

SUPPORTING INFORMATION

Tracing waterbird exposure to total mercury and selenium: a case study at the solar saltworks of Thyna (Sfax, Tunisia)

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INCLUDING MATERIAL

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- 16 **Page S3.- The saltworks of Thyna.** Detailed information on the study area and on the
- 17 potential sources of contaminants affecting the area.
- 18 **Page S3.- The strategy of sampling eggs.** Detailed information on sampling strategy.
- 19 **Pages S4-S5.- Stable isotope and total mercury and selenium determination.**
- 20 Detailed information of methodologies employed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and THg and Se
- 21 determinations.
- 22 **Pages S6-S7.- Reconstructing bird diets.** Details of the alternative mass-balanced
- 23 mixing model applied in this study.
- 24 **Figure S1.-** The saltworks of Thyna and detail of the eight different points where
- 25 Mediterranean Toothcarps were sampled.
- 26 **Figure S2.-** Trends for fish concentrations of Se across the salinity gradient.
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THE SALTWORKS OF THYNA

The saltworks of Thyna (central-eastern coast of Tunisia, 34°39'N 10°42'E) cover ~1500ha along 13Km of the Mediterranean coast, are separated from the sea by a seawall and consist of a series of interconnecting shallow ponds (20-70cm) of different and increasing salinity, from that of seawater (~34g/L) to saturation. They are surrounded by the industrial zone of Sfax, which is home to a soap factory, a lead foundry, and the Phosphogypsum and Manure Production Factory SIAPE (Société Industrielle d'Acide Phosphorique et d'Engrais). Industrial activity surrounding the area results in a flow of leachate enriched in hydrated calcium sulfate, hydrofluoric acid, and salts of several metals to the adjacent marine environment (the Gulf of Gabes, 1, 2).

THE STRATEGY OF SAMPLING EGGS

The study was based on egg sampling, which have been considered suitable material to monitor bird exposure to pollutants in their breeding areas (3) since their chemical compositions mainly reflects that of the female diet during the period of clutch formation, at least in those birds with an income strategy for breeding (i.e. those that mainly adjust their food intake concurrently with breeding, without reliance on stores, see 4). In this regard, waterbirds have been widely considered to mainly rely on exogenous resources for clutch production (reviewed in 5). On the other hand, the strong effect of female body burden on egg contaminant concentrations validates the strategy of sampling one egg per nest (6). Thus, sampling was restricted to a single egg per nest to avoid pseudoreplication.

STABLE ISOTOPE AND TOTAL MERCURY AND SELENIUM DETERMINATIONS

Subsamples of powdered egg contents or fish were weighed to the nearest μg and placed into tin capsules for carbon and nitrogen isotope ratio determination. Isotope analyses were carried out at the Serveis Científic-Tècnics of the University of Barcelona (Spain) by means of elemental analysis-isotope ratio mass spectrometry using a ThermoFinnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyzer coupled to a delta isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Finnigan MAT, Bremen, Germany) and applying IAEA standards (IAEA CH_6 , IAEA CH_7 and USGS 24 for C, and IAEA N1, IAEA N2 and IAEA NO_3 for N) to calibrate the system. Stable isotope ratios were expressed in the standard δ -notation (‰) relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$). Replicate assays of standards indicated analytical measurement errors of $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$.

THg and Se concentrations were determined at the Serveis Científic-Tècnics of the University of Barcelona (Spain) following an acid digestion protocol: $\sim 0.1\text{g}$ of sample was digested by 3ml of HNO_3 and 2ml of H_2O_2 using a Teflon reactor for 12 hours at 90°C . The digested product was diluted in 15ml of distilled water and trace elements were determined by ICP-MS (Induction Coupled Plasma-Mass Spectrometer, Perkin Elmer Optima 6000). The accuracy of the analysis was checked by measuring CRM certified reference tissues (Human Hair CRM 397, Lobster hepatopancreas Tort-2 and Dogfish liver Dolt-3; National Research Council Canada). Mean recoveries on reference tissues account for 102% for THg and 101% for Se, and no corrections were applied to the original results. THg and Se concentrations are expressed in $\mu\text{g/g}$ dw. ICP-MS LOD

76 values were 0.2ppb for THg and 1ppb for Se, which correspond approximately to
77 0.18µg/g dw (THg) and 0.95µg/g dw (Se) in the tissue samples analyzed. Routinely,
78 concentrations of lead (Pb) and cadmium (Cd) are also determined at this laboratory.
79 However, these pollutants show little transfer from females to eggs (7), and were
80 detected neither for egg contents, nor for fish. Present work is therefore based on
81 obtained results for THg and Se concentrations.

RECONSTRUCTING BIRD DIETS

Average contribution of different dietary resources (f_i) to the diet of Common Terns and Slender-billed Gulls were estimated through a dual-isotope ($\delta^{13}\text{C}$ - $\delta^{15}\text{N}$), three end-point (marine fish, saltworks fish and saltworks invertebrates) mixing model, according to the following equation system (see 8):

$$\delta^{13}\text{C}_M = f_A \cdot \delta^{13}\text{C}_A + f_B \cdot \delta^{13}\text{C}_B + f_C \cdot \delta^{13}\text{C}_C$$

$$\delta^{15}\text{N}_M = f_A \cdot \delta^{15}\text{N}_A + f_B \cdot \delta^{15}\text{N}_B + f_C \cdot \delta^{15}\text{N}_C$$

$$f_A + f_B + f_C = 1$$

where subscripts refer to mixture (M) and dietary resources (A, B and C). Average isotopic values for marine fish, saltworks fish and saltworks invertebrate predators (*i.e.* Yellow-legged Gulls, Little Egrets and Pied Avocets, respectively) were incorporated as isotopic end-points instead of the isotopic composition of potential dietary sources. In this way, we aimed to integrate the isotopic variability for different dietary sources while avoiding the use of isotope discrimination factors, which in the case of eggs are particularly scarce frustrating their applicability in the wild (9, 10). In addition, dietary reconstructions were performed at the individual level, which is particularly advantageous since population responses are ultimately determined by individual decisions (11 - 13). In this regard, the isotopic composition of eggs likely reflects daily dietary intake during the period of clutch production. Thus, mixing models can be applied at the individual level (14) avoiding typical biases of conventional methods due to the short time period over which dietary information can be inferred (generally <1day, 15).

To ensure the reliability of our mixing paradigm, *i.e.* to evaluate whether the isotopic composition of the mixtures could be matched through any combination of isotopic values for dietary end-points, we estimated 95% confidence intervals (95% CI) for individual dietary contributions (f_i) following (8). Briefly, a first order Taylor series approximation was used to evaluate the variance of estimated f_i ($\sigma_{f_i}^2$) which accounted for the isotopic variability of dietary end-points (standard deviation of isotopic values for Yellow-legged Gulls, Little Egrets and Pied Avocets). Approximate 95% CI were then constructed as $f_i \pm t_{0.05, \gamma} \sigma_{f_i}$, where σ_{f_i} was the square root of $\sigma_{f_i}^2$ and $t_{0.05, \gamma}$ represented the two-tailed Student's t for $\alpha=0.05$ and γ degrees of freedom calculated using Satterthwaite approximation (16). We considered that individual dietary reconstructions would be unreliable whenever estimated 95% CI for the most negative and the most positive contributions were $<0\%$ and $>100\%$, respectively (which would indicate that consumers isotopic values fell significantly outside of the mixing triangle). Otherwise, negative contributions predicted by the model would be readjusted by setting the most negative values to 0% and recomputing the other percentages according to the original proportions (14).

Figure S1.- The saltworks of Thyna consist of a series of interconnecting shallow ponds differing in their salinities. Eight different sampling points (differing in salinities) were chosen for the study in order to capture Mediterranean toothcarps. Salinity was measured using a multi-field meter WTW multi 340i, Geotech Environmental Equipment, Colorado, USA). Grey scale in ponds refers to the two ranges of salinities (> and < of 60g/L) for which fish' $\delta^{13}\text{C}$ values varied likely due to inorganic carbonate mineral precipitation (17, 18).

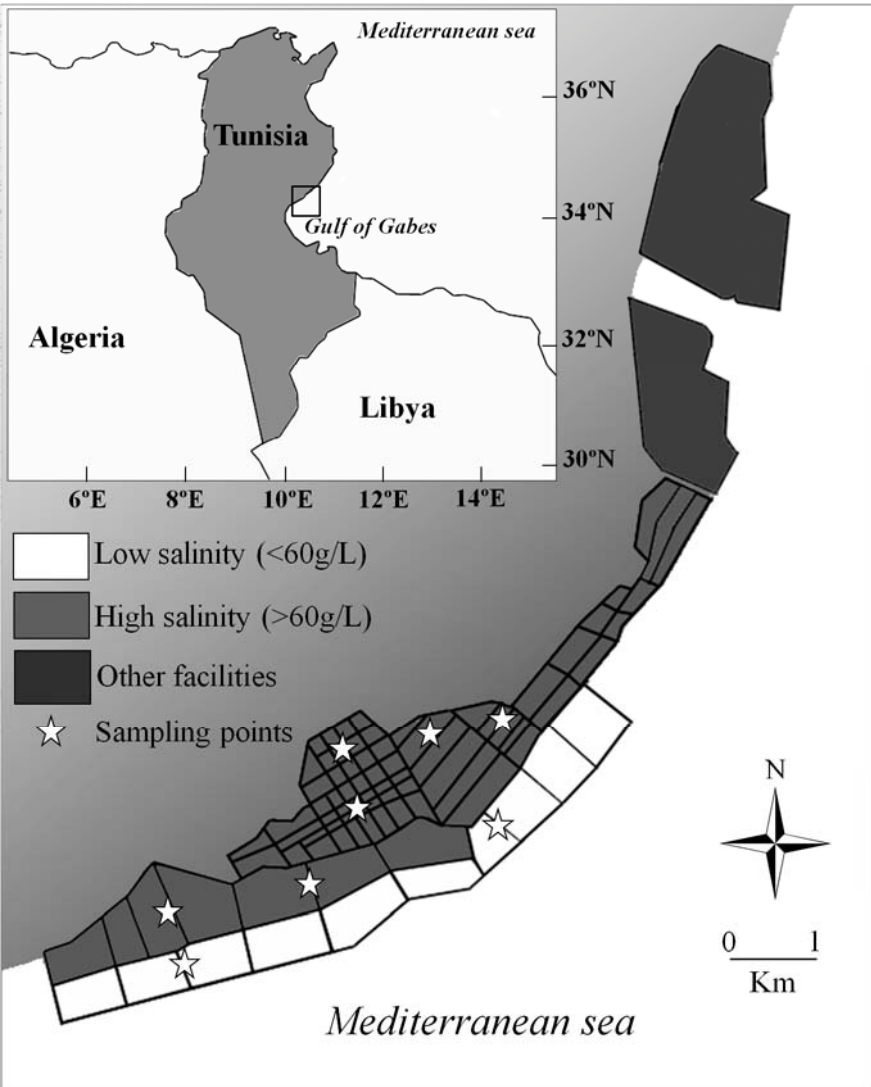
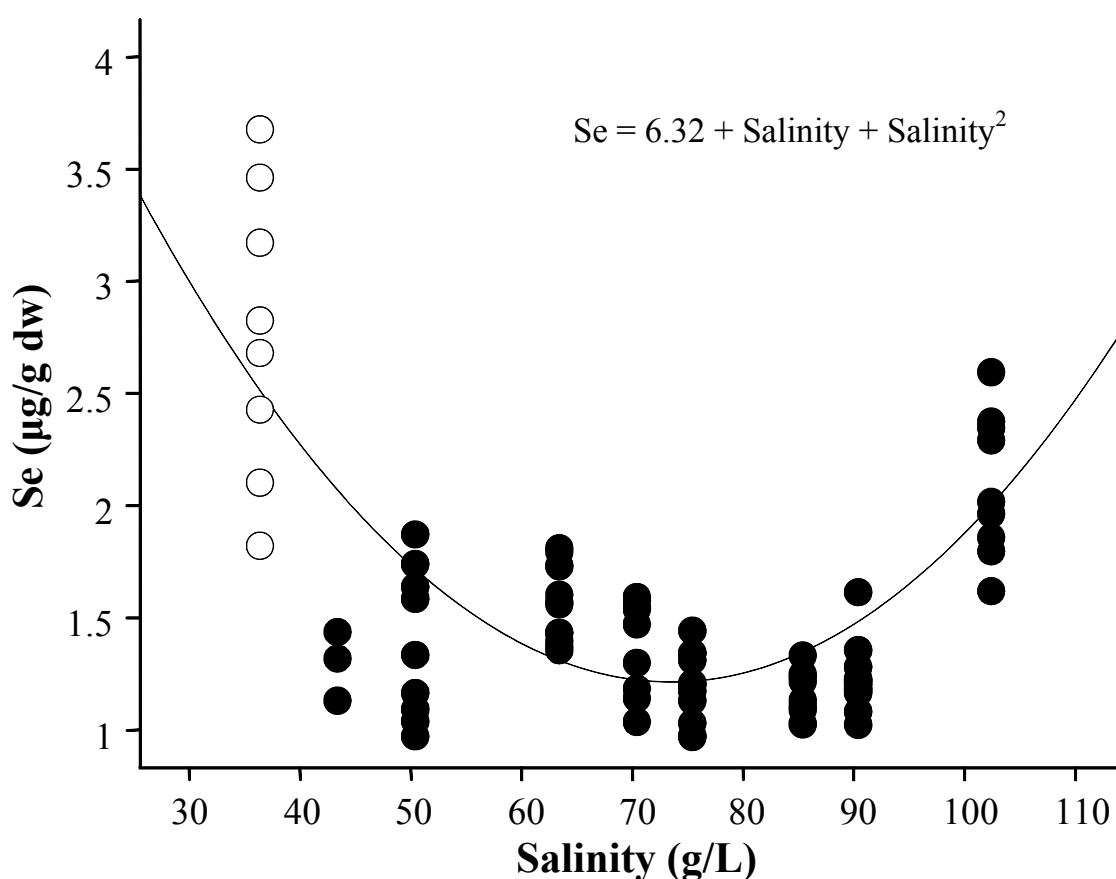


Figure S2.- Trends for fish concentrations of Se across the salinity gradient. Solid circles correspond to concentrations of Se for a pool of Mediterranean toothcarps (10 individuals per pool) randomly selected (the number of pools per sampling point has been summarized in Table 1). Concentrations of Se for marine fish (bogues and sardines) sampled in the Gulf of Gabes are also represented (open circles).



139 **Table S1.-** Relative contributions (Mean and 95% Confidence interval) of marine fish (MF), saltworks fish (SWF) and saltworks invertebrates
140 (SWI) to the diet of individuals within Slender-billed Gulls (SBG) and Common Terns (CT) predicted by the mixing model (see Materials &
141 methods for parameters incorporated in the model).

sp	iden	Mean			95% Confidence Interval					
		MF	SWF	SWI	upper 95% CI (MF)	lower 95% CI (MF)	upper 95% CI (SWF)	lower 95% CI (SWF)	upper 95% CI (SWI)	lower 95% CI (SWI)
SBG	SBG_01	0.47	0.09	0.43	0.61	0.34	0.19	0.00	0.51	0.36
	SBG_02	0.31	0.30	0.39	0.45	0.18	0.39	0.21	0.45	0.32
	SBG_03	0.18	0.57	0.24	0.33	0.04	0.67	0.48	0.32	0.17
	SBG_04	0.98	0.21	-0.18	1.18	0.77	0.36	0.06	-0.06	-0.31
	SBG_05	-0.42	0.97	0.44	-0.26	-0.57	1.09	0.85	0.55	0.34
	SBG_06	0.05	0.76	0.19	0.21	-0.10	0.87	0.65	0.27	0.10
	SBG_07	0.25	0.39	0.36	0.39	0.12	0.47	0.30	0.43	0.30
	SBG_08	0.21	0.68	0.11	0.37	0.05	0.79	0.57	0.19	0.03
	SBG_09	0.09	0.73	0.18	0.24	-0.06	0.83	0.62	0.26	0.10
	SBG_10	0.23	0.50	0.27	0.37	0.09	0.60	0.41	0.34	0.20
	SBG_11	0.14	0.70	0.16	0.29	-0.02	0.81	0.60	0.24	0.08
	SBG_12	0.34	0.38	0.28	0.48	0.20	0.48	0.29	0.35	0.21
	SBG_13	-0.06	0.70	0.35	0.09	-0.20	0.80	0.61	0.43	0.27
	SBG_14	-0.20	1.03	0.17	-0.03	-0.37	1.15	0.91	0.27	0.07
	SBG_15	0.24	0.38	0.38	0.37	0.11	0.46	0.29	0.45	0.32
	SBG_16	-0.12	0.24	0.88	-0.03	-0.22	0.31	0.17	0.94	0.82
	SBG_17	1.03	0.09	-0.12	1.23	0.82	0.24	-0.06	0.01	-0.24

sp	iden	Mean			95% Confidence Interval					
		MF	SWF	SWI	upper 95% CI (MF)	lower 95% CI (MF)	upper 95% CI (SWF)	lower 95% CI (SWF)	upper 95% CI (SWI)	lower 95% CI (SWI)
CT	CT_01	0.72	0.23	0.05	0.89	0.55	0.35	0.11	0.15	-0.04
	CT_02	0.55	0.61	-0.16	0.74	0.37	0.73	0.48	-0.06	-0.26
	CT_03	0.36	0.77	-0.13	0.54	0.18	0.89	0.65	-0.04	-0.23
	CT_04	0.84	0.33	-0.17	1.03	0.64	0.47	0.19	-0.06	-0.28
	CT_05	0.46	0.70	-0.15	0.64	0.27	0.82	0.57	-0.06	-0.25
	CT_06	0.44	0.72	-0.17	0.63	0.26	0.85	0.60	-0.07	-0.27
	CT_07	0.40	0.64	-0.04	0.57	0.23	0.75	0.52	0.05	-0.13
	CT_08	1.20	0.06	-0.26	1.43	0.98	0.23	-0.10	-0.12	-0.41
	CT_09	0.50	0.61	-0.11	0.68	0.32	0.73	0.49	-0.01	-0.20
	CT_10	0.91	0.30	-0.20	1.10	0.71	0.44	0.15	-0.08	-0.32
	CT_11	1.23	0.02	-0.25	1.45	1.00	0.19	-0.15	-0.10	-0.39
	CT_12	0.97	0.37	-0.34	1.18	0.76	0.52	0.21	-0.21	-0.47
	CT_13	0.68	0.66	-0.33	0.88	0.47	0.80	0.52	-0.22	-0.45

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