

Supplementary Material

Sources of Hg in aquatic top-predators in the Ebro Delta (NE Iberian Peninsula) by means of mercury stable isotopes: inter-specific and temporal differences

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Hg isotopic composition in feather samples of *I. audouinii*, *C. ridibundus* and *S. hirundo* fledglings

Results for Hg isotopic compositions are reported using the standard delta notation (in ‰) relative to the standard NIST 3133, according to the following equation (Bergquist and Blum 2007):

$$\delta^{xxx}\text{Hg} = [({}^{xxx}\text{Hg}/{}^{198}\text{Hg})_{\text{sample}}/({}^{xxx}\text{Hg}/{}^{198}\text{Hg})_{\text{NIST3133}} - 1] \times 1000$$

where, ‘xxx’ represents the mass number of Hg (199, 200, 201, 202, 204), $({}^{xxx}\text{Hg}/{}^{198}\text{Hg})_{\text{sample}}$ is the isotope ratio measured for the sample and $({}^{xxx}\text{Hg}/{}^{198}\text{Hg})_{\text{NIST3133}}$ is the mean isotope ratio for the standard NIST 3133. In this study, mass-dependent fractionation (MDF) is reported as $\delta^{202}\text{Hg}$ signature:

$$\delta^{202}\text{Hg} = [({}^{202}\text{Hg}/{}^{198}\text{Hg})_{\text{sample}}/({}^{202}\text{Hg}/{}^{198}\text{Hg})_{\text{NIST3133}} - 1] \times 1000$$

Mass-independent Hg isotopic fractionation (MIF) is reported using the capital delta notation, as $\Delta^{199}\text{Hg}$, $\Delta^{200}\text{Hg}$, $\Delta^{201}\text{Hg}$, $\Delta^{204}\text{Hg}$ (‰), calculated using the difference by the $\delta^{xxx}\text{Hg}$ value and a value predicted based on MDF (Bergquist and Blum 2007):

$$\Delta^{204}\text{Hg} \approx \delta^{204}\text{Hg} - (\delta^{202}\text{Hg} \times 1.493)$$

$$\Delta^{201}\text{Hg} \approx \delta^{201}\text{Hg} - (\delta^{202}\text{Hg} \times 0.752)$$

$$\Delta^{200}\text{Hg} \approx \delta^{200}\text{Hg} - (\delta^{202}\text{Hg} \times 0.502)$$

$$\Delta^{199}\text{Hg} \approx \delta^{199}\text{Hg} - (\delta^{202}\text{Hg} \times 0.252)$$

Where $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ are known as odd-MIF, and $\Delta^{200}\text{Hg}$ and $\Delta^{204}\text{Hg}$ as even-MIF.

Table S1. Measurements of Hg concentrations (THg ng/g dw), C, N and Hg stable isotopes signatures (‰) in feathers of fledglings from three Laridae species breeding in the Ebro Delta during the 2017 breeding season. Species codes: IA (*I. audouinii*, Audouin's gull), CR (*C. ridibundus*; black-headed gull), SH (*S. hirundo*; common tern).

Sample ID	Species	Year	$\delta^{204}\text{Hg}$	$\delta^{202}\text{Hg}$	$\delta^{201}\text{Hg}$	$\delta^{200}\text{Hg}$	$\delta^{199}\text{Hg}$	$\Delta^{204}\text{Hg}$	$\Delta^{201}\text{Hg}$	$\Delta^{200}\text{Hg}$	$\Delta^{199}\text{Hg}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	[THg]
LA-EDB17-12	IA	2017	0.35	0.16	0.86	0.03	1.06	0.12	0.74	-0.05	1.02	-16.83	14.22	7720.26
LA-EDB17-13	IA	2017	0.45	0.31	1.09	0.13	1.19	-0.02	0.85	-0.03	1.11	-17.16	13.46	8728.11
LA-EDB17-16	IA	2017	0.33	0.22	0.54	0.12	0.65	0.00	0.38	0.01	0.60	-19.69	13.28	8318.27
LA-EDB17-17	IA	2017	0.34	0.29	0.92	0.11	0.96	-0.08	0.70	-0.03	0.89	-16.91	13.66	9205.55
LA-EDB17-21	IA	2017	0.51	0.36	1.02	0.28	1.13	-0.03	0.75	0.1	1.04	-17.62	13.83	6439.79
LA-EDB17-23	IA	2017	0.57	0.43	1.12	0.27	1.04	-0.07	0.8	0.05	0.93	-17.81	13.55	6945.61
LA-EDB17-24	IA	2017	0.23	0.11	0.81	0.11	0.96	0.07	0.73	0.05	0.93	-17.30	13.81	7200.69
LA-EDB17-26	IA	2017	0.46	0.32	0.86	0.11	0.84	-0.02	0.62	-0.05	0.76	-18.04	13.94	9072.00
LA-EDB17-29	IA	2017	0.36	0.29	0.89	0.19	1.00	-0.08	0.68	0.04	0.93	-17.89	13.42	7533.32
LA-EDB17-30	IA	2017	0.20	0.13	0.63	0.07	0.72	0.01	0.54	0.00	0.69	-17.28	14.52	8666.11
LR-EDB17-01	CR	2017	0.93	0.65	1.39	0.38	1.28	-0.05	0.90	0.05	1.12	-19.71	13.79	3494.11
LR-EDB17-03	CR	2017	0.78	0.46	1.12	0.29	1.13	0.10	0.78	0.06	1.02	-22.29	13.65	1741.96
LR-EDB17-05	CR	2017	0.74	0.52	0.94	0.30	0.90	-0.04	0.54	0.03	0.77	-22.50	13.05	1337.37
LR-EDB17-08	CR	2017	0.58	0.38	0.88	0.23	0.86	0.01	0.59	0.04	0.77	-21.98	12.86	1213.24
LR-EDB17-09	CR	2017	0.55	0.37	1.13	0.16	1.11	-0.01	0.84	-0.02	1.01	-19.32	14.80	1325.87
LR-EDB17-11	CR	2017	0.59	0.43	1.12	0.26	1.05	-0.04	0.8	0.04	0.95	-21.64	12.82	1263.41
LR-EDB17-13	CR	2017	0.91	0.64	1.61	0.31	1.50	-0.04	1.13	-0.01	1.34	-20.22	14.19	1204.44
LR-EDB17-17	CR	2017	0.79	0.58	1.05	0.31	1.00	-0.08	0.61	0.01	0.86	-22.19	12.83	1549.64
LR-EDB17-18	CR	2017	1.37	0.84	1.65	0.39	1.50	0.11	1.01	-0.03	1.29	-21.48	13.29	1301.05
LR-EDB17-20	CR	2017	1.17	0.80	1.24	0.39	1.08	-0.02	0.63	-0.02	0.88	-21.93	13.76	1128.31
SH-EDB17-01	SH	2017	1.70	1.15	3.48	0.60	3.42	-0.02	2.61	0.03	3.13	-16.96	12.04	1381.47
SH-EDB17-05	SH	2017	1.37	1.02	3.05	0.54	2.98	-0.15	2.28	0.03	2.72	-16.82	12.38	2108.95
SH-EDB17-09	SH	2017	1.67	1.13	3.08	0.57	3.01	-0.01	2.23	0.00	2.73	-16.78	12.11	2100.57
SH-EDB17-12	SH	2017	1.55	1.03	3.22	0.62	3.13	0.01	2.44	0.10	2.87	-17.27	11.73	1793.80
SH-EDB17-16	SH	2017	1.47	1.02	3.12	0.55	3.11	-0.05	2.35	0.03	2.85	-16.58	11.64	1737.98
SH-EDB17-17	SH	2017	1.38	0.94	2.94	0.50	2.90	-0.03	2.23	0.03	2.67	-16.73	12.05	1933.22

SH-EDB17-20	SH	2017	1.65	1.20	3.17	0.66	3.12	-0.14	2.27	0.05	2.82	-16.60	12.03	1944.26
SH-EDB17-23	SH	2017	1.38	0.94	2.33	0.54	2.22	-0.02	1.62	0.07	1.98	-18.06	12.00	2379.45
SH-EDB17-24	SH	2017	1.60	1.13	3.07	0.60	3.05	1.54	2.22	0.03	2.76	-18.13	13.15	1661.40
SH-EDB17-28	SH	2017	1.45	0.97	2.78	0.52	2.75	0.00	2.05	0.04	2.50	-16.57	12.50	2120.32

Table S2. Measurements of Hg concentrations (THg ng/g dw), C, N and Hg stable isotopes signatures (‰) in feathers of fledglings from Audouin's gull (*I. audouinii*) breeding in the Ebro Delta during the 2010, 2013, 2014, 2017 breeding seasons.

Sample ID	Year	$\delta^{204}\text{Hg}$	$\delta^{202}\text{Hg}$	$\delta^{201}\text{Hg}$	$\delta^{200}\text{Hg}$	$\delta^{199}\text{Hg}$	$\Delta^{204}\text{Hg}$	$\Delta^{201}\text{Hg}$	$\Delta^{200}\text{Hg}$	$\Delta^{199}\text{Hg}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	[THg]
LA-EDB10-01	2010	0.30	0.28	1.15	0.17	1.25	-0.13	0.94	0.03	1.18	-18.17	12.77	3488.29
LA-EDB10-02	2010	0.79	0.55	1.54	0.37	1.47	-0.03	1.13	0.10	1.33	-17.76	12.48	2438.68
LA-EDB10-03	2010	0.38	0.27	1.18	0.11	1.27	-0.02	0.98	-0.02	1.20	-17.94	12.56	4300.13
LA-EDB10-04	2010	0.63	0.42	1.23	0.26	1.23	0.00	0.91	0.05	1.12	-18.09	12.39	4854.90
LA-EDB10-05	2010	0.37	0.41	1.20	0.23	1.32	-0.23	0.90	0.02	1.22	-17.57	13.08	2889.75
LA-EDB10-06	2010	0.90	0.82	1.77	0.45	1.63	-0.33	1.15	0.04	1.42	-18.73	12.58	5786.12
LA-EDB10-07	2010	1.19	0.88	1.65	0.51	1.49	-0.12	0.99	0.07	1.27	-17.46	13.27	5044.57
LA-EDB10-08	2010	1.23	0.75	1.73	0.39	1.58	0.12	1.16	0.01	1.39	-18.08	12.47	3635.68
LA-EDB10-09	2010	1.21	0.76	1.46	0.41	1.32	0.07	0.89	0.03	1.13	-17.83	12.76	4005.45
LA-EDB10-10	2010	1.14	0.78	1.74	0.44	1.64	-0.03	1.15	0.05	1.45	-18.11	12.38	6441.93
LA-EDB13-01	2013	0.31	0.32	1.20	0.16	1.31	-0.17	0.96	-0.01	1.23	-24.10	12.36	1588.99
LA-EDB13-04	2013	0.11	0.11	0.94	0.07	1.06	-0.06	0.85	0.01	1.03	-19.69	12.62	2471.91
LA-EDB13-06	2013	0.32	0.14	1.09	0.03	1.29	0.12	0.99	-0.04	1.25	-22.76	12.47	1607.24
LA-EDB13-07	2013	0.17	0.14	0.93	0.12	1.03	-0.04	0.82	0.05	0.99	-23.08	13.14	1655.81
LA-EDB13-10	2013	0.09	0.11	0.79	0.02	0.92	-0.07	0.71	-0.03	0.89	-19.84	12.67	2441.67
LA-EDB13-11	2013	0.37	0.29	1.00	0.12	1.11	-0.07	0.78	-0.03	1.04	-17.26	13.04	2710.85
LA-EDB13-12	2013	0.11	0.09	1.07	0.03	1.20	-0.04	1.00	-0.02	1.18	-17.95	12.44	2572.82
LA-EDB13-13	2013	0.12	0.18	1.09	0.10	1.26	-0.15	0.95	0.01	1.22	-18.00	12.53	2183.09
LA-EDB13-14	2013	-0.05	0.00	0.67	0.02	0.86	-0.05	0.67	0.02	0.86	-17.97	12.35	2427.23
LA-EDB13-15	2013	0.09	0.09	0.83	0.04	0.87	-0.04	0.76	-0.01	0.85	-17.58	12.89	2616.27
LA-EDB14-01	2014	0.70	0.50	1.33	0.31	1.29	-0.04	0.95	0.06	1.16	-16.40	13.43	7599.76
LA-EDB14-05	2014	0.86	0.57	1.42	0.31	1.36	0.02	0.99	0.03	1.22	-16.50	14.09	6619.52
LA-EDB14-06	2014	0.64	0.46	1.05	0.25	1.08	-0.04	0.71	0.02	0.96	-18.80	13.92	6421.69
LA-EDB14-07	2014	0.87	0.57	2.00	0.29	1.63	0.02	1.57	0.01	1.49	-18.20	13.41	11842.48
LA-EDB14-09	2014	0.74	0.56	1.27	0.33	1.20	-0.09	0.85	0.05	1.06	-17.30	13.21	9043.08
LA-EDB14-10	2014	0.72	0.54	1.03	0.31	1.06	-0.08	0.63	0.04	0.92	-19.60	14.25	6343.53
LA-EDB14-12	2014	1.09	0.74	1.57	0.39	1.40	0.00	1.02	0.02	1.21	-17.40	13.72	6458.19
LA-EDB14-14	2014	0.92	0.65	1.48	0.37	1.42	-0.05	1.00	0.04	1.26	-16.90	13.89	15009.73
LA-EDB14-17	2014	0.93	0.64	1.48	0.36	1.35	-0.02	0.99	0.04	1.19	-17.20	13.78	5898.27

LA-EDB14-18	2014	1.02	0.77	1.48	0.39	1.45	-0.13	0.90	0.01	1.25	-16.20	14.83	5084.04
LA-EDB17-12	2017	0.35	0.16	0.86	0.03	1.06	0.12	0.74	-0.05	1.02	-16.83	14.22	7720.26
LA-EDB17-13	2017	0.45	0.31	1.09	0.13	1.19	-0.02	0.85	-0.03	1.11	-17.16	13.46	8728.11
LA-EDB17-16	2017	0.33	0.22	0.54	0.12	0.65	0.00	0.38	0.01	0.60	-19.69	13.28	8318.27
LA-EDB17-17	2017	0.34	0.29	0.92	0.11	0.96	-0.08	0.70	-0.03	0.89	-16.91	13.66	9205.55
LA-EDB17-21	2017	0.51	0.36	1.02	0.28	1.13	-0.03	0.75	0.10	1.04	-17.62	13.83	6439.79
LA-EDB17-23	2017	0.57	0.43	1.12	0.27	1.04	-0.07	0.80	0.05	0.93	-17.81	13.55	6945.61
LA-EDB17-24	2017	0.23	0.11	0.81	0.11	0.96	0.07	0.73	0.05	0.93	-17.30	13.81	7200.69
LA-EDB17-26	2017	0.46	0.32	0.86	0.11	0.84	-0.02	0.62	-0.05	0.76	-18.04	13.94	9072.00
LA-EDB17-29	2017	0.36	0.29	0.89	0.19	1.00	-0.08	0.68	0.04	0.93	-17.89	13.42	7533.32
LA-EDB17-30	2017	0.20	0.13	0.63	0.07	0.72	0.01	0.54	0.00	0.69	-17.28	14.52	8666.11

Table S3. Mean \pm 2SD values of Hg isotopic composition (‰) obtained for reference materials NIST RM 8610 (UM-Almadén), NIES-13 (human hair) and internal reference material F-KP (penguin feathers).

Sample	Reference	n	$\delta^{204}\text{Hg}$ (‰)	$\delta^{202}\text{Hg}$ (‰)	$\delta^{201}\text{Hg}$ (‰)	$\delta^{200}\text{Hg}$ (‰)	$\delta^{199}\text{Hg}$ (‰)	$\Delta^{204}\text{Hg}$ (‰)	$\Delta^{201}\text{Hg}$ (‰)	$\Delta^{200}\text{Hg}$ (‰)	$\Delta^{199}\text{Hg}$ (‰)
NIST RM 8610 (UM-Almadén)	This study	18	-0.82 \pm 0.16	-0.53 \pm 0.14	-0.44 \pm 0.12	-0.26 \pm 0.09	-0.16 \pm 0.09	-0.04 \pm 0.13	-0.05 \pm 0.06	0.00 \pm 0.06	-0.02 \pm 0.07
	Reference values		-0.82 \pm 0.07	-0.56 \pm 0.03	-0.46 \pm 0.02	-0.27 \pm 0.01	-0.17 \pm 0.01	-	-0.04 \pm 0.01	0.00 \pm 0.01	-0.03 \pm 0.02
NIES-13	This study	4	3.14 \pm 0.13	2.16 \pm 0.12	3.18 \pm 0.12	1.15 \pm 0.07	2.39 \pm 0.09	-0.09 \pm 0.08	1.55 \pm 0.06	0.06 \pm 0.03	1.84 \pm 0.07
	Yamakawa et al. (2016)	11	2.76 \pm 0.16	1.89 \pm 0.10	2.77 \pm 0.10	0.98 \pm 0.08	2.13 \pm 0.07	-0.04 \pm 0.11	1.36 \pm 0.07	0.04 \pm 0.04	1.65 \pm 0.06
F-KP	This study	4	2.32 \pm 0.16	1.53 \pm 0.07	2.68 \pm 0.07	0.77 \pm 0.05	2.18 \pm 0.07	0.03 \pm 0.19	1.53 \pm 0.11	0.00 \pm 0.03	1.79 \pm 0.06
	Renedo et al. (2018)	17	2.40 \pm 0.25	1.58 \pm 0.22	2.67 \pm 0.21	0.80 \pm 0.16	2.11 \pm 0.18	0.04 \pm 0.24	1.48 \pm 0.13	0.01 \pm 0.09	1.71 \pm 0.15

Fig. S1. Map showing the location of the three species' breeding colonies sampled in this study: Audouin's gull (IA, in gray), black-headed gull (CR, in orange) and common tern (SH, in blue).

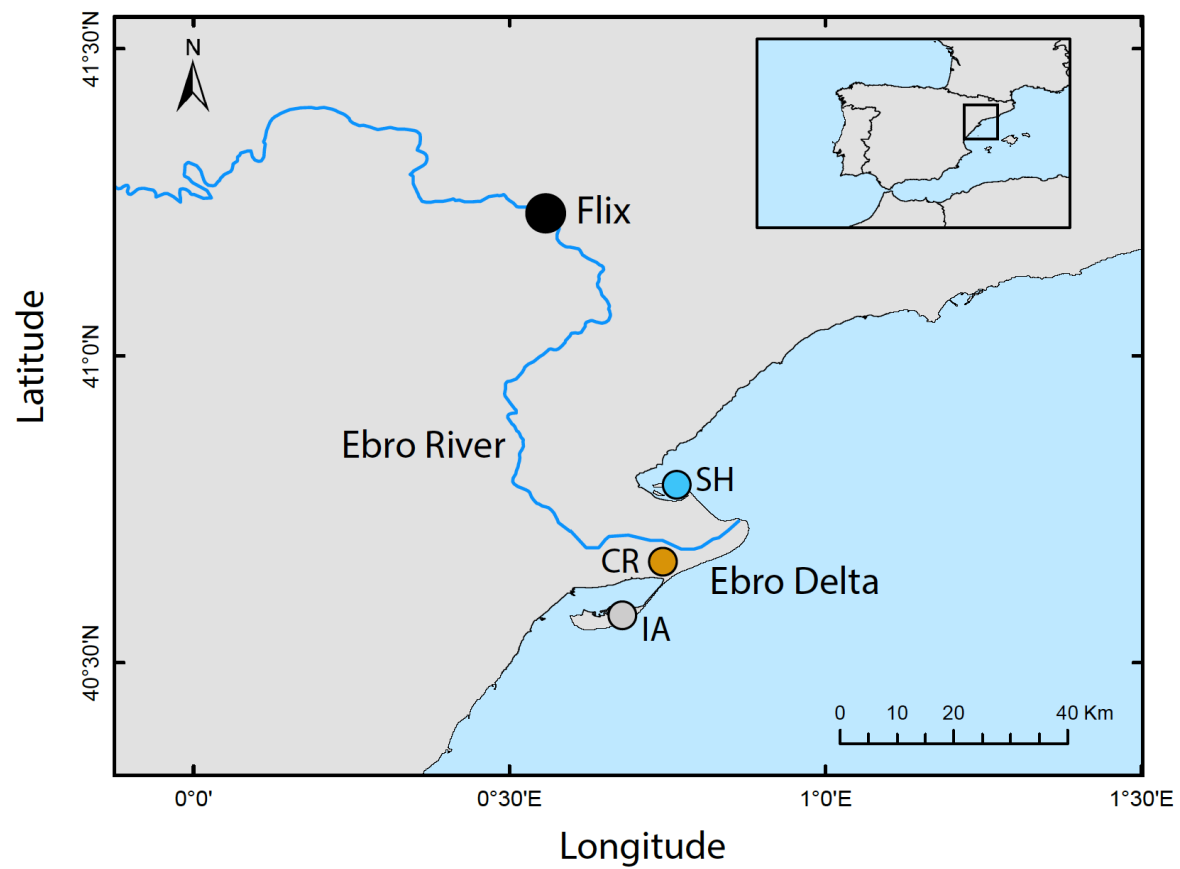


Fig. S2. Odd-MIF regression between $\Delta^{199}\text{Hg}$ vs. $\Delta^{201}\text{Hg}$ in feathers of Audouin's gulls (*I. audouinii*, in gray), black-headed gull (*C. ridibundus*, in orange) and common tern (*S. hirundo*, in blue) fledglings from the Ebro Delta in 2017. Individual data are plotted as dots. Dashed line represents linear regression between these variables for the three species: $y = 0.09558 + 1.1667x$; $R^2 = 0.9965$; $p < 0.0001$. Analytical error (2SD) for $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ is ± 0.07 and ± 0.06 , respectively, estimated from the 2SD of the mean Hg isotopic composition of the NIST RM 8610 (UM-Almadén) over multiple analytical sessions (see Table S3).

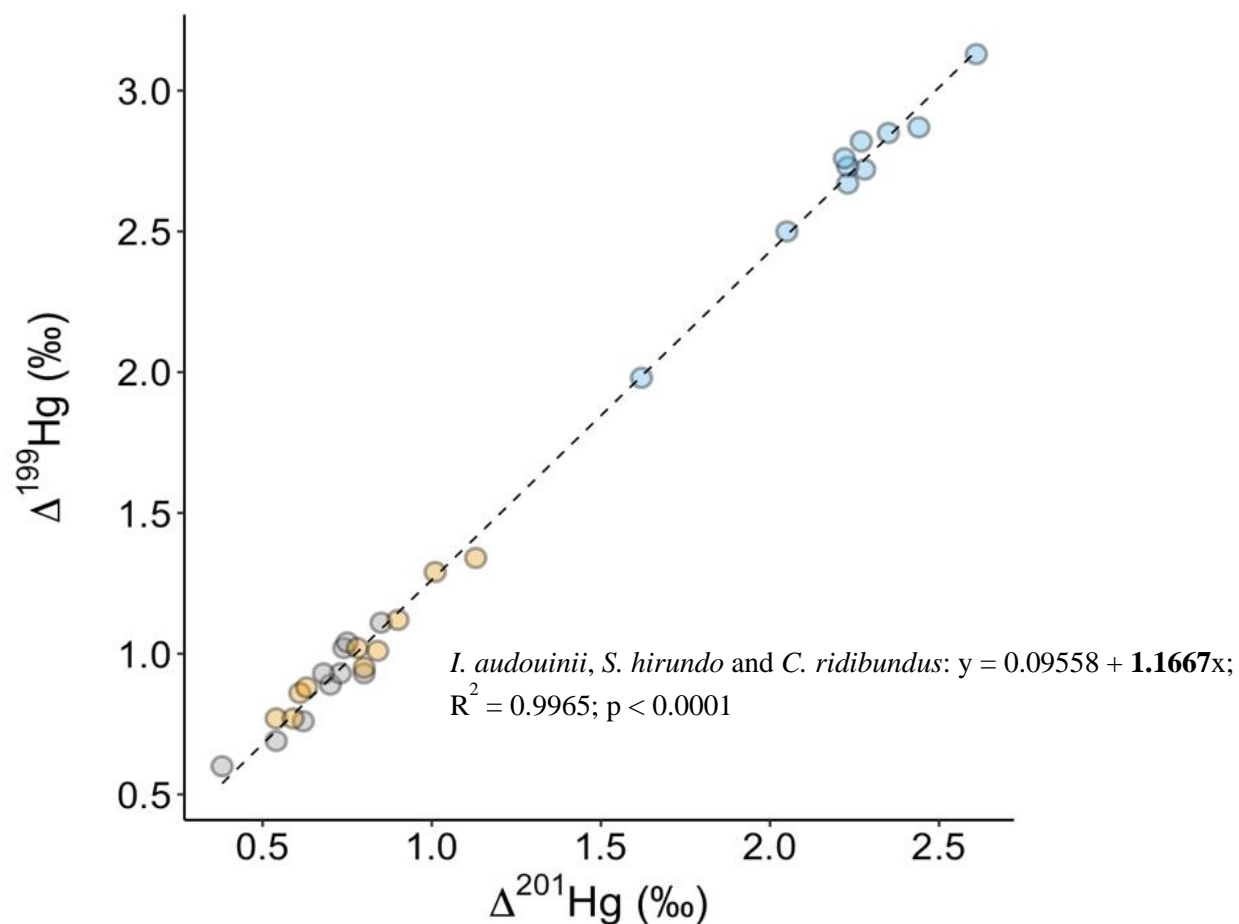


Fig. S3. Even-MIF plot between $\Delta^{200}\text{Hg}$ vs. $\Delta^{204}\text{Hg}$ in feathers of Audouin's gulls (*I. audouinii*, in gray), black-headed gull (*C. ridibundus*, in orange) and common tern (*S. hirundo*, in blue) fledglings from the Ebro Delta in 2017. Individual data are plotted as dots. Analytical error (2SD) for $\Delta^{200}\text{Hg}$ and $\Delta^{204}\text{Hg}$ is ± 0.06 and ± 0.13 , respectively, estimated from the 2SD of the mean Hg isotopic composition of the NIST RM 8610 (UM-Almadén) over multiple analytical sessions (see Table S3).

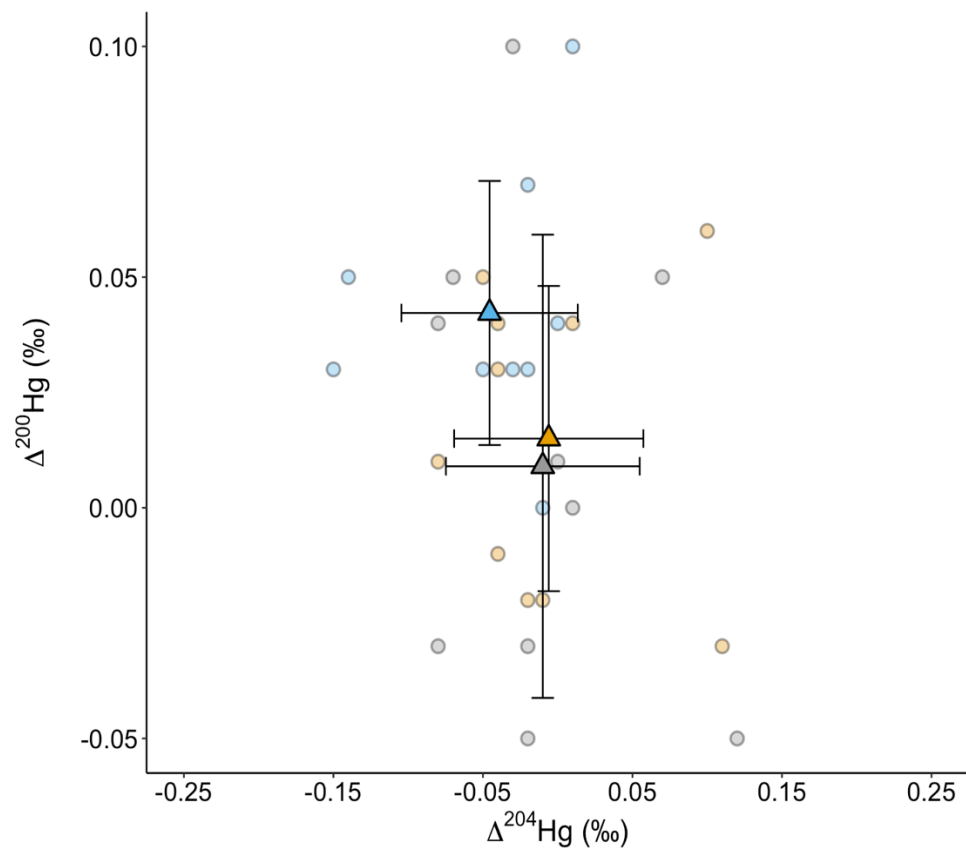


Fig. S4. Odd-MIF regression between $\Delta^{199}\text{Hg}$ vs. $\Delta^{201}\text{Hg}$ in feathers of Audouin's gulls (*I. audouinii*) fledglings from the Ebro Delta in 2010 (yellow), 2013 (green), 2014 (red) and 2017 (gray). Individual data are plotted as dots. Dashed line represents linear regression between these variables for each of the four years. Analytical error (2SD) for $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ is ± 0.07 and ± 0.06 , respectively, estimated from the 2SD of the mean Hg isotopic composition of the NIST RM 8610 (UM-Almadén) over multiple analytical sessions (see Table S3).

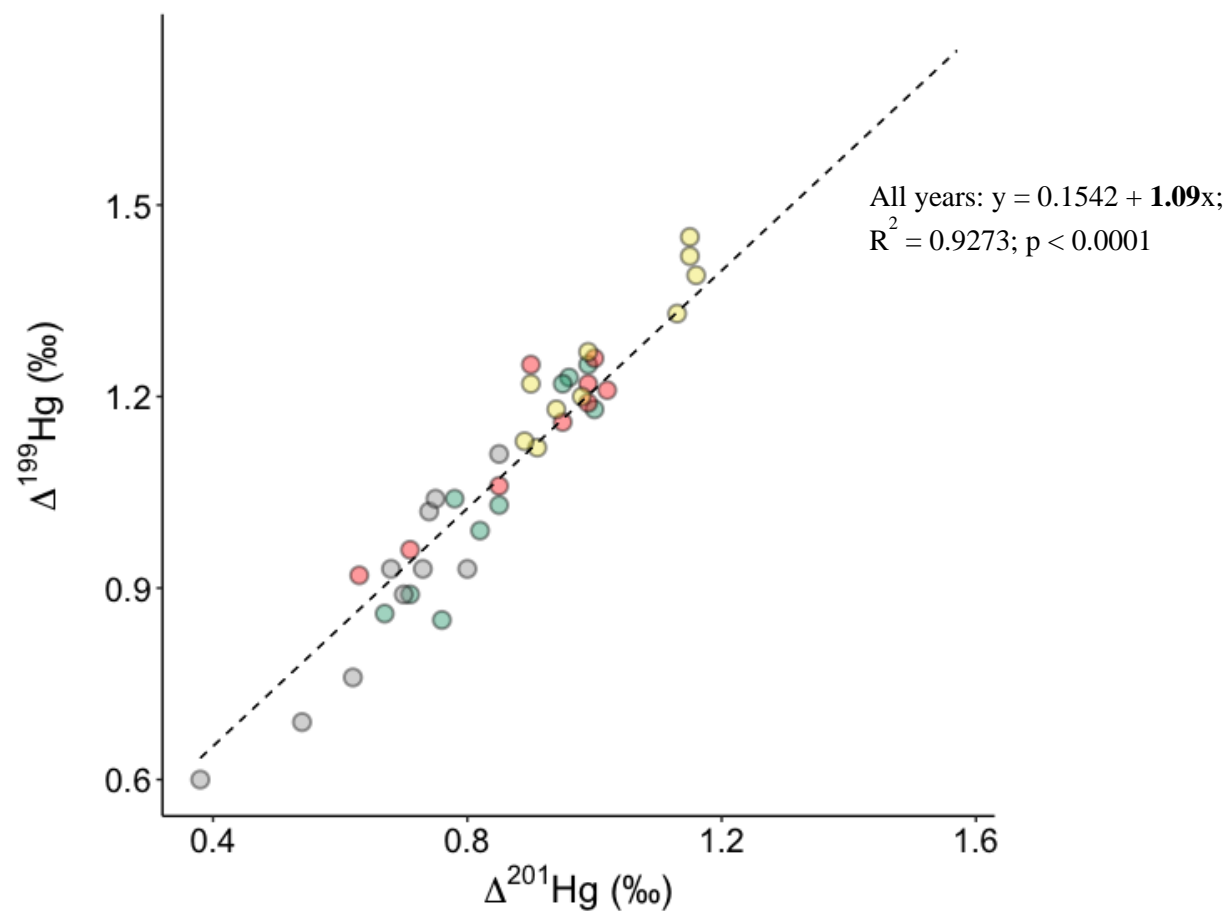
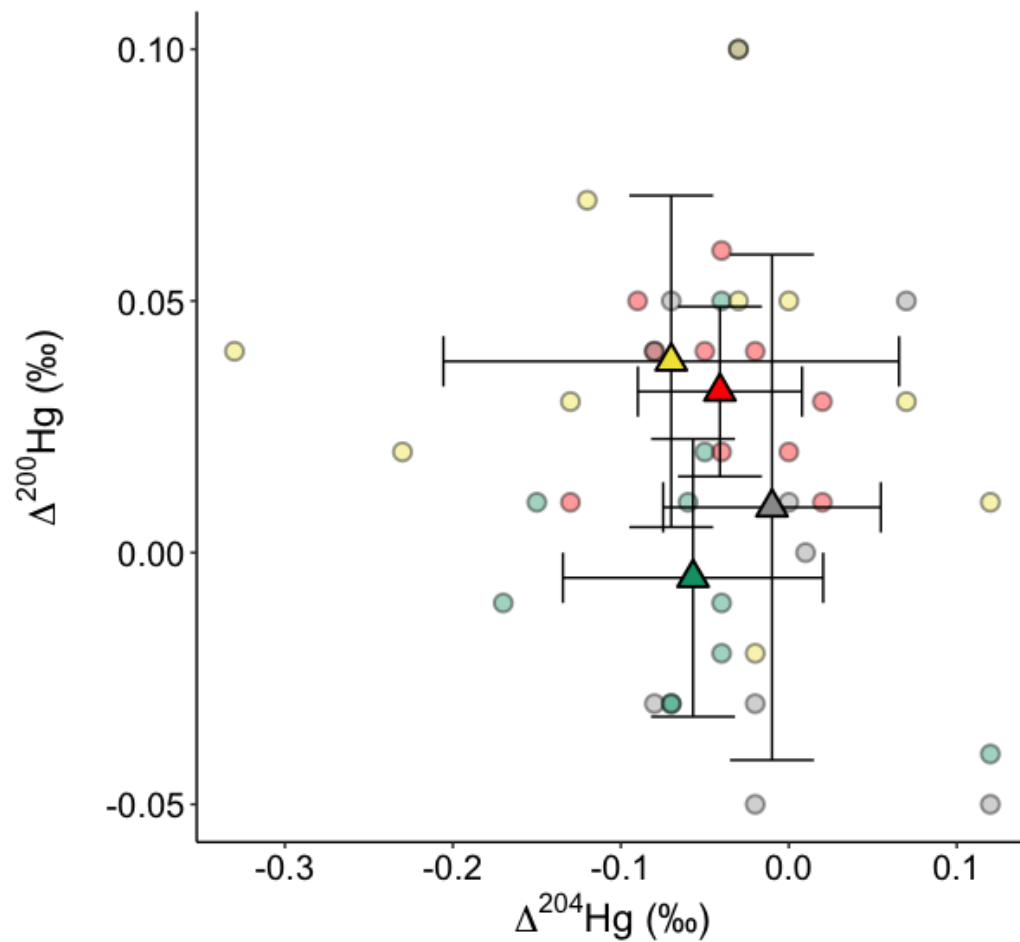


Fig. S5. Even-MIF plot between $\Delta^{200}\text{Hg}$ vs. $\Delta^{204}\text{Hg}$ in feathers of Audouin's gulls (*I. audouinii*) fledglings from the Ebro Delta in 2010 (yellow), 2013 (green), 2014 (red) and 2017 (gray). Individual data are plotted as dots. Analytical error (2SD) for $\Delta^{200}\text{Hg}$ and $\Delta^{204}\text{Hg}$ is ± 0.06 and ± 0.13 , respectively, estimated from the 2SD of the mean Hg isotopic composition of the NIST RM 8610 (UM-Almadén) over multiple analytical sessions (see Table S3).



References

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