidonias niger, Sterna sandvicensis* and *Sterna hirundo*).

		N	Mean	SD	Min	Max
$\delta^{15}N$ (‰)	Little Tern	24	14.6	0.9	12.6	16.4
	Gull-billed Tern	20	13.4	0.7	12.6	14.8
	Whiskered Tern	11	12.6	0.9	11.5	14.9
	Sandwhich Tern	20	12.1	0.3	11.3	12.7
	Common Tern 'Fangar'	18	10.9	0.3	10.2	11.4
	Common Tern 'Banya'	10	13.3	0.5	12.6	14.0
$\delta^{13}C$ (‰)	Little Tern	24	−14.6	2.1	−18.3	−11.8
	Gull-billed Tern	20	−24.8	0.5	−25.5	−23.4
	Whiskered Tern	11	−24.6	0.7	−26.2	−23.6
	Sandwhich Tern	20	−18.0	0.2	−18.3	−17.7
	Common Tern 'Fangar'	18	−17.5	0.2	−17.9	−17.1
	Common Tern 'Banya'	10	−17.9	0.2	−18.2	−17.6
$\delta^{34}S$ (‰)	Little Tern	24	9.4	3.3	4.3	15.9
	Gull-billed Tern	20	1.6	0.6	0.5	2.5
	Whiskered Tern	11	3.4	1.5	1.2	5.5
	Sandwhich Tern	20	18.5	0.3	17.8	18.8
	Common Tern 'Fangar'	18	17.0	0.6	16.0	17.8
	Common Tern 'Banya'	10	19.0	0.4	18.4	19.6
[Hg] ppb	Little Tern	24	4150.1	1973.7	1375.7	8621.9
	Gull-billed Tern	20	1770.1	699.9	879.2	3686.6
	Whiskered Tern	11	2576.6	1319.9	1119.6	5591.5
	Sandwhich Tern	20	2727.2	1264.3	1030.7	4999.8
	Common Tern 'Fangar'	18	1139.6	264.3	588.1	1663.8
	Common Tern 'Banya'	10	4148.9	1940.9	1138.5	7631.7

lower values, although these were very close to those reported for the Common Tern "Fangar". The Whiskered and Gull-Billed Terns showed lower δ^{13}C values. The mean difference between the two Common Tern colonies was quite small (0.42‰) (Fig. 2). Sulphur signatures showed almost the same groups outlined with carbon signatures but in a different sorting order. The Common Tern "Banya" and the Sandwich Tern showed the highest. The Common Tern "Fangar" showed a mean value close to that of the first group but significantly lower. The Little Tern showed intermediate values while the lowest δ^{34}S signatures corresponded to Whiskered and Gull-Billed Terns, which were similar but significantly different (Fig. 2). With reference to nitrogen, pairwise tests showed significant differences, again delineating four groups. The highest δ^{15}N values corresponded to the Little Tern, followed by the Gull-Billed Tern and the Common Tern "Banya" with values around 13.3‰. Significantly lower signatures were observed for the Sandwich Tern and the lowest δ^{15}N corresponded to the Common Tern "Fangar". The Whiskered Tern showed intermediate values with a relative high variability, and its mean value did not differ from those of the Sandwich Tern, Gull-Billed Tern or Common Tern "Banya" (Fig. 2).

Mean mercury concentrations were higher for the Little and Common Tern "Banya" (Table 1). Significant differences were found in mercury concentrations between the six tern colonies ($F_{5,97} = 19.841$, $p < 0.001$). We grouped terns in four categories (from higher to lower Hg concentrations): the Little Tern and Common Tern "Banya", the Sandwich Tern and Whiskered Tern, the Gull-Billed Tern, and finally the Common Tern "Fangar" (Fig. 3).

No significant linear relationships were found among the three stable isotopes analyzed when the Bonferroni correction was applied. δ^{15}N signatures and mercury concentrations showed a linear relationship when considering all species simultaneously. This relationship was mainly due to a species-cluster effect. From an intra-species view, only the Little Tern presented a positive correlation between nitrogen and mercury ($r = 0.63$, $P = 0.001$) (Fig. 4).

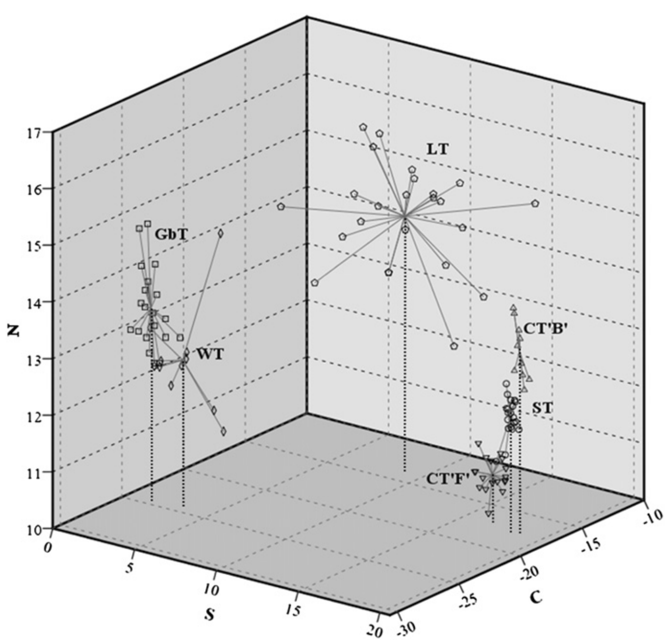

Fig. 2. 3D-scatterplot showing the distribution of δ^{15}N, δ^{34}S and δ^{13}C signatures of the five tern species: Little Tern (pentagons), Sandwich Tern (circles), Common Tern "Banya" (upwards triangles), Common Tern "Fangar" (downwards triangles), Gull-Billed Tern (squares) and Whiskered Tern (rhombus).

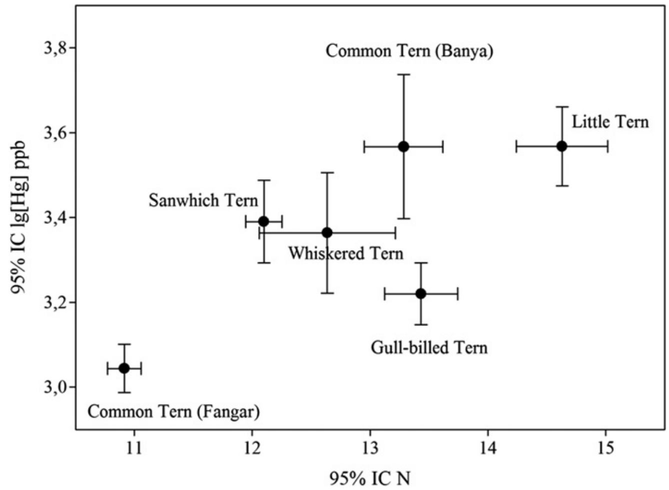

Fig. 3. Plot showing the mean position of the five tern species, according to log[Hg] ppb and δ^{15}N signatures. Dot indicates mean value, and lines their corresponding 95% confidence intervals.

4. Discussion

The isotopic values of δ^{13}C and δ^{34}S of the six tern colonies showed three distinct patterns. These were related mainly to differences in the foraging habitats exploited. The first group comprised the Sandwich and Common Tern. This isotopic pattern, characterized by high δ^{34}S and intermediate δ^{13}C signatures, was close to values reported for marine feeders in the Ebro Delta, such as those of the Audouin's Gull, a species that relies mainly on discarded fish (Sanpera et al., 2007a) and the Yellow-legged gull, which also uses mainly marine resources at this site (Ramos et al., 2009). The Sandwich Tern has typically been described as a marine feeder (Stienen et al., 2000) and no other foraging habitat has ever been reported for this species. Although the Common Tern also feeds over freshwater in some areas (Becker et al., 1997; Neubauer, 1998), it is reported mainly as a marine forager (Becker et al., 1993; Granadeiro et al., 2002) and isotopic signatures indicate this is the

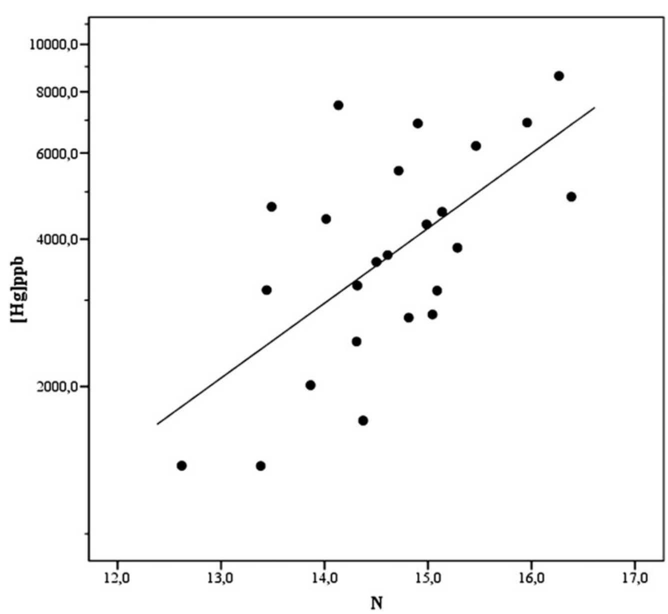

Fig. 4. Scatterplot of the dispersion between δ^{15}N signatures and mercury of the Little Tern. Relation between Hg and δ^{15}N is shown by the continuous line.

feeding strategy that this species follows in the Ebro Delta. This observation is consistent with the findings of other studies (Arcos et al., 2002; Guitart et al., 2003). However, although the slight differences in the isotopic values of δ^{13}C and δ^{34}S between the two Common Tern colonies pointed to local differences, the huge differences in δ^{15}N indicate that the colonies used different resources. In fact, while Arcos et al. (2002) reported that the Common Tern in the Ebro Delta fed mainly on epipelagic fish (Clupeiforms), Oro and Ruiz (1997) found that this species also fed on discarded fish. Both observations are consistent with the feeding strategies described by Guitart et al. (2003) and Mateo et al. (2004) for the colonies at "Punta del Fangar" (which rely mainly on epipelagic fish) and "Punta de la Banya" (where demersal resources were obtained from fish discards).

The Whiskered and Gull-Billed Terns showed low δ^{13}C and δ^{34}S signatures. The former feeds mainly on aquatic preys in wetlands (Latraube et al., 2005; Paillisson et al., 2007), and the rice fields, marshes and lagoons in the Ebro Delta are suitable foraging habitats for this species. Carbon isotopic data from heron species feeding in these habitats in the delta show very similar values (Purple heron *Ardea purpurea* $\delta^{13}C = -24.1$ $n = 16$ $SD = 0.87$; Squacco Heron *Ardeola ralloides* $\delta^{13}C = -22.9$ $n = 15$ $SD = 0.47$, unpublished data). Although reported to be a generalist feeder on a range of prey (Goutner, 1991), such as grasshoppers and caterpillars (Mathew et al., 1998), amphibians and beetles (Cabo and Sanchez, 1986), crayfish, insects and fish (Dies et al., 2005) or even fiddle crabs (*Uca tangeri*) (Stienen et al., 2008), the Gull-Billed Tern behaves like a freshwater forager in this locality, as shown by its low δ^{13}C range. The slight differences found in the δ^{34}S signatures between the two species would indicate that while the Gull-Billed Tern also exploits terrestrial habitats (with lower δ^{34}S values), in agreement with previous studies (Andersen, 1945; Bogliani et al., 1990; Sanchez et al., 1991), the Whiskered Tern may use brackish waters (increasing its S isotopic values). It is of interest to note that the Common Tern egg collected on "Punta del Fangar" that was excluded from the analysis presented isotopic values very similar to the freshwater foragers. This observation indicates individual foraging strategies, even when this foraging behavior has not been described previously for this tern population (Arcos et al., 2002; Mateo et al., 2004).

The Little Tern showed high δ^{13}C and intermediate δ^{34}S signatures. Previous studies carried out at Ria Formosa (Portugal) report on some of the factors that influence the selection of foraging habitats by this species, such as prey abundance, channels with strong currents, proximity to saltpans and conspecific information (Paiva et al., 2008a). Many studies have identified saltpans as a breeding habitat (Catry et al., 2004; Oro et al., 2004; Peste et al., 2004; Paiva et al., 2006a) and remark that this species is an opportunistic feeder and thus may use a wide variety of foraging grounds. The only limitation would be its small foraging range of 4–6 km when breeding (Fasola and Bogliani, 1990; Perrow et al., 2006). Observational data from transects in the Ebro Delta show that these birds forage in channels, lagoons and lagoon mouths, while salt marshes are avoided and rice fields are rarely visited (Bertolero et al., 2005); however, that study did not include transects in the saltpans (located at "Punta de la Banya" and where our samples were taken). δ^{13}C isotopic values for this species reflect a foraging habitat that differs to that of sea/freshwater/terrestrial habitats and that is very similar to those reported by Tavares et al. (2007) in Little Terns feeding in Portuguese saltpans. In coastal hypersaline habitats, changes in primary producers raise the δ^{13}C signature (Michener and Schell, 1994). This finding indicates that Little Terns at "Punta de la Banya" feed mainly in the saltpans, which is the main habitat around the colony. Moreover, the high variance in their isotopic values also indicates the use of other habitats to a lesser extent, thus pointing to individual foraging strategies. Furthermore, the δ^{15}N values of this species were the highest of the tern species examined. As the diet of the Little Tern is based mainly on fish and prawns (Norman, 1992; Bogliani et al., 1994; Paiva et al., 2006b), the high δ^{15}N signatures are probably the result of an increased basal signature in this habitat rather than differences in the trophic levels occupied.

Regarding mercury concentrations, our results indicate that the influence from riverine inputs from the Flix reservoir into the freshwater habitats of the Ebro Delta is lower than the mercury input to the marine foodwebs. Nevertheless, mercury derived from intensive agricultural activities in the wetland system (Mañosa et al., 2001) and from the industry located along the riverside, specially in the Flix Reservoir (Barata et al., 2010), may have accumulated in the sand banks of the Ebro Delta over the years, especially in the southern hemidelta, as materials carried by the Ebro river are mainly deposited there by the Liguro-Provençal-Catalan oceanic current (Guitart et al., 2003). However, riverine inputs have probably increased the mercury baseline of neighboring marine foodwebs, as has been reported for Audouin's Gull chicks in the Ebro Delta when compared with other colonies elsewhere in the Mediterranean (Sanpera et al., 2007a). Mercury concentrations in freshwater foragers (Whiskered Tern) are related mainly to the river contaminant input and are in the same range as those reported for the Purple Heron and the Little Egret at this site (Barata et al., 2010). In contrast, the Gull-Billed Tern, which shows partial terrestrial habitat use, showed lower mercury concentrations. Differences in mercury concentrations between the marine species may be related to their differential bioavailability in marine ecosystems, as demersal fish reach higher mercury loads than epipelagic ones (Arcos et al., 2002); thus, Common Terns sampled in "Punta de la Banya", which have access to fish discards, had the highest mercury concentrations of all the marine feeders. These concentrations were close to those reported for Audouin's Gull eggs at the same locality, a species that also feeds on fish discards (Morera et al., 1997). Higher mercury concentrations have been reported only for the Common Tern in the Elbe estuary (Becker et al., 1993a), a heavily polluted area. Other studies on mercury content of the Common Tern feeding mainly on epipelagic fish in several sites in Europe and North America ranged from 800 to almost 2000 ppb (Monteiro et al., 1999; Guitart et al., 2003; Becker and Munoz Cifuentes, 2004; Burger and Gochfeld, 2006; Bond and Diamond, 2009), with most values around 1000 ppb. Although the Sandwich Tern feeds on epipelagic fish, its larger size may allow capture of bigger prey with higher pollutant loads. Finally, the Little Tern presented the highest mercury concentrations. This observation is attributed to a different mercury baseline in the saltpan food webs. Relatively high concentrations of this pollutant have also been found in Little Terns feeding in saltpans in Portugal (Tavares et al., 2007; Paiva et al., 2008b). The concentrations in those birds were found to be higher than those of Little Terns in the Baltic Sea feeding mainly in marine ecosystems (Thyen et al., 2000).

Finally, the relatively high mercury concentrations in the Little Tern and Common Tern colonies in "Punta de la Banya" may be reaching critical values that endanger breeding success. However, embryos of Charadriformes species show an intermediate range sensitivity to mercury compared with other bird families such as Anseriformes (higher sensitivity) or Falconiformes (lower sensitivity) (Heinz et al., 2009). Thus studies to evaluate these effects are required. The striking variability observed in δ^{15}N signatures for Little Tern allowed us to detect a clear relationship between mercury and nitrogen signatures (i.e. trophic level), a predictable observation given the known underlying biomagnification process that occurs for mercury.

In summary, the tern community in the Ebro Delta reveals outstanding differences in habitat use as a result of niche segregation. These differences allowed us to interpret their mercury concentrations. In this regard, mercury load was found to be unexpectedly higher in terns feeding in marine habitats than in freshwater ones. We propose that this finding is associated with decades of accumulation of polluted sediments from the river in the sand banks of the Ebro Delta. Potential alterations induced by changes in the fisheries, saltpan and/or crop management should be adequately evaluated to preserve the diverse tern community inhabiting this area. Moreover, further research into mercury concentrations in seabirds feeding in marine habitats and saltpans is recommended. Given the mercury concentrations we found in eggs of Little and Common Terns, further studies are of particular relevance in order to monitor whether the mercury load of these species has become a conservation concern.

Acknowledgments

The authors wish to acknowledge the kindly cooperation of the personnel at the Parc Natural del Delta de l'Ebre for the facilities during fieldwork. Thanks are given to Sergi Ferrer and the staff at the *Serveis científico-tècnics* (UB, Spain) for their help during the sample collection and laboratory analysis. Javier Cotín was supported by a BRD grant from the *Universitat de Barcelona* (Spain). The present work was funded by the project 'Metalls pesants i organoclorats en el curs inferior i delta del riu Ebre: La ornitofauna com a bioindicadora (*Ministerio de Medio Ambiente*, Spain, *Agència Catalana de l'Aigua*, *Departament de Medi Mabient i Habitatge*, *Generalitat de Catalunya*) and by Fundación BBVA (BIOCON06/113; project EMECO).

References

Andersen, J., 1945. The food of the Gull-Billed Tern, *Gelochelidon nilotica* (Gm.). Dansk Ornithologisk Forenings Tidsskrift 39, 198–205.

Arcos, J.M., Ruiz, X., Bearhop, S., Furness, R.W., 2002. Mercury levels in seabirds and their fish prey at the Ebro Delta (NW Mediterranean): the role of trawler discards as a source of contamination. Marine Ecology Progress Series 232, 281–290.

Barata, C., Fabregat, M.C., Cotin, J., Huertas, D., Sole, M., Quiros, L., Sanpera, C., Jover, L., Ruiz, X., Grimalt, J.O., Pina, B., 2010. Blood biomarkers and contaminant levels from feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). Environmental Pollution 158, 704–710.

Becker, P.H., Frank, D., Sudmann, S.R., 1993. Temporal and spatial pattern of Common Tern (*Sterna hirundo*) foraging in the Wadden sea. Oecologia 93, 389–393.

Becker, P.H., Frank, D., Wagener, M., 1997. Luxury in freshwater and stress at sea? The foraging of the Common Terra *Sterna hirundo*. Ibis 139, 264–269.

Becker, P.H., Schuhmann, S., Koepff, C., 1993a. Hatching failure in Common Terns (*Sterna hirundo*) in relation to environmental chemicals. Environmental Pollution 79, 207–213.

Becker, P.H., Furness, R.W., Henning, D., 1993b. Mercury dynamics in young Common Tern (*Sterna hirundo*) chicks from a polluted environment. Ecotoxicology 2, 33–40.

Becker, P.H., Munoz Cifuentes, J., 2004. Contaminants in bird eggs. Recent spatial and temporal trends. Wadden Sea Ecosystem 18, 5–24.

Bertolero, A., Oro, D., Martinez Vilalta, A., Lopez, M.A., 2005. Selection of foraging habitats by Little Terns *Sterna albifrons* at the Ebro delta (NE Spain). Revista Catalana d'Ornitologia 21, 37–42.

Bogliani, G., Fasola, M., Canova, L., Saino, N., 1990. Food and foraging rhythm of a specialized Gull-Billed Tern population *Gelochelidon nilotica*. Ethology Ecology & Evolution 2, 175–181.

Bogliani, G., Fasola, M., Canova, L., Saino, N., 1994. Prey selection by parents and chicks of the Little Tern *Sterna albifrons*. Avocetta 18, 9–11.

Bond, A.L., Diamond, A.W., 2009. Mercury concentrations in seabird tissues from Machias Seal Island, New Brunswick, Canada. Science of the Total Environment 407, 4340–4347.

Bridge, E.S., Jones, A.W., Baker, A.J., 2005. A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. Molecular Phylogenetics and Evolution 35, 459–469.

Burger, J., Gochfeld, M., 2006. Temporal trends in mercury, cadmium and lead in eggs of Common Terns in New Jersey, USA, 1971–2005. Journal of Ornithology 147, 144.

Burger, J., Nisbet, I.C.T., Gochfeld, M., 1992. Metal levels in regrown feathers – Assessment of contamination on the wintering and breeding grounds in the same individuals. Journal of Toxicology and Environmental Health 37, 363–374.

Cabo, J.M., Sanchez, J.M., 1986. Recent Information on *Gelochelidon nilotica* in Morocco and its diet. Alauda 54, 207–212.

Catry, T., Ramos, J.A., Catry, I., Allen-Revez, M., Grade, N., 2004. Are salinas a suitable alternative breeding habitat for Little Terns *Sterna albifrons*? Ibis 146, 247–257.

Catry, T., Ramos, J.A., Le Corre, M., Kojadinovic, J., Bustamante, P., 2008. The role of stable isotopes and mercury concentrations to describe seabird foraging ecology in tropical environments. Marine Biology 155, 637–647.

Cherel, Y., Le Corre, M., Jaquemet, S., Menard, F., Richard, P., Weimerskirch, H., 2008. Resource partitioning within a tropical seabird community: new information from stable isotopes. Marine Ecology Progress Series 366, 281–291.

Connolly, R.M., Guest, M.A., Melville, A.J., Oakes, J.M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia 138, 161–167.

Dies, J.I., Marin, J., Perez, C., 2005. Diet of nesting Gull-Billed Terns in eastern Spain. Waterbirds 28, 106–109.

Fasola, M., Bogliani, G., 1990. Foraging ranges of an assemblage of Mediterranean seabirds. Colonial Waterbirds 13, 72–74.

Fernandez, M., Alonso, C., Gonzalez, M., Hernandez, L., 1999. Occurrence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). Chemosphere 38, 33–43.

Ferrer, X., Martinez-Vilalta, A., 1986. Fluctuations of the gull and tern populations in the Ebro Delta, north-east Spain (1960–1985). In: Medmaravis, Monbailliu, X. (Eds.), Mediterranean Marine Avifauna, vol. 12. Springer-Verlag, Berlín y Heidelberg, pp. 273–284.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226, 497–509.

Forero, M.G., Gonzalez-Solis, J., Hobson, K.A., Doncazar, J.A., Bertellotti, M., Blanco, G., Bortolotti, G.R., 2005. Stable isotopes reveal trophic segregation by sex and age in the southern giant petrel in two different food webs. Marine Ecology-Progress Series 296, 107–113.

Forero, M.G., Hobson, K.A., 2003. Using stable isotopes of nitrogen and carbon to study seabird ecology: applications in the Mediterranean seabird community. Scientia Marina 67, 23–32.

Gonzalez-Solis, J., Sokolov, E., Becker, P.H., 2001. Courtship feedings, copulations and paternity in Common Terns, *Sterna hirundo*. Animal Behaviour 61, 1125–1132.

Goutner, V., 1991. Food and feeding ecology of Gull-Billed terns (*Gelochelidon nilotica*) in Greece. Revue d'Ecologie (La Terre Et La Vie) 46, 373–384.

Goutner, V., 1990. Habitat selection of Little Terns in the Evros delta, Greece. Colonial Waterbirds 13, 108–114.

Granadeiro, J.P., Monteiro, L.R., Silva, M.C., Furness, R.W., 2002. Diet of Common Terns in the Azores, Northeast Atlantic. Waterbirds.

Guitart, R., Mateo, R., Sanpera, C., Hernandez-Matias, A., Ruiz, X., 2003. Mercury and selenium levels in eggs of Common Terns (*Sterna hirundo*) from two breeding colonies in the Ebro Delta, Spain. Bulletin of Environmental Contamination and Toxicology 70, 71–77.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R., Kondrad, S.L., Erwin, C.A., 2009. Species differences in the sensitivity of avian embryos to methylmercury. Archives of Environmental Contamination and Toxicology 56, 129–138.

Hobson, K., 2006. Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. Ardea 94, 359–369.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120, 314–326.

Hobson, K.A., Piatt, J.F., Pitocchelli, J., 1994. Using stable isotopes to determine seabird trophic relationships. Journal of Animal Ecology 63, 786–798.

Hobson, K.A., Sirois, J., Gloutney, M.L., 2000. Tracing nutrient allocation to reproduction with stable isotopes: a preliminary investigation using colonial waterbirds of Great Slave Lake. Auk 117, 760–774.

Koiadinovic, J., Menard, F., Bustamante, P., Cosscon, R.P., Le Corre, M., 2008. Trophic ecology of marine birds and pelagic fishes from Reunion Island as determined by stable isotope analysis. Marine Ecology-Progress Series 361, 239–251.

Latraube, F., Trotignon, J., Bretagnolle, V., 2005. Breeding biology of Whiskered tern *Chlidonias hybridus* in Brenne (France). Alauda 73, 425–429.

Llorente, G., Farran, A., Ruiz, X., Albaiges, J., 1987. Accumulation and distribution of hydrocarbons, polychlorobyphenyls, and DDT in tissues of 3 species of Anatidae from the Ebro Delta (Spain). Archives of Environmental Contamination and Toxicology 16, 563–572.

Mañosa, S., Mateo, R., Guitart, R., 2001. A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. Environmental Monitoring and Assessment 71, 187–205.

Mateo, R., Gil, C., Badia-Vila, M., Guitart, R., Hernandez-Matias, A., Sanpera, C., Ruiz, X., 2004. Use of fatty acids to explain variability of organochlorine concentrations in eggs and plasma of Common Terns (*Sterna hirundo*). Ecotoxicology 13, 545–554.

Mathew, K.L., Lukose, C., Pethani, K.V., Dave, H.R., 1998. Foraging behaviour of the Gull-Billed Tern, *Sterna nilotica*. Pavo 36, 15–18.

Michener, R.H., Schell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha, K., Michener, R.H. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications, London, pp. 138–157.

Monteiro, L.R., Granadeiro, J.P., Furness, R.W., Oliveria, P., 1999. Contemporary patterns of mercury contamination in the Portuguese Atlantic inferred from mercury concentrations in seabird tissues. Marine Environmental Research 47, 137–156.

Morera, M., Sanpera, C., Crespo, S., Jover, L., Ruiz, X., 1997. Inter and intraclutch variability in heavy metals and selenium levels in Audouin's gull eggs from the Ebro Delta, Spain. Archives of Environmental Contamination and Toxicology 33, 71–75.

Neubauer, W., 1998. Habitat choice of the Common Tern *Sterna hirundo* in eastern Germany. Vogelwelt 119, 169–180.
Nisbet, I.C.T., Montoya, J.P., Burger, J., Hatch, J.J., 2002. Use of stable isotopes to investigate individual differences in diets and mercury exposures among Common Terns *Sterna hirundo* in breeding and wintering grounds. Marine Ecology Progress Series.
Nisbet, I.C.T., 1973. Courtship-feeding, egg-size and breeding success in Common Terns. Nature 241, 141–142.
Norman, D., 1992. The growth rate of Little Tern *Sterna albifrons* chicks. Ringing and Migration 13, 98–102.
Oro, D., Bertolero, A., Vilalta, A.M., Lopez, M.A., 2004. The biology of the Little Tern in the Ebro delta (northwestern Mediterranean). Waterbirds 27, 434–440.
Oro, D., Ruiz, X., 1997. Exploitation of trawler discards by breeding seabirds in the north-western Mediterranean: differences between the Ebro Delta and the Balearic islands areas. ICES Journal of Marine Science 54, 695–707.
Paillisson, J.M., Reeber, S., Carpentier, A., Marion, L., 2007. Reproductive parameters in relation to food supply in the Whiskered Tern (*Chlidonias hybrida*). Journal of Ornithology 148, 69–77.
Paiva, V.H., Ramos, J.A., Catry, T., Pedro, P., Medeiros, R., Palma, J., 2006a. Influence of environmental factors and energetic value of food on Little Tern *Sterna albifrons* chick growth and food delivery. Bird Study 53, 1–11.
Paiva, V.H., Ramos, J.A., Machado, D., Penha-Lopes, G., Bouslama, M.F., Dias, N., Nielsen, S., 2006b. Importance of marine prey to growth of estuarine tern chicks: evidence from an energetic balance model. Ardea 94, 241–255.
Paiva, V.H., Ramos, J.A., Martins, J., Almeida, A., Carvalho, A., 2008a. Foraging habitat selection by Little Terns *Sternula albifrons* in an estuarine lagoon system of southern Portugal. Ibis 150, 18–31.
Paiva, V.H., Tavares, P.C., Ramos, J.A., Pereira, E., Antunes, S., Duarte, A.C., 2008b. The influence of diet on mercury intake by Little Tern chicks. Archives of Environmental Contamination and Toxicology 55, 317–328.
Pastor, D., Sanpera, C., Gonzalez-Solis, J., Ruiz, X., Albaiges, J., 2004. Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). Chemosphere 55, 567–576.
Perrow, M.R., Skeate, E.R., Lines, P., Brown, D., Tomlinson, M.L., 2006. Radio telemetry as a tool for impact assessment of wind farms: the case of Little Terns *Sterna albifrons* at Scroby Sands, Norfolk, UK. Ibis 148, 57–75.
Peste, F., Trigo, S., Luis, A., 2004. Foraging behaviour of breeding Little Tern *Sterna albifrons* at Ria de Aveiro. Airo 14, 55–62.
Quiros, L., Ruiz, X., Sanpera, C., Jover, L., Pina, B., 2008. Analysis of micronucleated erythrocytes in heron nestlings from reference and impacted sites in the Ebro basin (NE Spain). Environmental Pollution 155, 81–87.
Ramos, R., Ramirez, F., Sanpera, C., Jover, L., Ruiz, X., 2009. Feeding ecology of Yellow-legged Gulls *Larus michahellis* in the western Mediterranean: a comparative assessment using conventional and isotopic methods. Marine Ecology Progress Series 377, 289–297.
Ruiz, X., Jover, L., Pedrocchi, V., 1998. Audouin's gull intraspecific variability in yolk and albumen egg contents: why is yolk size so constant? Biologia e Conservazione della Fauna, 327–334.
Sanchez, J.M., Munoz del Viejo, A., de la Cruz, C., 1991. Segregacion alimentaria entre adultos y pollos de Gelochelidon nilotica (Gm., 1789) en la laguna de Fuente de Piedra. Ardeola 38, 21–27.
Sanpera, C., Moreno, R., Ruiz, X., Jover, L., 2007a. Audouin's gull chicks as bioindicators of mercury pollution at different breeding locations in the western Mediterranean. Marine Pollution Bulletin 54, 691–696.
Sanpera, C., Ruiz, X., Moreno, R., Jover, L., Waldron, S., 2007b. Mercury and stable isotopes in feathers of Audouin's Gulls as indicators of feeding habits and migratory connectivity. Condor 109, 268–275.
Stienen, E.W.M., Van Beers, P.W.M., Brenninkmeijer, A., Habraken, J.M.P.M., Raaijmakers, M.H.J.E., Van Tienen, P.G.M., 2000. Reflections of a specialist: patterns in food provisioning and foraging conditions in Sandwich Terns *Sterna sandvicensis*. Ardea 88, 33–49.
Stienen, E.W.M., Brenninkmeijer, A., Klaassen, M., 2008. Why do Gull-Billed terns *Gelochelidon nilotica* feed on fiddler crabs *Uca tangeri* in Guinea-Bissau. Ardea 96, 243–250.
Tavares, P.C., Kelly, A., Lopes, R.J., Pereira, M.E., Duarte, A.C., Furness, R.W., 2007. The influence of dietary specialization and trophic status on mercury levels in two species using common coastal wetlands, *Himantopus himantopus* and *Sterna albifrons*. Ardeola 54, 275–288.
Tavares, P.C., McGill, R., Newton, J., Pereira, E., Duarte, A., Furness, R.W., 2009. Relationships between carbon sources, trophic level and mercury exposure in generalist shorebirds revealed by stable isotope ratios in chicks. Waterbirds 32, 311–321.
Thyen, S., Becker, P.H., Behmann, H., 2000. Organochlorine and mercury contamination of Little Terns (*Sterna albifrons*) breeding at the western Baltic Sea, 1978–1996. Environmental Pollution 108, 225–238.
Wiggins, D.A., Morris, R.D., 1986. Criteria for female choice of mates – Courtship feeding and paternal care in the Common Tern. American Naturalist 128, 126–129.