

The Influence of Diet on Mercury Intake by Little Tern Chicks

Vitor H. Paiva · Paula C. Tavares · Jaime A. Ramos · Eduarda Pereira ·
Sandra Antunes · Armando C. Duarte

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Abstract We assessed mercury levels in the feathers of little tern (*Sternula albifrons*) chicks from hatching to fledging and in their prey captured by adults in three main foraging habitats: lagoon, salinas, and adjacent sea. These data were used to model mercury concentration in chick feathers through food ingestion, in order to explore the effects that changes in diet would have on the mercury burden of chicks as they aged. The mercury concentration in feathers of chicks raised in sandy beaches was higher than in those raised in salinas. Lagoon prey had a significantly higher mercury concentration ($0.18 \pm 0.09 \mu\text{g g}^{-1}$ dry weight [d.w.]) than prey from salinas and the adjacent sea (both $0.06 \pm 0.03 \mu\text{g g}^{-1}$ d.w.). In relation to prey species group, mercury content was significantly higher for bottom fish ($0.17 \pm 0.10 \mu\text{g g}^{-1}$ d.w.) than for pelagic ($0.08 \pm 0.06 \mu\text{g g}^{-1}$ d.w.), euryhaline fish ($0.04 \pm 0.02 \mu\text{g g}^{-1}$ d.w.), and crustacea ($0.08 \pm 0.03 \mu\text{g g}^{-1}$ d.w.). To understand the importance of mercury content of each prey group, we ran several theoretical scenarios assuming that chicks were fed on only one species at a time. Considering a diet restricted to lagoon (mostly benthic) prey, A- and B-chicks may encounter health problems with an excess of mercury. On the contrary, a diet restricted to marine (mostly pelagic) prey would decrease the mercury concentration in chick feathers;

the fast growth rate and the related mercury dilution effect in little tern chicks seem to decrease mercury levels in their feathers. Our study supports the fact that marine pelagic prey are important for estuarine seabirds because they provide a food resource with lower contamination levels. This model may have a wider application in similar seabird species and coastal environments.

Mercury is a toxic heavy metal that bioamplifies in food webs (Bearhop et al. 2000; Burger and Gochfeld 2002, 2004). Deposition in feathers represents the major elimination pathway for accumulated mercury in birds; mercury in feathers is almost entirely in the form of methyl-mercury, and levels correlate well with body burdens (Thompson and Furness 1989a, 1989b; Furness and Camphuysen 1997). Mercury contamination in chicks is influenced by egg contamination, hatching order, chick age, and mercury uptake through food ingestion (Becker et al. 1993a, 1993b, 1994; Monteiro and Furness 1995; Wenzel et al. 1996; Stewart et al. 1997; Goutner et al. 2001). Mercury levels in hatchlings are influenced by the mercury burdens present in the female, because 20% of body contamination is released into the egg in the week before laying (Lewis et al. 1993). As chicks' age increases, the importance of diet for mercury burden in chick tissues also increases (Wenzel et al. 1996). In general, mercury decreases in chicks as they grow due to a fast increase in body mass, which represents a dilution effect (Becker et al. 1994). This effect is likely to be very important during the initial fast growing period in chicks (Konarzewski et al. 1998). Tavares et al. (2005) obtained a significant negative correlation between mercury levels in feathers and chick size in little tern *Sternula albifrons*, which suggests a strong dilution effect. However, their samples did not

V. H. Paiva (✉) · J. A. Ramos · S. Antunes
IMAR-Instituto do Mar, Department of Zoology,
University of Coimbra, 3004-517 Coimbra, Portugal
e-mail: vitorpaiva@ci.uc.pt

P. C. Tavares · E. Pereira · A. C. Duarte
Department of Chemistry, University of Aveiro,
3810-193 Aveiro, Portugal

P. C. Tavares
CVRM, IST, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

include intermediate chick size classes. Thyen et al. (2000) studied mercury contamination of little tern chicks on the western coast of the Baltic Sea but only sampled chicks 0–3 days of age. Studies on the dynamics of mercury in chicks reared in the laboratory were performed for some seabird species such as the black-headed gull *Larus ridibundus* (Lewis and Furness 1991) and common loon *Gavia immer* (Kenow et al. 2003). Monteiro and Furness (2001) fed Cory's shearwater *Calonectris diomedea borealis* chicks with increasing doses of mercury and analyzed their growing feathers to evaluate the dilution effect.

Mercury intake through food ingestion is considered to be the main factor determining mercury contamination in fledglings, and the influence of egg contamination is considered negligible for old chicks (Becker et al. 1993a, 1993b; Monteiro and Furness 1995; Wenzel et al. 1996). Chicks may be useful as biomonitors of mercury contamination in a restricted area because they are confined to a particular site before independence, their parents deliver prey caught around the breeding grounds (Cramp 1985; Allcorn et al. 2003), and their feathers are easy to sample. Breeding little terns feed within 4 km of their colonies (Fasola and Bogliani 1990; Allcorn et al. 2003), where they take the most abundant prey (Catry et al. 2006). Hence, they are likely to be a good bioindicator of environmental contamination in the coastal area around the colonies. Therefore, it is important to understand the exact details of the relationships between mercury in chick prey and mercury in their feathers (Arcos et al. 2002).

In this paper we examine little tern chick provisioning from hatching to fledging and assess mercury levels in the feathers of those same chicks, and in their prey, at the ages of 0, 4, 9, 14, and 17 days, in order to answer the following questions: (a) Do the mercury levels in growing chicks relate to those of their prey? (b) How important is the dilution effect in determining mercury contamination in large chicks? and (c) What is the contribution of different prey items to the mercury burden in feathers of little tern chicks? To examine the latter point we built a model of mercury release into chick feathers to assess the capacity of the chicks to dilute and excrete into feathers the mercury ingested from their prey.

Methods

Study Area

Ria Formosa Natural Park is situated on the south coast of Portugal, Algarve (37°06'N, 7°38'W), and consists of a complex tidal system of natural and seminatural channels (lagoon), marshland, and barrier islands covering an area of 18,400 ha along 60 km of coast. In the margins of the

marshland area, there are manmade ponds which represent salinas (salt pans) and extensive fish farms. Close to the park's northern borders are the three large towns Faro, Olhão, and Tavira. Our observations took place in both natural (sandy beaches of the barrier islands Armona, Faro, and Barreta) and alternative (salinas around Santa Luzia, Tavira) breeding habitats (Catry et al. 2004). The closest foraging areas were the lagoon and the sea for birds breeding in natural habitats and the lagoon and salinas for those breeding in alternative habitats.

Food Delivery

During regular visits to the colonies on sandy beaches (Armona, Barreta, Praia de Faro) and salinas (Santa Luzia I, Santa Luzia II, Arraial Ferreira Neto, Vale Carangueijo) in 2005; 42 dropped prey items (these include prey that were not ingested) were collected and each prey item was immediately placed in a new plastic bag. All specimens were identified to the lowest taxonomic level based on available identification keys. Food delivery to 10 fenced broods in salinas was observed from 10 June to 3 July 2005. Two portable hides, placed 2–7 m from these nests, were used to observe and identify prey delivered to chicks, using 10 × 40 binoculars. A total of 90 h of observations was made, divided into periods of 1 to 10 h (mean = 2 h). The observations were randomly spread across the 3-week period, the daylight hours, and the tidal phases. Each nest was sampled equally (same number of hours per nest; see Paiva et al. [2006a] for further details on food delivery procedures). During the breeding season of 2005, the main prey items delivered to chicks were *Sardina pilchardus*, *Atherina* spp., and shrimp (*Palaemon* spp. and *Palaemonetes* spp.), comprising almost 74% of the diet. Also, prey items comprising each < 5% of the diet were *Belone belone*, *Fundulus* spp., *Pomatoschistus* spp., and *Scorpaenopsis scorpaenoides* (Paiva et al. 2006b). The length of each prey delivered was determined in relation to the mean little tern adult bill length (Paiva et al. 2006a) and transformed into mass using regression equations (Table 1). Further details on chick growth and food delivery to chicks are given by Paiva et al. (2006a, 2006b).

Sample Collection and Preparation

Samples of down and breast feathers were collected from 64 little tern chicks from different colonies in salinas and sandy beaches, during the 2004 and 2005 breeding seasons. Sampled chicks were weighed (g), and their wing length and tarsus length were measured (mm). In 2005 we sampled down and feathers from chicks of 14 fenced nests in salinas of Sta. Luzia at 0, 4, 9, 14, and 17 days of age.

Table 1 Regression equations for the species used in the model: total length (TL ; cm) and weight (W ; g, fresh weight)

Species	TL - W regression	Reference
<i>Atherina</i> spp.	$W = 0.0069 \times TL^3$	Fishbase (2007)
<i>Sardina pilchardus</i>	$W = 0.0060 \times TL^3$	Fishbase (2007)
<i>Belone belone</i>	$W = 0.0020 \times TL^{2.87}$	Fishbase (2007)
<i>Fundulus</i> spp.	$W = 0.0142 \times TL^3$	Fishbase (2005)
<i>Scomberesox saurus</i>	$W = 0.0015 \times TL^{3.19}$	Fishbase (2007)
<i>Pomatoschistus</i> spp.	$W = 0.0142 \times TL^3$	Fishbase (2007)
Shrimp	$W = 0.0042 \times TL^{2.4}$	Unpublished data

Feather samples were collected from *A*-, *B*-, and *C*-chicks ($n = 12, 11,$ and $5,$ respectively). Chicks were designated *A*, *B*, or *C* according to hatching order (when two chicks hatched on the same day, we considered the *A*-chick as the one hatching from the largest egg, which had a greater body mass and greater tarsus length). Body mass (g) for each fenced chick was obtained daily (Paiva et al. 2006b). Prey samples were collected directly from food delivered to chicks in the breeding ground, stored at -20°C , and dried until constant weight. To give strong consistency to the mean mercury level of each prey species, we analyzed prey collected in several colonies at Ria Formosa as laboratory replicates and, also, analyzed mercury levels for prey collected in salinas of other coastal Portuguese wetlands where little terns breed, the Tejo and Sado estuaries.

Mercury Determinations

Total mercury concentration was determined in down, feather, and prey samples by thermal atomization followed by atomic absorption spectroscopy using an AMA254 spectrophotometer (Altec, Czech Republic). Accuracy of the method was within 10% of the reference value, and it was monitored through reference materials, NIES-5 (human hair, $4.4 \pm 0.4 \mu\text{g g}^{-1}$) and TORT-2 (lobster hepatopancreas, $0.27 \pm 0.06 \mu\text{g g}^{-1}$), with a 95% confidence interval. The uncertainty was assessed by performing successive measurements with the same sample, and relative standard deviations in the range of 5% were found. The detection limit was established as 0.01 ng Hg for 0.100 g samples (0.1 ng g^{-1}) by Altec. Mercury concentrations were given on a fresh weight (f.w.) basis in down and feather samples and a dry weight (d.w.) basis in prey samples.

Model Building

The model takes into account the total mercury content of the main prey items (formerly described), derived from

their biomass and specific mercury concentration, and the prey delivery rate to chicks, contributing to a pool of mercury to be divided among *A*-, *B*-, and *C*-chicks based on a competition factor. This factor assumes that larger chicks have a higher chance of getting food because they have a higher fitness than smaller chicks (Shew and Ricklefs 1998); this was confirmed during our observations as well. The competition also included a hatching delay factor: the *B*- and *C*-chicks hatched 0.29 ± 0.03 and 0.87 ± 0.05 day later than the *A*-chicks (mean value \pm SD obtained from the fenced nests in 2003). Those values were set in the model to create a delay in the available energy flow to *B*- and *C*-chicks (Paiva et al. 2006b). Each chick allocates some portion of mercury to down and new grown feathers ($\sim 38\%$ [Becker et al. 1993a]). The maximum allocation of mercury to feathers or other chick tissues is regulated by the balance between mercury inputs through food ingestion and a dilution effect associated with chick growth (Becker et al. 1994). In order to take this into account we used an energetic balance growth model previously developed by Paiva et al. (2006b) for little tern chicks and collected feathers of the same individual chicks from hatching to fledging. To build the model of mercury release into feathers we used the chick growth rate and the maximum weight of chicks from the growth model constructed by Paiva et al. (2006b). Although we had no independent data to validate our model according to different initial conditions and respective outputs, we believe that the output from the theoretical scenarios tested with this model may help in understanding how little tern chicks allocate the mercury ingested and assimilated from prey delivered by their parents, and how the parents' choices may influence chicks' mercury burdens and, thus, their potential health and body fitness. The model was constructed using STELLA (High Performance Systems Inc. 1997) v7.03 software.

The model building procedure then followed 11 main steps. (1) From significant regressions obtained in the literature and in Fishbase (2007) (Table 1), it was possible to determine the mass of each prey from its length (Table 2). (2) The biomass of each prey was multiplied by the mean mercury concentration of each prey species, which gives the mercury content relative to each prey. (3) To calculate the mean mercury rate delivered per day, the value delivered per hour for each species (Table 2) was multiplied by 13 h, the number of daylight hours at this time of the year; parents do not deliver food at night (Davies 1981). (4) Total daily mercury available from all ingested prey was calculated from the sum of individual mercury content of each prey. (5) The total mercury available was then divided among three chicks from the same brood (*A*-, *B*-, and *C*-chicks), creating a pool of body mercury that enters each

Table 2 Total mercury concentration ($\mu\text{g g}^{-1}$ d.w.) of different prey species from little tern chicks

Taxon (place of collection)	Hg concentration (sample size)	Mean foraging trip duration (h)	Total length (cm)	Mass of meal delivered (mass of prey/chick/h)
Pelagic fish				
<i>Sardina pilchardus</i> (Tejo)	0.17 \pm 0.03 (9)			
<i>Sardina pilchardus</i> (Sado)	0.18 \pm 0.06 (7)			
<i>Sardina pilchardus</i> (Ria Formosa)	0.09 \pm 0.03 (9)	0.63 \pm 0.75 (140)	3.94 \pm 1.04 (161)	2.80 \pm 0.45 (49)
<i>Atherina</i> spp. (Ria Formosa)	0.19 \pm 0.09 (3)	0.47 \pm 0.77 (303)	5.10 \pm 1.86 (365)	0.88 \pm 0.43 (68)
<i>Belone belone</i> (Ria Formosa)	0.03 \pm 0.01 (5)	0.55 \pm 0.69 (48)	7.57 \pm 2.06 (54)	1.70 \pm 0.80 (26)
<i>Scorpaenopsis scorpaenoides</i> (Ria Formosa)	0.04 \pm 0.01 (6)	0.75 \pm 0.74 (52)	4.94 \pm 2.46 (60)	0.24 \pm 0.08 (24)
Mugilidae (Tejo)	0.15 \pm 0.04 (5)			
Mugilidae (Sado)	0.11 \pm 0.00 (3)			
<i>Trachurus trachurus</i> (Ria Formosa)	0.09 \pm 0.00 (1)			
Euryhaline fish				
<i>Fundulus</i> spp. (Ria Formosa)	0.04 \pm 0.02 (2)	0.50 \pm 0.55 (51)	3.01 \pm 0.27 (57)	0.42 \pm 0.10 (25)
Crustacea				
<i>Palaemonetes</i> spp. (Ria Formosa)	0.08 \pm 0.03 (3)	0.40 \pm 0.51 (204)	2.57 \pm 0.51 (248)	0.24 \pm 0.10 (50)
<i>Palaemonetes</i> spp. (Sado)	0.08 \pm 0.07 (17)			
<i>Palaemonetes</i> spp. (Tejo)	0.08 \pm 0.06 (29)			
<i>Palaemonetes</i> spp. (Aveiro)	0.08 (1)			
Bottom/demersal fish				
<i>Pomatoschistus</i> spp. (Ria Formosa)	0.31 \pm 0.09 (3)	0.37 \pm 0.42 (53)	2.85 \pm 0.38 (131)	0.33 \pm 0.11 (21)
<i>Gambusia holbrooki</i> (Tejo)	0.28 \pm 0.24 (5)			
<i>Symphodus melops</i> (Ria Formosa)	0.16 \pm 0.02 (4)			
<i>Diplodus</i> spp. (Ria Formosa)	0.08 \pm 0.01 (3)			
Blenniidae (Ria Formosa)	0.21 \pm 0.00 (1)			
<i>Spondyliosoma cantharus</i> (Ria Formosa)	0.12 \pm 0.03 (2)			

Mean values \pm SD, with samples size in parenthesis. Mean foraging trip duration, total length, and mean mass of meal delivered for all species except *Pomatoschistus* spp. and *Scorpaenopsis scorpaenoides* (this study) were taken from Paiva et al. (2006b)

chick daily. (6) Then some part of this body mercury uptake (43%, 39%, and 38% for A-, B-, and C-chicks, respectively; values obtained by calibration) was allocated to new growing feathers as a way of mercury excretion. These values were set considering that Laridae chick feathers normally contain 38%–65% of the total mercury body burden (Lewis and Furness 1991; Becker et al. 1993a). (7) The logistic curve of mercury decrease, which has a sigmoid shape (very similar to an inverted logistic growth curve [Paiva et al. 2006b]), enables the description of mercury decrease in feathers of chicks as they grow (Wenzel et al. 1996). (8) The decrease rate constant ($K_{\text{Hg}} = 0.1566$) was obtained by calibration with data collected in 2005. (9) We used the logistic growth rate constant obtained by Paiva et al. (2006b) for little tern chicks ($K_{\text{G}} = 0.0249 \pm 0.01$) to associate the mercury decrease in chick feathers with growth. This represents in the model the dilution of the dietary mercury intake due to the increase in body mass. The association of the logistic growth rate with mercury dynamics enabled us to simulate

the phase when chicks stop begging for food and parents respond by reducing feeding rate, as the food ingested and the amount of mercury uptake by the chicks will be limited by chick growth and satiation. (10) We used a minimum mercury concentration in feathers for A-, B-, and C-chicks as the value they usually attain by 17 days of age, 2.78 ± 0.47 , 2.84 ± 0.43 , and $3.16 \pm 0.34 \mu\text{g g}^{-1}$ f.w. (\pm SD), respectively (obtained from fieldwork, as chick feathers would never be completely free from mercury). Also, the asymptotic mass attained by A-, B-, and C-chicks, on days 20, 19, and 22, was 43.0 ± 1.34 , 42.4 ± 1.32 , and 43.0 ± 0.50 g (\pm SD), respectively. Most chicks began the asymptotic part of their growth at 15 (t_i) days of age (Konarzewski et al. 1998; Starck and Ricklefs 1998). (11) Mercury concentrations in down feathers of recently hatched chicks were 10.37 ± 3.89 , 6.93 ± 1.38 , and $6.57 \pm 0.41 \mu\text{g g}^{-1}$ f.w. for A-, B-, and C-chicks, correspondingly. Hatching mass corresponded to measured mean values, 6.50 ± 0.17 , 6.40 ± 0.26 , and 6.16 ± 0.31 g for A-, B-, and C-chicks, respectively.

Sensitivity Analysis

To check for the most sensitive parameters (Table 3), we calculated the “individual parameter perturbation” (Madenjian and Gabrey 1995) described in the STELLA software manual (High Performance Systems, Inc. 1997), which examines the sensitivity of model performance to variation in model’s parameter values. Changes of $\pm 10\%$ were imposed on the model parameters and the consequent variations of mercury content in feathers of A-, B-, and C-chicks were analyzed.

Theoretical Scenarios

To understand the importance of the mercury content of each prey species, we ran the model assuming that chicks were fed only one single species at a time. For example, considering that an adult foraging trip for *Atherina* spp lasted 0.47 h (Table 2), and assuming that parents could forage during the 13 h of daylight, they could feed their chicks 6.11 *Atherina* spp. items per day. We also analyzed the effects of a diet restricted to lagoon prey on chicks’ mercury levels, in relation to the effects of a diet of only marine items (but accounting for the extra foraging time parents’ gain by not foraging on the omitted prey). In addition, we compared the effects of a diet of shrimp (crustacea) vs one of fish, and the effects of pelagic prey vs bottom prey, on chicks’ mercury levels. Finally, we tested the effect of a typical diet of adults for that area on chicks’ mercury levels, using the proportions of each prey type: 45.9% of *Pomatoschistus* spp., 40.5% of *Atherina* spp. and 13.6% of shrimp (*Pomatoschistus* spp. and *Paleomonetes* spp.), as obtained by Catry et al. (2006). A maximum value of $20 \mu\text{g g}^{-1}$ for the total mercury concentration in feathers, detected in the salinas of Santa Luzia in 2005, was used as a theoretical physiological limitation of chicks to passively allocate mercury to their feathers. In the references no threshold mercury value was found above which body condition and chick survival would be compromised in this species.

Table 3 Sensitivity analysis of the principal parameters entered in the model

Parameter	Value	A-chicks	B-chicks	C-chicks
Constant of mercury decay	0.1566	13.4346	12.8502	8.9701
Intercept of mercury decay	0.272	10.6470	11.4290	7.6760
Feeding, h/day	13	1.9798	0.6559	0.0872

Note. See text for explanation of sensitivity computations

Statistical Analysis

Data were checked for normality (Kolmogorov-Smirnov test) and homocedasticity (Levene’s test). Analysis of covariance (ANCOVA), with chick age as a covariate, followed by post hoc Tukey tests, was used to assess the influence of (1) breeding habitat (sandy beaches and salinas), (2) breeding location (Barreta barrier island, Armona barrier island, Ancão peninsula, and salinas of Sta. Luzia), and (3) study year (2004 or 2005) on the mercury concentration in down feathers of chicks <5 days old. To test the null hypothesis that there were no differences in mean mercury concentration among prey species across (1) habitats (sandy beaches and salinas) and (2) prey species group (crustacean, pelagic, bottom, and euryhaline species), two-way analysis of variance (ANOVA), followed by post hoc Tukey tests for unequal sample size, was used. A Spearman correlation was applied to analyze the relationship between the amount of different prey groups in the diet (pelagic, bottom, and euryhaline fish and crustacea) and the mercury concentration in breast feathers of chicks aged 4 and 9 days. A one-way ANOVA was used to investigate differences in mercury content among A-, B-, and C-chicks at 0 days of age (hatching day). For model calibration with data collected during the 2005 breeding season, Model II regressions, a recommended procedure whenever both variables are subject to error, was used (Sokal and Rohlf 1995). The significance of the regressions was checked with ANOVA because analysis of variance is the only means of testing it in Model II regression (Fowler et al. 1998). The null hypothesis that the intercept of the estimated regressions is not significantly different from 0, and the slope is not significantly different from 1, was tested using the Dent and Bleckie (DBK; 1979) regression test, which simultaneously tests the slope and the intercept. All analyses were performed with statistica v. 6.0 (Statsoft 1996) with a significance level of $p < 0.05$. Data are presented as mean \pm standard deviation.

Results

Influence of Breeding Habitat and Location on Mercury Concentration in Chicks

Considering samples taken in 2004 (different beaches and salinas), there was no significant effect of breeding location (ANCOVA: $F_{3,24} = 1.64$, $p = 0.20$) and habitat ($F_{1,26} = 0.67$, $p = 0.42$) on the mercury concentration of chicks <5 days old. When we compared 2004 and 2005, and removed salinas at Arraial Ferreira Neto (not sampled in 2005) and the barrier island of Armona (not sampled in 2004), the result was not significant ($F_{1,64} = 0.49$,

$p = 0.48$). For 2005 only, we found significant differences in the mercury concentration of chicks <5 days of age between sandy beaches and salinas ($F_{1,61} = 4.64$, $p = 0.04$) and among breeding locations ($F_{3,59} = 10.18$, $p = 0.00$; Fig. 1). The mean mercury concentration of chicks from Armona was approximately twofold higher than that of chicks from other areas (Fig. 1).

Mercury in Prey

Lagoon prey had a significantly higher mercury concentration ($0.18 \pm 0.09 \mu\text{g g}^{-1}$ d.w.) than prey from salinas ($0.06 \pm 0.03 \mu\text{g g}^{-1}$ d.w.) and the sea ($0.06 \pm 0.03 \mu\text{g g}^{-1}$ d.w.), 1-Way ANOVA: $F_{2,40} = 16.49$, $p = 0.00$). Comparing species groups, the mercury content was significantly higher for bottom fish ($0.17 \pm 0.10 \mu\text{g g}^{-1}$ d.w.) than for pelagic fish ($0.08 \pm 0.06 \mu\text{g g}^{-1}$ d.w.), euryhaline fish ($0.04 \pm 0.02 \mu\text{g g}^{-1}$ d.w.), and crustacea ($0.08 \pm 0.03 \mu\text{g g}^{-1}$ d.w.; $F_{3,39} = 5.88$, $p = 0.00$) (Fig. 2). These results are also in agreement with those for *Sardina pilchardus* (pelagic marine prey), Mugillidae (pelagic lagoon prey), and *Paleomonetes varians* (salinas prey), which had a lower mean mercury content than *Gambusia holbrooki*, a characteristic benthic prey (Table 2).

Contribution of Diet to Mercury Content in Chicks

Because mercury in early hatched chicks is mainly due to the egg mercury content (Becker et al. 1993), and consequently a reflection of female's mercury levels, we began to analyze differences of mercury concentration in chick feathers from 4 days of age onward, because these levels should be a reflection of their diet. There was a significant negative correlation between the mercury concentration in chick feathers and the percentage of pelagic fish on the diet of chicks at 4 days of age ($r_s = -0.69$, $p = 0.03$; $n = 10$). Also, the amount of crustacean plus bottom fish in the diet

of chicks at 9 days of age was significantly correlated with the mercury concentration in chick feathers ($r_s = 0.67$, $p = 0.05$; $n = 9$).

Mercury Calibration Model

The mercury concentration in feathers of A-chicks decreased faster than that in B- and C-chicks, but all chicks attained a similar amount of mercury from 14 days onward. At hatching, the A-chicks had a higher mercury content than B- and C- chicks did (one-way ANOVA: $F_{2,17} = 7.25$, $p = 0.001$) (Fig. 3).

The mercury decrease in feathers of little tern chicks predicted by the model for A-, B-, and C-chicks agreed well with the observed data for 2005. This decrease was better predicted for A-chicks than for B- and C-chicks, because all points predicted for A-chicks were within standard deviation lines. This model had significant Model II regressions between predicted and observed values for A-, B-, and C-chicks in 2005 (ANOVA: $F_{1,3} = 74.15$, $p = 0.003$, $r^2 = 0.96$; $F_{1,3} = 18.20$, $p = 0.02$, $r^2 = 0.86$; and $F_{1,3} = 32.56$, $p = 0.01$, $r^2 = 0.92$). Furthermore, the slope of the regression was not significantly different from 1 and the intercept was not significantly different from 0 for A-, B-, and C-chicks (DBK regression test: $F_{1,3} = 60.45$, $p = 0.005$; $F_{1,3} = 15.32$, $p = 0.03$; and $F_{1,3} = 29.90$, $p = 0.01$), giving credibility to our model (Fig. 4).

Sensitivity Analysis

Sensitivity analysis revealed that the constant of mercury decrease was the most sensitive parameter, followed by the intercept of mercury decrease (both were obtained from the logarithmic calibrated formula for mercury decrease applied in the model) and the number of daylight hours that parents used for delivering the food to their chicks (Table 3). Changes of $\pm 10\%$ in each of these three parameters caused

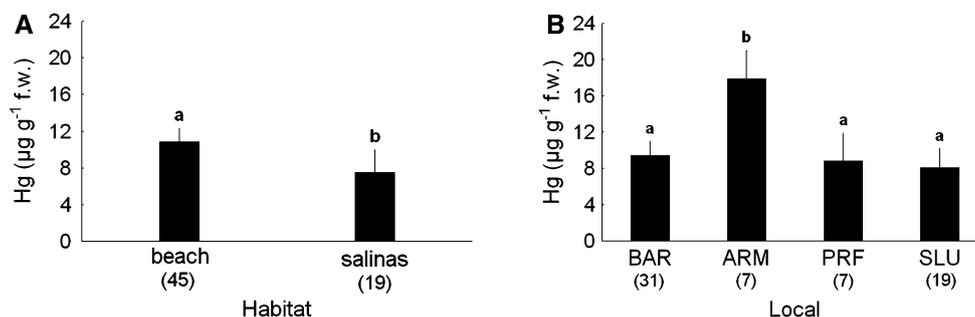


Fig. 1 Mercury concentrations ($\mu\text{g g}^{-1}$ f.w.) in feathers of little tern chicks <5 days old from 2005 in relation to breeding habitat (A) and location (B). Mean value \pm SD, with sample size in parentheses. BAR, Barreta barrier island; ARM, Armona barrier island; PRF,

Ancão peninsula; SLU, Sta. Luzia salinas. Different lowercase letters indicate significant differences in mercury concentration. One-way ANCOVA with age as a covariate followed by post hoc Tukey test for unequal Ns at $p < 0.05$

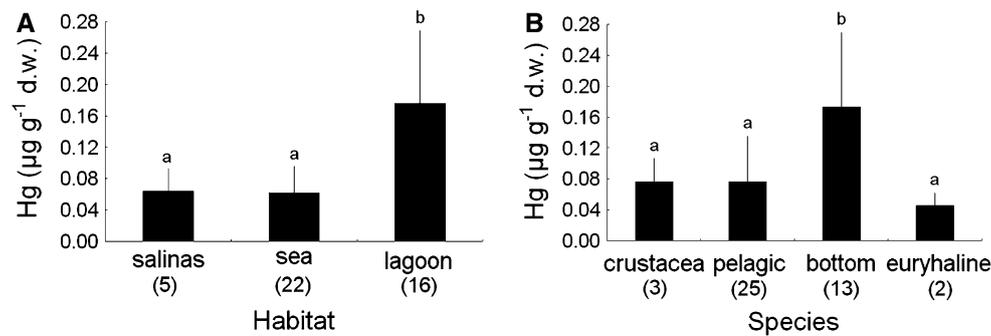


Fig. 2 Mercury concentrations ($\mu\text{g g}^{-1}$ d.w.) in different prey of little tern chicks in relation to habitat (A) and species group (B). Mean value \pm SD, with sample size in parentheses. Different lowercase

letters indicate significant differences in mercury concentration. One-way ANOVA followed by post hoc Tukey test for unequal Ns at $p < 0.05$

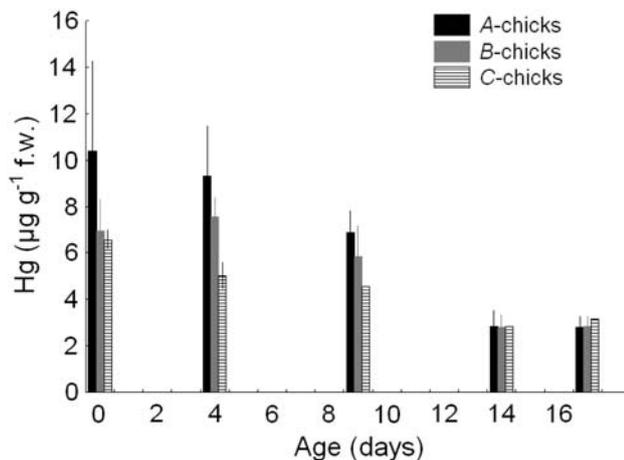


Fig. 3 Mercury concentration (fresh weight; f.w.) in feathers of little tern A-, B-, and C-chicks from Sta. Luzia salinas, in relation to age. Mean value \pm SD

$\pm 10\%$ variation in the mercury concentration in the chick feathers. A-chicks were more sensitive to the constant of mercury decrease, followed by B- and C-chicks. The same pattern was noticed for the effect of alterations on the feeding hours per day for all three groups of chicks, whereas changes in the intercept of mercury decrease affected more B-chicks than A- and C-chicks.

Theoretical Scenarios

Considering a diet restricted to the lagoon prey (*Pomatoschistus* spp., *Fundulus* spp., and *Atherina* spp.) and accounting for the specific foraging time of the former three prey plus an extra foraging time that is gained by not foraging on the omitted prey (Stienen and Brenninkmeijer 2002) (Table 2), A- and B-chicks may potentially have to deal with an excess of mercury on their body, because the capacity of dilution (by chick growth) and excretion to feathers would be exceeded. Across this scenario, only C-

chicks would be maintained in a good situation, as they have much less mercury content at hatching. On the contrary, a diet restricted to marine prey (*Sardina pilchardus*, *Belone belone*, and *Scorpaenopsis scorpaenoides*) would allow a decrease in the mercury concentration in chick feathers during chick growth (Fig. 5). Similarly a diet restricted to shrimp (crustacea, *Palaemon* spp., and *Palaemonetes* spp.) would enable low levels of mercury for chicks at 17 days of age. On the contrary, a diet of exclusively fish would lead to abnormal high levels of mercury, at least for A-chicks (Burger and Gochfeld 1997) (Fig. 5).

A diet solely of pelagic fish (*Sardina pilchardus*, *Atherina* spp., *Belone belone*, and *Scorpaenopsis scorpaenoides*) would result in an increase in mercury levels in feathers as chicks aged. However, only an exclusive diet of bottom prey (*Pomatoschistus* spp. and *Fundulus* spp.) would increase the mercury levels for B-chicks (above the typical mercury decrease curve for 2005 chicks) and likely reach abnormal high levels for A-chicks (from 17 days onward; Fig. 5).

We identified the prey that contributed the most to reaching abnormally high mercury contamination levels in little tern chicks by restricting the diet of chicks to only one of the major prey delivered by parents on 2005. Assuming that parents feed their chicks solely on *Pomatoschistus* spp. (spending 0.37 h to deliver one item of this species to the nest) or on *Atherina* spp. (foraging trip duration = 0.47 h), the whole brood (A-, B-, and C-chicks) would reach possibly adverse levels of mercury contamination. Assuming that parents deliver to their chicks the same proportion of each prey that they feed themselves (Catry et al. 2006), the good condition of all chicks in the brood could be compromised (Fig. 5).

Discussion

Our results have shown that the mercury concentration in feathers of chicks raised in sandy beaches was higher than

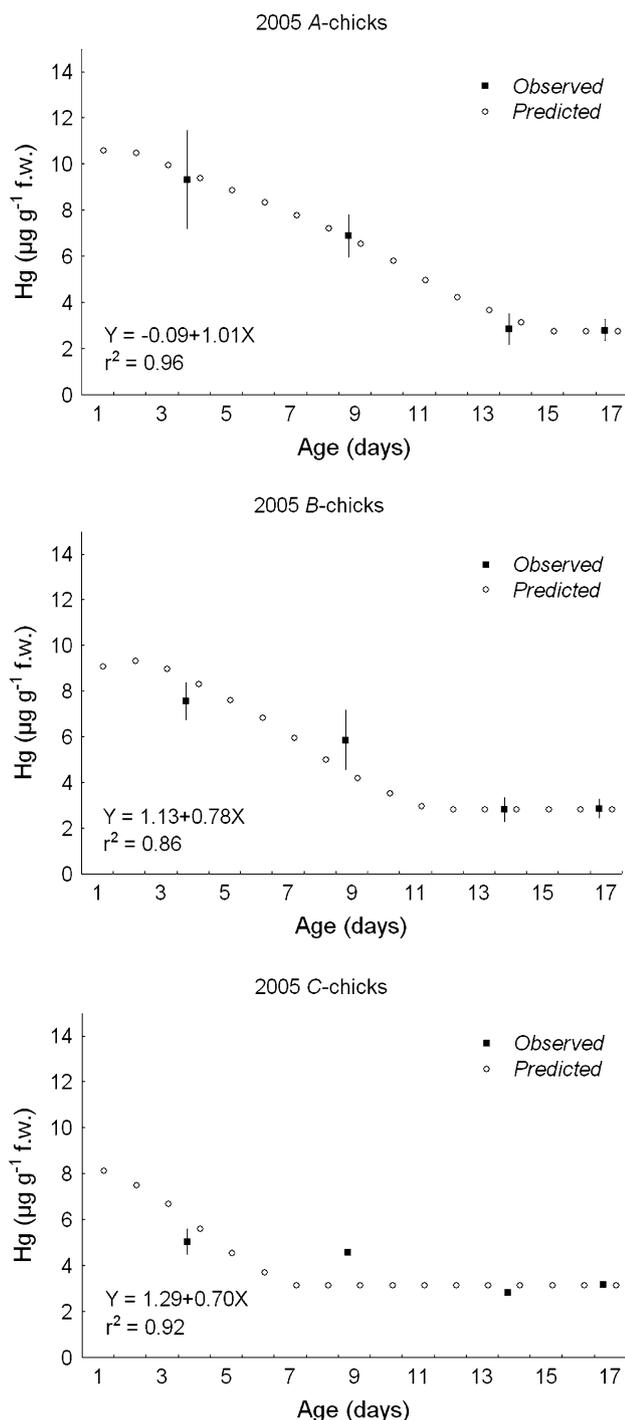


Fig. 4 Graphical representation of observed data (collected in 2005) and model predictions for mercury concentration (fresh weight; f.w.) in feathers of A-, B-, and C-chicks as they aged. Also shown are the regression results and equations for the three types of chicks within the brood (model calibration)

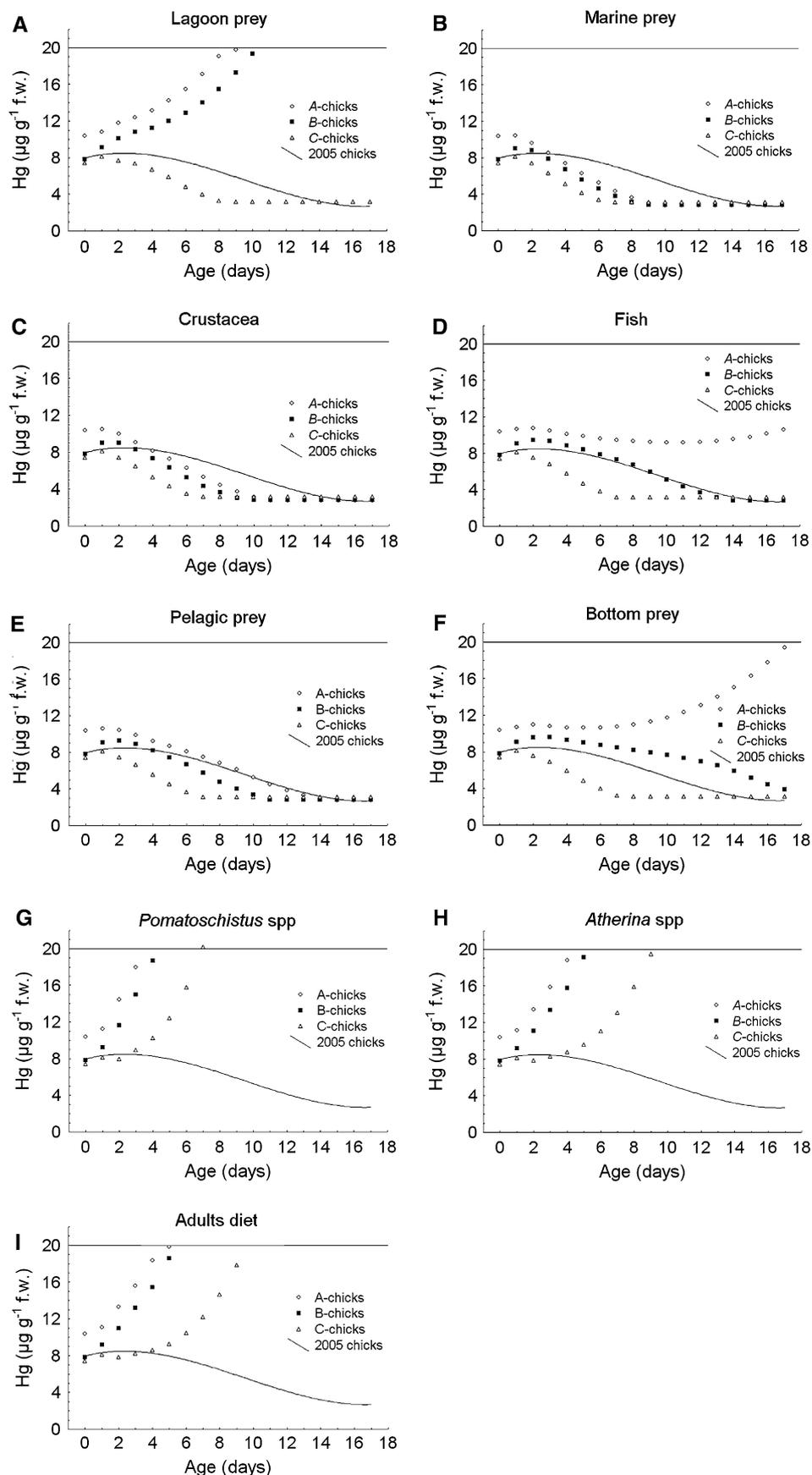
that of chicks raised in salinas. This difference was mainly explained by the significantly higher mercury levels of chicks from Armona barrier island. In chicks <5 days of age, mercury levels were related not only to items

delivered by their parents (Goutner and Furness 1997) but also to mercury burdens present in their eggs (transmitted from their female parents [Becker et al. 1993]). Our results may be explained by the fact that adults breeding on Armona barrier island foraged much more in the lagoon than in the sea (Paiva et al. 2007). The same did not occur for other sandy beaches, Barreta barrier island, or Ancão peninsula, where birds were frequently sighted feeding at sea (Paiva et al. 2007; personal observation). Adult foraging around a sanitary discharge on the mainland in front of Armona may also explain the higher mercury content of chicks from Armona.

Overall, our results from chick feathers that mainly fed on prey from the lagoon are in agreement with the higher concentration of mercury present in prey from the lagoon than in prey from the adjacent sea. In Ria Formosa and other Portuguese wetlands typical prey groups from the lagoon (mostly benthic) had significantly higher levels of mercury than prey groups from the adjacent sea (mostly pelagic). According to Braune (1987), birds feeding on pelagic invertebrates accumulate significantly lower mercury levels than birds feeding on benthic organisms or piscivorous birds. This is associated with mercury bioamplification along the food web. A high degree of variation may also be found within piscivorous birds. The coefficient between predator and prey contamination levels, the biomagnification factor (BMF), given as the ratio between the concentration in the tissues of an organism and that in respective prey(s), may vary with species, habitat, prey availability, age, and other factors influencing type of prey captured (Gray 2002). Several studies have related potential prey, trophic level, and mercury levels. As the mercury bioaccumulates in prey, its concentration increases in predator species (Jarman et al. 1996; Muir et al. 1999; Dietz et al. 2000; Borga et al. 2006), revealing a deeper gradient in the water column from the pelagic area to the benthic zone (Thompson et al. 1998; Monteiro et al. 1999). Monteiro and Furness (2001) estimated that a dose of approximately 1 µg Hg f.w. in food items was necessary to increase by a factor of nine the feather's burden of mercury (0.8 µg g⁻¹ f.w.) in chicks of *Calonectris diomedea*. Monteiro et al. (1998) observed that mean values for BMF varied between 125 and 225 in several seabirds and that the mean value for BMF in *Calonectris diomedea* was 132. In our study, little tern chicks feeding on prey with mercury levels of 0.03–0.31 µg g⁻¹ d.w. revealed mercury levels in feathers of 1.94–16.53 µg g⁻¹ f.w., which gives a BMF varying from 53.32 to 64.67.

The mercury concentration in feathers decreased strongly from A- to B- and C-chicks, as they aged from 0 to 17 days. In addition, the mercury concentration in feathers of newborn little tern A-chicks (at their hatching age, 0 days) was significantly higher than that in newborn B-

Fig. 5 Various theoretical scenarios of the amount of mercury (fresh weight; f.w.) that little tern A-, B-, and C-chicks might allocate to their breast feathers if their diet could be restricted to some prey items. The typical logistic mercury decline curve for 2005 chicks is presented for comparison. The solid top line represents the maximum value of $20 \mu\text{g g}^{-1}$ f.w. of mercury in feathers detected in 2005 in the salinas of Sta. Luzia. Lagoon prey: *Pomatoschistus* spp (POM), *Fundulus* spp (FUN), and *Atherina* spp (ATH). Marine prey: *Sardina pilchardus* (SAR), *Belone belone* (BEL), and *Scomberesox saurus* (SCO). Crustacea (shrimp; SHR): *Paleomon* spp. and *Paleomonetes* spp. Pelagic prey: SAR, ATH, BEL, and SCO. Bottom prey: POM and FUN. Adult diet: 45.9% POM, 40.5% ATH, and 13.6% SHR



and C-chicks (Becker et al. 1994), strengthening the idea that the mother excretes the majority of accumulated mercury into the first egg laid, as subsequent eggs normally have a much lower mercury content (Becker 1992). This high level of mercury could be supplanted by a higher dilution effect generated by the faster body growth in the case of A-chicks (Paiva et al. 2006a). A-chicks were fed alone for almost 1 day (Paiva et al. 2006b) and therefore assimilated all food delivered by parents. Also, larger chicks may exhibit advantages over other chicks during food delivery. Little tern chicks revealed a large reduction in mercury concentration with age in relation to other birds, e.g., aquatic birds like *Ardeola ralloides* (Goutner et al. 2001) or seabirds such as *Sterna paradisaea*, *Rissa tridactyla*, and *Uria aalge* (Stewart et al. 1997), but were similar to their Azorean relatives (*Sterna dougallii* and *Sterna hirundo* [Monteiro et al. 1995]).

We successfully modeled mercury decline in feathers of growing A-, B-, and C-chicks. This allowed us to examine some diet scenarios by manipulating the diet inputs (changing the quantity and quality of prey items) and examining the amount of mercury content in plumage. Other studies used several seabird species to establish relationships between diet and body mercury concentrations (Monteiro et al. 1998) or manipulated the diet of chicks raised partly or totally in captivity (e.g., Monteiro and Furness 2001; Kenow et al. 2003). Naturally, the model was more sensitive to parameters related to the mercury decline formula, namely, the constant that drove mercury levels in feathers and the intercept value of that formula. The number of hours that parents have available to actively feed their chicks was also important to explain mercury levels in chicks, as this will linearly increase the numbers of prey delivered and therefore the amount of mercury that chicks receive. We used a constant number of daylight hours for parents to deliver food at a constant rate, which varied among prey species. This does not match the reality because parents will probably reduce their feeding rate when chicks are well-fed. The only way to better simulate reality would be to account for chicks being food-limited. Data on the amount of food that drives chicks to stop begging were not available for this species. Theoretical scenarios tested with the constructed mercury model agreed with the results of mercury contamination in the different prey species groups, suggesting that, for example, if chicks were raised only on lagoon prey, they might encounter problems in their condition. At least A- and B-chicks would reveal adverse effects if they were fed solely with lagoon prey, as mercury contamination would have not been diluted through excretion to feathers and body growth as referred to by Becker et al. (1994). On the contrary, chicks will not experience large adverse effects if they are fed with prey captured in the sea, which are also

the most important (in energetic terms) for their growth (Paiva et al. 2006b).

The importance of salinas as alternative breeding and feeding sites (Catry et al. 2004; Paiva et al. 2006b) is highlighted by the effect that crustacean prey from salinas (*Paleomonetes* spp.) had on chicks' mercury levels, which results from the balance between mercury intake by food ingestion and the dilution effect of growth, compared to a diet restricted only to fish (at least A-chicks will suffer from a fish-only diet). Becker et al. (2002) discussed the effect of the ingestion of crustacean (mainly shrimp) in decreasing the amount of mercury input by food intake in chicks. Seabird chicks such as those of little terns need to be supplied with a specific and varied diet, different from that of their parents (Catry et al. 2006). This has been attributed to their developmental restriction to ingest only smaller and thinner prey items (Bogliani et al. 1994; Ramos et al. 1998; Paiva et al. 2006a). However, our model also suggests that if little tern chicks were fed solely on a diet similar to that of the adults, they would ingest such high mercury levels that their normal development could be affected. This is because *Pomatoschistus* spp. and *Atherina* spp., the most important prey for adults (Catry et al. 2006), had the highest mercury concentration.

Studies relying on molecular biomarkers have contributed new information on mercury effects at a cellular level. Mercury concentrations between 2 and 4 $\mu\text{g g}^{-1}$ d.w. were associated with DNA damage in organisms of lower trophic levels like invertebrates (Benton et al. 2002). Moreover, the relation between contaminants like mercury and the variation of DNA content in great blue heron eggs was investigated (Custer et al. 1997). Other studies have already considered that mercury levels $\geq 5 \mu\text{g g}^{-1}$ d.w. in feathers may represent deleterious effects in birds' metabolism (Burger and Gochfeld 1997).

Conclusions

The findings of this study raise again the importance of pelagic marine prey for estuarine birds, not only for their "normal" growth (Paiva et al. 2006b) but also for provision of a food resource with lower contamination levels. This enhances the importance of sandy beaches for breeding little terns (Catry et al. 2004), where birds have better access to marine prey (Paiva et al. 2006b). Crustacean prey obtained in salinas, *Paleomonetes* spp., are less important in energetic terms (Paiva et al. 2006b), but they may also contribute to the lower mercury levels of chicks raised in salinas. Prey captured in the lagoon, mainly benthic fish, may be considered an important contributor to flux of mercury to chicks of little terns breeding in salinas. The contribution of this type of food may have

consequences, in particular, during the first period (from 0 to 4 days) of chick growth, especially for *A*- and *B*-chicks, to which mercury levels may be harmful.

The fast growing rates in little tern chicks and the related dilution effect seem to decrease mercury levels, which may prevent some deleterious effects in completely grown chicks if the habitat is not largely polluted. However, if pollution levels are high and persistent in the lagoon, and chicks are mainly fed prey from the lagoon, the mercury entering the chick body by food ingestion may not be sufficiently balanced by the dilution effect. This model may have a wider application in similar seabird species breeding in coastal environments.

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