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THE INFLUENCE OF DIETARY SPECIALIZATION AND TROPHIC STATUS ON MERCURY LEVELS IN TWO SPECIES USING COMMON COASTAL WETLANDS, *HIMANTOPUS HIMANTOPUS* AND *STERNA ALBIFRONS*

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Summary.—*The influence of dietary specialization and trophic status on mercury levels in two species using common coastal wetlands,* Himantopus himantopus *and* Sterna albifrons.

Aims: Coastal wetlands provide a diversity of prey to birds and thus variable diet-mediated mercury budgets. The main purpose of the study was to test the effect of dietary specialization and trophic status on mercury levels of birds using common coastal wetlands in a different manner, as revealed by stable isotopes signatures.

Location: Seven saltpans and a large area of ricefields in Portugal.

Methods: Total mercury concentration and stable isotope ratios were measured in chick feathers of black-winged stilt *Himantopus himantopus* and little tern *Sterna albifrons*, from Portuguese coastal wetlands during breeding seasons.

Results and Conclusions: Variation in trophic status explained the inter-specific variation of mercury levels, the intra-specific variation of mercury levels in *H. himantopus*, but not the intra-specific variation of mercury levels in *S. albifrons*. A different diet specialization and a mixture of prey groups, teleosts and invertebrates from different saltpan ponds and trophic status are suggested as being the basis for intra-specific variation of mercury levels not being related with the trophic status in *S. albifrons*. *H. himantopus* only uses invertebrates, but a wide range of stable isotope signatures and mercury levels were observed. Because chicks are precocial, the feeding area may be associated with a confined area in the ricefield, in the saltpan or in the saltpan pond. Differences of $\delta^{13}C$ in chick feathers of *H. himantopus* between ricefields and saltpans, and also between saltpan ponds suggested that different contributions from marine versus freshwater sources exist among preys. Prey exhibited differences in $\delta^{15}N$ and mercury levels, and significant correlation between mercury concentration and $\delta^{15}N$ revealing that prey occupy different trophic status. Differences between ponds for $\delta^{13}C$ in prey also support the idea that variation in food resources may occur within saltpans in the basis of the trophic chain.

Key words: bird, coastal wetland, himantopus, mercury, saltpan, stable isotopes, sterna

Resumen.—*La influencia de la especialización en la dieta y la posición trófica en los niveles de mercurio de dos especies que utilizan frecuentemente los humedales,* Himantopus himantopus *y* Sterna albifrons.

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Objetivos: Los humedales costeros proporcionan una diversidad de presas a las aves que pueden proporcionar distintos niveles de mercurio. El principal obejtivo de este estudio fue contrastar el efecto de la especialización de la dieta y de la posición en la cadena trófica en los niveles de mercurio en dos especies de aves que normalmente utilizan humedales costeros.

Localidad: Siete saladares y un gran arrozal en Portugal.

Métodos: La concentración total de mercurio y la proporción de isótopos estables fue medida en plumas de pollos de cigueñuela común *Himantopus himantopus* y de charrancito común *Sterna albifrons*, en los humedales portugueses durante el periodo reproductor.

Resultados y Conclusiones: Diferencias en la posición trófica de estas especies explicaba la variación interespecífica en los niveles de mercurio, la variación intraespecífica en *H. himantopus*, pero no la variación intraespecífica en *S. albifrons*. Una dieta especializada diferente y una mezcla en los grupos de presas, peces teleósteos e invertebrados de los diferentes saladares y la situación en la cadena trófica se sugieren como la base de la variación intraespecífica en niveles de mercuio y no deben estar relacionados con la posición trófica de *S. albifrons*. *H. himantopus* sólo consume invertebrados, pero se observó un amplio rango de trazas de isótopos estables y de niveles de mercurio. Debido a que los pollos son nidífugos, el área de búsqueda de alimento de estos es difícil de determinar. Diferencias en el $\delta^{13}C$ de las plumas de los pollos de *H. himantopus* entre los arrozales y saladares sugiere que las contribuciones entre agua dulce y salada existen entre presas. Las presas poseían diferencias en $\delta^{15}N$ y en los niveles de mercurio. La correlación significativa entre la concentración de mercurio y $\delta^{15}N$ indica que las presas ocupaban diferentes posiciones en la cadena trófica. Diferencias entre zonas en los niveles de $\delta^{13}C$ en las presas corrobora la idea de que la variación en las fuentes de comida puede ocurrir en los saladares en la base de la cadena trófica.

Palabras clave: ave, humedales costeros, *Himantopus*, mercurio, saladar, isótopos estables, Sterna.

Introduction

Coastal wetlands are important feeding and breeding habitats to fish and birds. They are highly productive areas thus providing large diversity and biomass of prey. Since coastal areas have been highly polluted, and some pollutants are persistent in the environment and easily bioaccumulate in biota, i.e. mercury, these areas provide also variable diet-mediated contaminant budgets. The main purpose of this study was to use stable isotope signatures to investigate differences in dietary specialization and trophic status that may be related with a different mobilization of mercury into birds using common coastal wetlands in a different manner, black-winged stilt *Himantopus himantopus* and little tern *S. albifrons*. The hypothesis that a highly confined habitat may have different contributions to chick contamination depending on the diet was tested. Chicks of *H. himantopus* obtain invertebrates directly from the breeding ground, and chicks of *S. albifrons* use fish and invertebrates obtained by adults in surrounding areas.

The first reason to follow these objectives is related to the fact of coastal wetlands in south-west Europe represent important breeding grounds for birds, namely saltpans (Britton and Johnson, 1987). Some of those habitats are localised in areas that have revealed hotspots for anthropogenic pollution and also geographic variation for mercury levels in chicks of *H. himantopus* (Tavares *et al.*, 2004) and *S. albifrons* (Tavares *et al.*, 2005). *S. albifrons* is a widespread but scarce western Palearctic species utilising coastal habitats including coastal shallow saline. Over the past two decades, the species has undergone declines in some areas and protective measures are necessary (Tucker and Heath, 1994). *H. himantopus* is also a widespread western

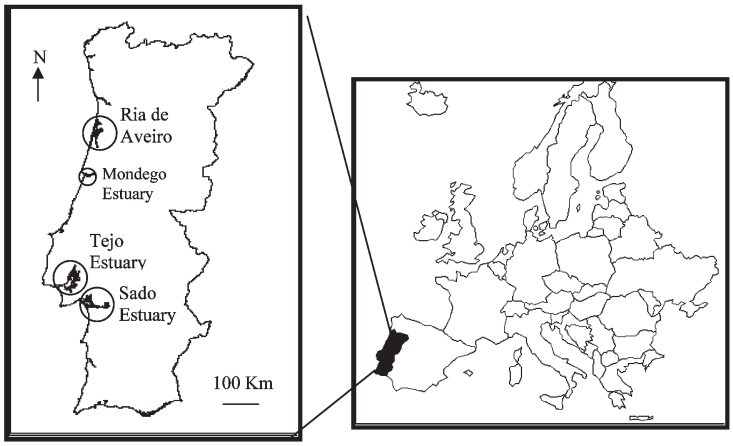

FIG. 1.—Map showing the study areas in Portugal.
[Mapa mostrando las áreas de estudio en Portugal.]

Palearctic species using shallow water habitats (Cramp and Simmons, 1983).

The second reason to follow these objectives is because the conventional dietary analysis, such as analysis of stomach contents, regurgitates or pellets, has been unable to demonstrate some particular changes on diet, due to the biases associated with these techniques (Duffy and Jackson, 1986) and it was necessary to relate diets and mercury contamination in a particular habitat. Nitrogen and carbon stable isotope analysis offer an alternative to conventional dietary analyses to infer trophic status (*e.g.*, Hobson and Clark, 1992; Thompson *et al.*, 1998). The technique is based on the premise that the ratios of $^{15}N/^{14}N$ ($\delta^{15}N$) and $^{13}C/^{12}C$ ($\delta^{13}C$) in consumer proteins reflect those in their diet in a predictable manner (DeNiro and Epstein, 1978, 1981; Petersen and Fry, 1987). For nitrogen, $\delta^{15}N$ exhibits a stepwise enrichment of 3 - 5 ‰ at each successive level within a food chain (Schoeninger and DeNiro, 1984; Hobson and Clark, 1992) with predators occupying high trophic levels having correspondingly high $\delta^{15}N$ values. In the case of carbon, $\delta^{13}C$ also increases with trophic level but to a lesser degree with a stepwise enrichment of between 0.5 - 1 ‰ (Rau *et al.*, 1983; Fry and Sherr, 1984; Hobson and Welch, 1992). In addition, $\delta^{13}C$ values can provide useful information on the sources of carbon in food webs and can be used to discriminate between animals living in different biomes i.e. freshwater versus marine (Mizutani *et al.*, 1990; Bearhop *et al.*, 1999) and inshore/benthic feeding versus pelagic (Hobson *et al.*, 1994; Sydeman *et al.*, 1997).

MATERIAL AND METHODS

Study area

Seven saltpans and a large area (600 ha) of ricefields were represented in the study. Saltpans belonged to four Portuguese coastal wetlands (Fig. 1): Sado Estuary (Vaia saltpans); Tejo Estuary (Atalaia, Esteiro-Furado, Vasa-Sacos and Vau saltpans); Mondego Estuary (Gala saltpans) and Ria de Aveiro (Lota saltpans). The ricefields' area belonged to Tejo Estuary. These saltpans have been severely affected by human pressure, habitat fragmentation, anthropogenic pollution sources and also habitat destruction over the past few

decades. In the case of *H. himantopus* saltpans and ponds were considered since chicks use different ponds and saltpans. In the case of *S. albifrons* such comparison was not required, and Vaia saltpans were used, since adults bring food from several saltpan ponds converted into extensive aquacultures.

Sample collection

Breast feathers were collected from chicks of *H. himantopus* and *S. albifrons*. Sampling occurred during the breeding seasons, approximately between April and July of 2000 and 2001. Samples of food resources used by these species were also collected at Vau, Vaia and Lota saltpans. A 1000 μm mesh size was used along horizontal transects of 10 meters including the water column and the surface sediments of each site. Spatial field replicates were used at each collection date at each site. Temporal field replicates were considered in the same breeding season for each site. Teleosts provided by *S. albifrons* adults to chicks were collected in the same sampling periods. Prey samples were preserved at -20ºC, and dried until constant weight at 60ºC prior to analysis.

Stable isotope analysis

Each sample was ground to a homogeneous powder at liquid nitrogen temperature in a grinding mill (Spex Certiprep 6570, Glen Creston Ltd., UK). Approximately 0.7 mg of sample was sealed into a tin capsule for combustion. Stable isotope analyses were carried out by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a Carlo Erba C/N/S analyser coupled to a Finnigan Tracer Matt mass spectrometer. Isotope ratios are expressed in the standard δ notation in parts per thousand (‰) according to the following equation: $\delta X = [(R_{sample}/R_{standard})\text{-}1] \times 1000$, where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. $R_{standard}$ for ^{13}C is PDB (Pee Dee Belemnite) and atmospheric nitrogen for ^{15}N. Typical analytical precision was better than ± 0.3 ‰ for $\delta^{15}N$ and ± 0.1 ‰ for $\delta^{13}C$.

Mercury analysis

Total mercury concentration was determined by thermal atomization followed by atomic absorption spectroscopy using the spectrofotometer AMA254, Althec. All reagents used through out the work namely in calibration curves were of analytical grade. All containers were previously decontaminated by immersion in a HNO_3 1:5 solution and then washed with ultra-pure water. The uncertainty of the method was assessed by performing successive measurements with the same sample. Relative standard deviations in the range of 5 % were found. Accuracy of the method was within 10% of the reference value and it was monitored analysing reference material e.g. Tort2 with a certified mean value and 95 % confidence interval of 0.27 ± 0.06 μg/g. The mean value and 95% confidence interval obtained for the reference material with a total of 26 replicates was 0.26 ± 0.04 μg/g. The limit of detection (LD) was given as 0.01 ng Hg and in the case of 0.100 g samples as 0.1 ng Hg / g (0.1 ppb) by Altec, and it was monitored as twice the standard deviation of triplicate analysis of blanks (Saltzman *et al.*, 1983). Mercury concentrations are expressed in μg/g on a fresh weight basis.

Statistical analysis

Statistical analyses were carried out using generalised linear models in GLIM 3.77 or Statistica software package StatSoft 1995. Tests were performed with $\alpha = 5$ % as threshold for significance. The goodness of fit to normal distribution (Kolmogorov-Smirnov one-sample tests) and the homogeneity of variance were checked (Zar, 1984).

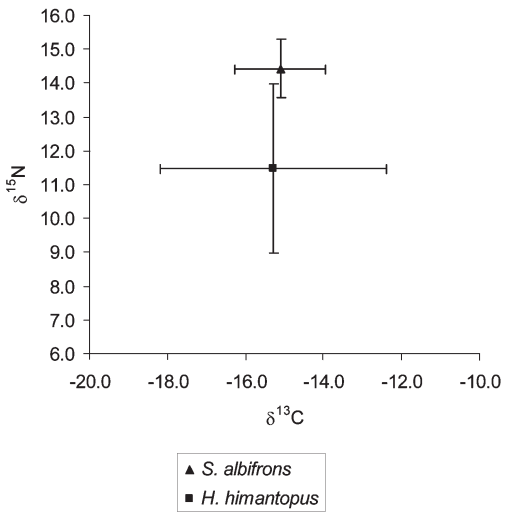

FIG. 2.—Dual isotope plot for $d^{13}C$ and $d^{15}N$ in chick feathers of *H. himantopus* and *S. albifrons*. There was no difference between the species in $d^{13}C$. However, $d^{15}N$ was more enriched in *S. albifrons* indicating that *S. albifrons* were feeding at a higher trophic level than *H. himantopus*. Error bars = 1 SD.
[Gráfico mostrando los niveles de $\delta^{13}C$ y $\delta^{15}N$ para las plumas de pollos de H. himantopus *y* S. albifrons*. No existen diferencias entre especies en los niveles de $\delta^{13}C$. Sin embargo, los niveles de $\delta^{15}N$ fueron mayores en* S. albifrons*, indicando que esta especie se alimentó en niveles tróficos superiores que* H. himantopus*. Barras de error = 1 DT.]*

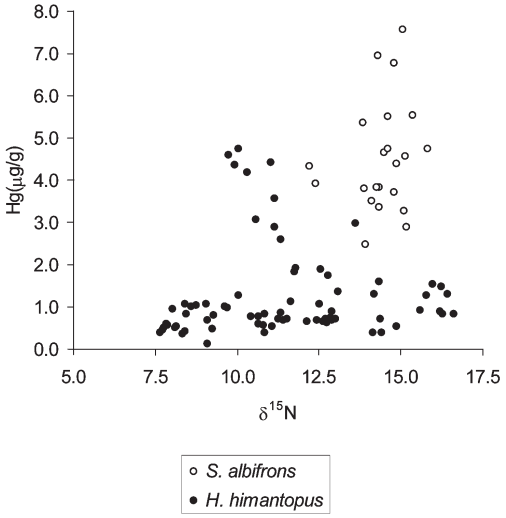

FIG. 3.—The relationship between $\delta^{15}N$ and mercury concentration in the feathers of *H. himantopus* and *S. albifrons* chicks.
[La relación entre los niveles de $\delta^{15}N$ y la concentración de mercurio en las plumas de los pollos de H. himantopus *y* S. albifrons*.]*

RESULTS

Inter-specific variation in birds

Significant differences were observed between *H. himantopus* and *S. albifrons* in Vaia during 2000 (t-test, $t = 16.8$, df = 18, $P < 0.001$) and during 2001 ($t = 4.2$, df = 19, $P < 0.001$) for $\delta^{15}N$ in chick feathers. Significant differences were also observed between the species in Vaia during 2000 ($t = 7.9$, df = 18, $P < 0.001$), in Vaia during 2001 ($t = 9.5$, df = 19, $P < 0.001$), in E. Furado during 2001 ($t = -4.1$, df = 24, $P < 0.001$) and in Passa during 2002 ($t = -3.4$, df = 21, $P < 0.005$) for total mercury concentration in chick feathers. $\delta^{15}N$ varied between 7.7 and 16.6 ‰ for *H. himantopus* (overall average of 11.5 ‰), and between 12.2 and 16.0 ‰ for *S. albifrons* (overall average of 14.4 ‰). Total mercury concentration in chick feathers ranged from 0.86 to 31.95 μg/g in *S. albifrons* (overall average of 4.5 μg/g) and from 0.15 to 4.81 μg/g in *H. himantopus* (overall average of 1.2 μg/g; Fig. 2 and 3). Significant positive correlation was observed between $\delta^{15}N$ and mercury levels during 2000 (Pearson $r = 0.44$, $P < 0.005$) and during 2001 ($r = 0.38$, $P < 0.01$) for chick feathers of *H. himantopus* and *S. albifrons*. Significant differences were observed between the species in Vaia during 2000 for $\delta^{13}C$ in chick feathers ($t = -7.2$, df = 18, $P < 0.001$). Values of $\delta^{13}C$ in chick feathers varied between –24.0 and –10.3 ‰ for *H. himantopus* (overall average of –15.3 ‰) and between –17.3 and –12.6 ‰ for *S. albifrons* (overall average of –15.1‰; Fig. 2 and 4). Arithmetic means and standard

deviations for total mercury concentration and stable isotope signatures by species and site are given in Table 1. No significant correlation was observed between $\delta^{13}C$ and mercury levels in chick feathers (all $P > 0.05$).

Intra-specific variation in H. himantopus

No significant correlation was observed between chick biometry and $\delta^{15}N$ in chick feathers, between chick biometry and mercury levels in chick feathers or between chick biometry and $\delta^{13}C$ in chick feathers for *H. himantopus* (all $P > 0.05$). There was significant differences between sites in $\delta^{13}C$ ($F_{7, 71} = 7.9$, $P < 0.01$) and $\delta^{15}N$ ($F_{7, 71} = 5.8$, $P < 0.01$; Fig 6). Ricefields revealed the lowest value for $\delta^{13}C$ (Tukey, $P < 0.05$), as presented in Table 1. In saltpans, pond type was a significant factor in determining $\delta^{13}C$ for *H. himantopus* ($F_{2, 72} = 26.7$, $P < 0.01$). There was no significant difference in $\delta^{15}N$ between pond types ($F_{2, 72} = 1.1$, $P > 0.05$). Significant variation was found between 2000 and 2001 at Vaia for $\delta^{15}N$ in chick feathers of *H. himantopus* ($t = 7.1$, df = 17, $P < 0.001$). Significant variation was also found between 2000 and 2001 at Vaia for $d^{15}N$ in chick feathers of *S. albifrons* ($t = -2.2$, df = 20, $P < 0.05$). Significant positive correlation was observed between mercury and $\delta^{15}N$ for chick feathers of *H. himantopus* during 2000 ($r = 0.38$, $P < 0.05$; Fig. 4). There was no significant relationship between mercury and $\delta^{13}C$ (Fig. 3).

Intra-specific variation in S. albifrons

No significant correlation was observed between chick biometry and $\delta^{15}N$ for *S. albifrons* chicks (all $P > 0.05$). Significant negative correlation was observed between chick biometry and total mercury concentration in chick feathers for *S. albifrons* in Vaia ($r = -0.47$, $P < 0.05$). Significant positive correlation was observed between chick biometry and $\delta^{13}C$ in chick feathers for *S. albifrons* in Vaia ($r = 0.56$, $P < 0.05$). There was no significant difference in $\delta^{13}C$ or $\delta^{15}N$ between feather types (one-way ANOVA, $P > 0.05$). Neither feather type nor pond type could explain the variation in mercury levels and there was no difference between years (all $P > 0.05$). Differences between individuals could not explain the variability in mercury levels (one-way ANOVA, $F_{20, 20} = 1.0$, $P > 0.05$). There was no significant relationship between mercury and $\delta^{13}C$ or between mercury and $\delta^{15}N$ (all $P > 0.05$) in chick feathers of *S. albifrons*.

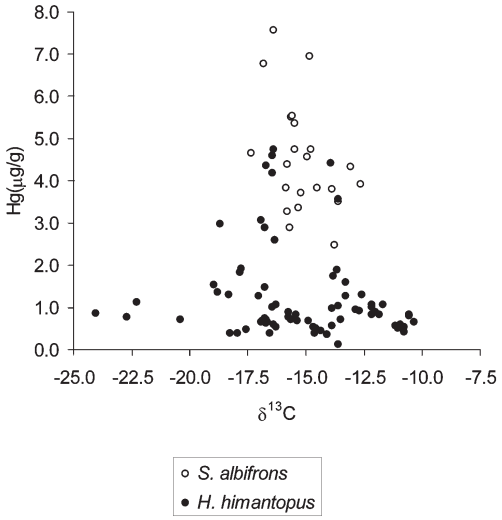

FIG. 4.—The relationship between $\delta^{13}C$ and mercury concentration in the feathers of *H. himantopus* and *S. albifrons* chicks.
[La relación entre los niveles de $\delta^{13}C$ *y la concentración de mercurio en las plumas de los pollos de* H. himantopus *y* S. albifrons*.]*

Inter-specific variation in potential prey

Significant variation was observed between all potential prey types, macroinvertebrates and teleost fish, for $\delta^{15}N$ (one-way ANOVA, $F_{17, 85} = 5.7$, $P < 0.001$). *Atherina presbyter* showed

TABLE 1

Total mercury concentration (μg/g) and stable isotope ratios for nitrogen and carbon (‰) in chick feathers of *H. himantopus* and *S. albifrons* during the breeding seasons of 2000 and 2001. Values refer to arithmetic mean ± standard deviation. Sample size (*n*) and coefficient of variation (CV; %) were also given.
[Concentración de mercurio (μg/g) y ratio de isótopos estables de nitrógeno y carbono (‰) en las plumas de pollos de H. himantopus *y* S. albifrons *en las primaveras de los años 2000 y 2001. Los valores se expresan como media ± desviación típica, además se da el tamaño muestral (*n*) y el coeficiente de variación (CV; %).]*

Year	Site	*n*	Hg (μg/g)	CV	δ15N	CV	δ13C	CV
S. albifrons								
2000	Vaia	12	4.40 ± 1.31	29.7	14.0 ± 0.9	6.3	-14.7 ± 1.4	9.8
2001	Vaia	10	4.67 ± 1.38	29.5	14.9 ± 0.6	3.8	-15.5 ± 0.5	3.1
H. himantopus								
2000	Atalaia	7	0.64 ± 0.30	46.6	11.8 ± 2.0	17.2	-16.4 ± 1.2	7.5
	E. Furado	9	0.92 ± 0.33	36.0	14.8 ± 2.0	13.5	-12.4 ± 1.1	8.5
	Giganta	6	0.78 ± 0.18	23.6	11.7 ± 0.7	6.1	-20.5 ± 3.2	15.5
	V. Sacos	6	1.30 ± 0.86	66.1	12.4 ± 1.1	8.8	-16.8 ± 1.5	3.3
	Vaia	8	0.66 ± 0.21	32.4	8.3 ± 0.5	5.5	-10.9 ± 0.4	9.3
2001	Gala	8	0.77 ± 0.49	63.3	8.9 ± 1.5	17.1	-15.1 ± 1.5	10.2
	Lota	9	1.74 ± 1.39	80.2	10.5 ± 1.5	14.3	-13.8 ± 0.4	3.2
	Vaia	11	0.68 ± 0.21	30.4	12.3 ± 1.8	14.9	-16.2 ± 2.1	12.7
	Vau	11	2.96 ± 1.33	44.8	12.1 ± 2.6	20.5	-16.9 ± 0.8	4.4

significantly higher mean value than other prey groups (Tukey, $P < 0.05$). No significant variation was observed for $\delta^{15}N$ between teleost groups or between sites in teleosts (one-way ANOVA, $P > 0.05$).

Significant variation was also observed between all potential prey types for total mercury concentration at Vaia in 2000 (one-way ANOVA, $F_{6, 34} = 38.2$, $P < 0.001$), Alhos-Vedros in 2000 (one-way ANOVA, $F_{6, 24} = 2.9$, $P < 0.05$). *A. presbyter* was significantly more highly contaminated than Mugilidae, *Sardina pilchardus*, and macroinvertebrate groups at Vaia in 2000 (Tukey, $P < 0.05$). *S. pilchardus* was also significantly more highly contaminated than Mugilidae, and macroinvertebrate groups at Vaia in 2000 (Tukey, $P < 0.05$). Significant variation was observed between teleost groups for total mercury concentration (one-way ANOVA, $F_{3, 42} = 9.0$, $P < 0.001$), and *A. presbyter* showed a significantly higher mean value than *S. pilchardus*, Mugilidae and *Gambusia holbrooki*. (Tukey, $P < 0.05$). No significant differences were obtained for mercury levels between teleosts collected in water column of breeding grounds and teleosts provided by *S. albifrons* adults ($P > 0.05$). Significant correlation was observed between $\delta^{15}N$ and total mercury concentration ($r = 0.73$, $P < 0.001$). Significant variation was observed between all potential prey types for $\delta^{13}C$ (one-way ANOVA, $F_{9, 50} = 2.6$, $P < 0.05$). Significant variation was also observed between macroinvertebrates and teleosts for $\delta^{13}C$ ($t = 3.1$, df = 58, $P < 0.001$). $\delta^{13}C$ in macroinvertebrates varied between -21.7 and -8.9 ‰ (overall average of -15.7 ‰) and $\delta^{13}C$ in teleosts varied between -27.6 and -14.6 ‰ (overall av-

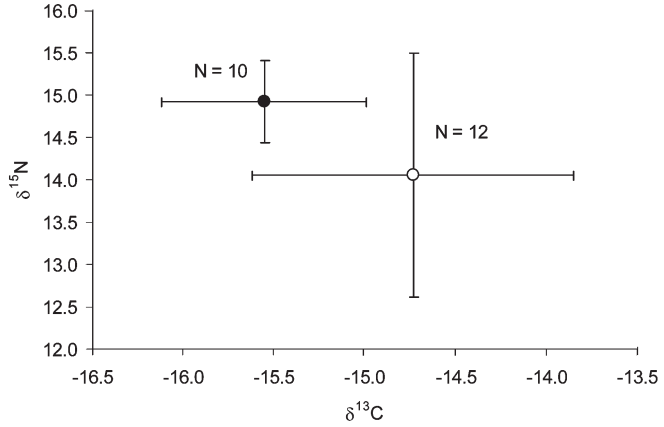

FIG. 5.—Dual isotope plot for $\delta^{13}C$ and $\delta^{15}N$ in feathers of *S. albifrons* chicks sampled at Vaia (Sado Estuary) in consecutive years (closed circle = 2000; open circle = 2001). Error bars = 1 SD.
[Gráfico mostrando los niveles de $\delta^{13}C$ y $\delta^{15}N$ para las plumas de pollos de S. albifrons *en Vaia (estuario de Sado) en dos consecutivos años (círculos negros = 2000; círculos blancos = 2001). Barras de error = 1 DT.]*

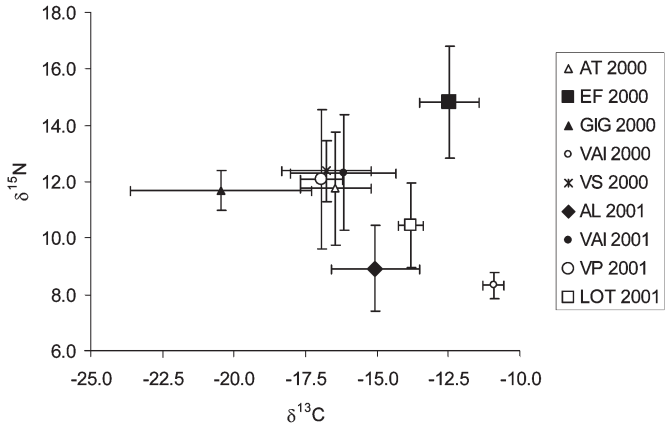

FIG. 6.—Dual isotope plot showing between-site variation of $\delta^{15}N$ in the feathers of *H. himantopus* chicks. Error bars = 1 SD.
[Gráfico mostrando los niveles de $\delta^{15}N$ para las plumas de pollos de H. himantopus *en las distintas áreas de estudio. Barras de error = 1 DT.]*

TABLE 2

Total mercury concentration (μg/g) and stable isotope ratios for nitrogen and carbon (‰) in potential prey during the breeding seasons of 2000 and 2001. Values refer to arithmetic mean ± standard deviation (sample size).

[Concentración de mercurio (μg/g) y ratio de isótopos estables de nitrógeno y carbono (‰) en presas potenciales en las primaveras de los años 2000 y 2001. Los valores se expresan como media ± desviación típica (tamaño muestral).]

Year	Site	Potential prey	Hg	$\delta^{15}N$	$\delta^{13}C$
2000	Vaia	Corixidae[b]	0.07 ± 0.01 (3)	4.55 ± 0.40 (3)	-12.97 ± 0.43 (3)
		Chironomidae[b]*	0.046	7.23	-12.04
		Mugilidae n.i.[a]	0.11 ± 0.01 (3)	8.3±2.8 (3)	-16.2 ± 1.9 (3)
		S. pilchardus[a]*	0.18	4.3	-26.5
		A. presbyter[a]	0.37 ± 0.09 (3)	13.2 ± 0.3 (3)	-19.7 ± 0.15 (3)
2001	Vaia	Corixidaeb	0.05 ± 0.02 (3)	4.87 ± 0.35 (3)	-11.75 ± 0.16 (3)
		Chironomidae[b]*	0.03	5.92	-13.26
		Hydrophilidae[b]	0.09 ± 0.01 (3)	5.66 ± 1.52 (3)	-13.52 ± 0.04 (3)
2001	Lota	Chironomidae[b]*	0.036	5.53	-14.99
		Ephydridae[b]*	0.06	5.59	-17.17
2001	Vau	Corixidae[b]	0.15 ± 0.02 (3)	6.93 ± 2.01 (3)	-18.34 ± 5.25 (3)
		Chironomidae[b]	0.14 ± 0.02 (2)	6.24 ± 2.10 (2)	-18.39 ± 0.06 (2)
		G. holbrooki[a]	0.15 ± 0.03 (2)	7.7 ± 3.9 (2)	-23.5 ± 4.1 (2)

[a] Teleosts (only juveniles), [b] Macroinvertebrates (larvae stages, except for Corixidae), * Sample size is limited to the occurrence of each prey group in each field sample, and marked groups were present in a single field sample in respective sites.

*[[a] Teleósteos (juveniles), [b] Macroinvertebrados (estado larvario, excepto para Corixidae), * Tamaño muestral limitado a la presencia de cada grupo de presas en cada muestra de campo, y grupos marcados estaban presentes en una única muestra de campo en los sitios respectivos.]*

erage of -20.1 ‰). Significant variation was observed between macroinvertebrates for $\delta^{13}C$ (one-way ANOVA, $F_{9, 62} = 2.4$, $P < 0.05$) but no significant variation was observed between teleosts for $\delta^{13}C$ (one-way ANOVA, $P > 0.05$). Arithmethic means and standard deviations for mercury levels and stable isotope signatures by prey group and site are given in Table 2.

DISCUSSION

Inter and intra-specific variation in the mercury contamination levels were found in *S. albifrons* and *H. himantopus* chicks. In both years, the average mercury concentrations in the feathers of *S. albifrons* chicks were about four times higher than in *H. himantopus* chicks and this was reflected by their trophic level as determined by $\delta^{15}N$, which is consistent with the prediction that species feeding at higher trophic levels will accumulate higher levels of mercury (Cabana and Rasmussen, 1994; Atwell *et al.*, 1998). Studies about the inter-specific variation of mercury in birds (Braune, 1987; Walsh, 1990) referred to a wide range of factors such as migratory habits, moult patterns, dietary preferences, foraging areas and age. Chick

feathers were studied with the purpose of focusing on dietary preferences in a particular type of habitat. The difference between the two species in the trophic level, as determined by $\delta^{15}N$, and in the mercury levels can be explained by differences in diets and foraging sites within the wetland. *S. albifrons* chicks feed predominantly on small fish, but they may also use invertebrates as in the case of *H. himantopus*. However, although *H. himantopus* chicks are limited to shallow waters within the breeding ground and feed in small family groups alongside their parents, *S. albifrons* chicks use the food presented to them by their parents, which may forage in surrounding areas and ponds with higher water column.

Differences between years in the $\delta^{15}N$ of *S. albifrons* chicks at Vaia saltpan (Sado Estuary), suggested that some changes occurred in diets between years. Despite fish being the main food resource for the most part of the time, maybe invertebrates made up a larger proportion of the diet in 2000 than in 2001. This is supported by the larger variance in 2000 (Fig. 5) which suggests a wider variety of prey.

For *H. himantopus*, there was considerable spatial variation between saltpans in $\delta^{13}C$ and $\delta^{15}N$, indicating that the localization of the breeding ground is an important factor. This is not surprising given that chicks feed inside the breeding ground until fully fledged. Pond type was a significant factor in determining $\delta^{13}C$, but not $\delta^{15}N$ in the present data. The spatial variation in $\delta^{13}C$ may be due to differential input of carbon from freshwater and marine sources, and that suggested the availability of different preys since $\delta^{13}C$ may also differ between prey groups. The $\delta^{13}C$ values of marine animals are more enriched in ^{13}C than freshwater animals by up to 7‰ (Chisholm *et al.*, 1982; Fry *et al.*, 1983) and this difference has been used to discriminate between animals living in freshwater or marine ecosystems (e.g. Fry and Sherr, 1984; Bearhop *et al.*, 1999). Low $\delta^{13}C$ values observed in Giganta ricefields can be explained on that basis, since the majority of water incomes to ricefields are obtained upwards in the Tejo river and thus are mainly influenced by freshwater. Also, the pond effect of $\delta^{13}C$ in chick feathers indicated that $\delta^{13}C$ differences occur within saltpans, probably as a response to water incomes, which affect abiotic conditions and thus, prey types.

The spatial variation in $\delta^{15}N$ is likely due to inter-site differences in diet. It is interesting that, although *S. albifrons* has a potentially larger range of foraging sites and therefore the potential to feed their chicks a more variable diet, intra-specific variation was larger in *H. himantopus* chicks for both mercury contamination and stable isotope ratios. Moreover, there was a relationship between $\delta^{15}N$ and mercury levels in feathers for *H. himantopus* chicks, but not for *S. albifrons* chicks. Thus trophic level, as determined by $\delta^{15}N$, seems to explain intra-specific variation of mercury levels in *H. himantopus* chicks, but not the intra-specific variation in mercury levels in *S. albifrons* chicks. The feeding ecology and the breeding phenology of *H. himantopus* enables that chick's foraging area may be associated with a confined area in the wetland, and therefore the species represent a better model in the study of intra-specific variation.

Causes of intra-specific variation in mercury tissue concentrations may include foraging area, dietary preferences, age, migration, moult and physiology (Walsh, 1990). By sampling chicks, migration and moult effects were discounted since the chicks sampled had not yet fledged. *H. himantopus* chicks were feeding in the respective breeding ground, and *S. albifrons* parents were providing food also from saltpan ponds converted into extensive aquaculture adjacent to the breeding colony. It seems that there is unlikely to be sufficient differences between individuals in physiology that would determine intra-specific variation in mercury concentration but this possibility cannot be excluded. It could be argued that older chicks will have accumulated more mercury than younger con-specifics since they will have had more

time to incorporate mercury from the diet. Although the age of chicks of either species was not measured, biometry was measured to study the effect of chick size. Negative correlation between biometry and mercury levels in *S. albifrons* chicks revealed that older chicks exhibited lower contamination levels than younger chicks (Tavares *et al.*, 2005). Large mass increments during faster growing period can balance the mercury inputs in less polluted sites reducing mercury contamination with increasing chick ages. Although biometry seems to influence mercury levels there was no relationship with $\delta^{15}N$, and thus biometry does not seem to influence trophic status. In *H. himantopus* no correlation was observed between biometry and other variables. Foraging area and dietary preferences would appear to be the most important factors influencing mercury levels in individuals. Bearhop *et al.* (2000b) found positive correlations between $\delta^{15}N$ and the mercury levels for chicks of *Stercorarius skua* and suggested that different dietary specialization was important in determining intra-specific variability in mercury burdens. In adults of skuas, trophic status, as determined by $\delta^{15}N$, had an influence on mercury levels in blood and feathers, but this was relatively minor compared to the effects of foraging area (Bearhop *et al.*, 2000a). Fevold *et al.* (2003) reported correlations in mercury levels in the blood of *Gavia immer* between chick and adult blood, reflecting the influence of mercury content of prey from the natal lake on chick mercury levels.

It was clear that there was inter-specific variation in trophic status and mercury levels between the two species which exhibited differences in their respective diets, and those factors such as chick size, foraging site and potential prey can contribute to the observed intra-specific variation. The data presented here for chicks feeding in coastal wetlands also demonstrated that chicks can reveal different patterns of mercury contamination within the same trophic level. Variation of foraging sites and more or less specialized diets may have effects on the mercury levels in birds in similar scales to those recently associated to different trophic status, even in the absence of variation in the trophic status. Results obtained for potential prey are curious since variation was observed between potential prey groups for $\delta^{15}N$ and mercury levels. Moreover, $\delta^{15}N$ and mercury were significantly positively correlated for potential prey, which indicated that there is variation in trophic status between preys. However, since chicks can consume prey from several groups with each group giving different contributions to $\delta^{15}N$ and mercury levels, inferences from the prey should be done with caution.

The inter-specific variation in birds appears to be the result of differences in the diet between the chicks of the two species. Diet of *H. himantopus* chicks (Serrano *et al.*, 1983) is different enough from *S. albifrons* chicks to show different trophic status between species. In relation to intra-specific variation, despite *S. albifrons* being a piscivorous species, chicks are fed with different prey including teleosts and invertebrates. On the other hand, *H. himantopus* chicks only use invertebrate but exhibited a larger range of $\delta^{15}N$ and mercury levels in relation to *S. albifrons* chicks, they exhibited significant differences between ponds for $\delta^{13}C$ within saltpans, and positive correlation between $\delta^{15}N$ and mercury was only observed in *H. himantopus*.

The well marked pattern in intra-specific variation of mercury contamination in *H. himantopus* chicks that make the species a good model may be related to the fact that different ponds and saltpans were considered separately due to breeding phenology of this species. The localization of the breeding ground inside a certain pond seems to influence mercury in chicks through their diet. Different ponds may contribute with different prey for *H. himantopus* chicks depending on water incomes and abiotic conditions. Chicks may use several ponds during their growing period, but most of them stayed in the same saltpan until start flying. Variation in the

macroinvertebrate groups between saltpans and between ponds within each saltpan may cause some effect either on the trophic status or mercury levels, since $\delta^{15}N$ and mercury correlated significantly.

Although a variation was detected between years in $\delta^{15}N$ for *S. albifrons* chicks indicating a change in trophic status, no relationship was observed between $\delta^{15}N$ and mercury for this species and thus mercury patterns cannot be associated to variation in trophic status but only to variation in diets. Although prey provided to *S. albifrons* chicks were captured in a larger area than in the case of *H. himantopus* chicks, a lower range of $\delta^{15}N$ and mercury values were found in chick feathers of *S. albifrons* which indicated that a mixture of foraging sites and prey groups in the diet can mask the effect of single prey in the isotope signatures and mercury levels of chick feathers.

In *H. himantopus* chicks, the contribution of each foraging site was possible to identify because the species phenology enabled to distinguish that. Chicks are precocial, obtaining food by themselves. Thus, the saltpan pond where each chick is obtaining its food is known, and different isotope signatures and mercury levels may be compared between different foraging sites within the same wetland and within the same saltpan. In the case of *S. albifrons* chicks, the overall contribution of several foraging sites to each chick is known, but it is not possible to associate a chick with a single saltpan pond because adults use several adjacent ponds to feed their chicks. Moreover, *S. albifrons* may use either teleost or invertebrates to feed the chicks, which also makes the interpretation of $\delta^{15}N$ data complex. The effect of foraging area and the level of dietary specialization on chick mercury levels may be easily controlled in *H. himantopus* chicks in relation to *S. albifrons* chicks. *H. himantopus* chicks may provide information about the different contribution of each pond, and *S. albifrons* chicks may only provide information about the overall contribution of several ponds, to the mercury contamination in a certain area.

Because some preys have a higher trophic status and diet composition may vary among chicks, it was expected that differences in trophic status among preys could also be marked in chicks. No differences could be observed for trophic status among chicks of *S. albifrons* colonies, but intra-specific differences were clearly observed in mercury levels. It is usually accepted that increasing trophic status results in higher mercury levels because of mercury biomagnification. However, the converse does not appear to be true since large variation in mercury levels can be caused directly by foraging site which affect diets without a clear variation in the trophic status. That seems to be the situation reported here.

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Authors of this study have focused their work on ecology and biodiversity (IMAR and IBLS) or environmental pollution with particular interest in mercury (IBLS and Univ. Aveiro). **R.W. Furness** and **P.C. Tavares** have developed work in both areas, and are particularly interested on factors affecting contaminant mobilization through the trophic chain, and the potential of birds as bioindicators.