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Influence of trophic ecology and spatial variation on the isotopic fingerprints of seabirds

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ABSTRACT: Notwithstanding the potential applications of stable isotopes in feeding and migration studies, the simultaneous influence of diet, foraging behavior and spatial variation on the stable isotope signatures of seabirds is poorly understood. Many studies have interpreted their isotopic signatures without considering local baseline and prey isotopic signatures; consequently, the main factors causing isotopic differences between populations have frequently not been discerned. To examine the influence of these factors on the stable isotopic signatures of seabirds, we analyzed the $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and Hg concentrations of chick feathers of the European shag *Phalacrocorax aristotelis*, its main fish prey and baseline indicator organisms (mussels), all sampled in 2 sectors of northwest coastal Spain with marked differences in primary productivity. Our results show that the $\delta^{15}N$ signature and Hg concentration of shags are influenced by both feeding ecology and spatial variation. The $\delta^{13}C$ and $\delta^{34}S$ signatures, however, mainly related to spatial differences and can thus be used as reliable geographic markers. Our findings also highlight the importance of assessing spatio-temporal variation in baseline isotopic signatures and their progressive integration through the food web. Omission of potential prey and baseline values, or application of only a single baseline to the food webs of the 2 sectors, assuming isotopic homogeneity because of geographical proximity, would have led to significantly distorted interpretations of feeding ecology of shag chicks.

KEY WORDS: $^{34}S \cdot ^{13}C \cdot ^{14}N \cdot$ Mercury · Upwelling · Feeding ecology · Atlantic Ocean · European shag

INTRODUCTION

Biogeochemical markers such as stable isotopes applied to marine ecosystems have provided new insights into feeding ecology and animal migration research (e.g. Oppel & Powell 2008, Newsome et al. 2009, Ramos et al. 2009a, Votier et al. 2010). Since isotope ratios in consumer tissues reflect those of their prey in a predictable manner, isotopic signatures have been used as indicators of trophic position and food web interactions (Hobson et al. 1994, Weiss et al. 2009). Also, because differences in biogeochemical processes result in geographical isotopic variation, stable isotope analysis has permitted inferences of animal movements between water masses with different isotopic baselines (e.g. Rooker et al. 2008, Phillips et al. 2009).

Nevertheless, in spite of the potential applications of isotopic signatures of top consumers, many studies have interpreted these markers without local baseline and prey isotopic signatures, making it impossible to discern the main factors causing isotopic

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differences between populations. For this reason, interpretations based solely on isotopic differences between foraging locations or on geographic isotopic patterns described in the literature may be misleading; such differences, or lack thereof, could have a geographic basis, or be caused by other factors. Given that diet (Inger et al. 2006), foraging behavior (Cherel & Hobson 2007) and natural biogeochemical gradients (Phillips et al. 2009) simultaneously influence the isotopic composition of marine animal tissues, sorting out the relative contributions of these factors to the variation in animal isotopic signatures remains a challenge.

In this context, several stable isotopes have been shown to offer great discriminatory power in getting at the sources of isotopic variability (Wunder et al. 2005, Moreno et al. 2010). Approaching the question in a multidisciplinary fashion is helpful (Barrett et al. 2007), and complementary tools such as Hg analysis have also proven useful in marine animal feeding studies. Since methyl-Hg is biomagnified in aquatic food webs, the consumption of prey in a higher trophic status has been related to higher Hg consumer burden (Atwell et al. 1998, Monteiro et al. 1998, Bearhop et al. 2000, Arcos et al. 2002, Anderson et al. 2010).

In the present study we examined the influence of feeding ecology and baseline spatial variation on the stable isotopic signature of seabirds. For this purpose, we chose the food web of a coastal seabird species breeding along the northwest coast of Spain, the European shag *Phalacrocorax aristotelis*, analysing the $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and Hg concentrations in chick feathers and fish prey species. The food webs of this area provide an ideal opportunity for analysing the simultaneous influence of spatial variation and feeding and foraging ecology on isotopic values for the following reasons: (1) the European shag breeds in small colonies scattered throughout the study area and is relatively accessible to sampling during the breeding season; (2) the European shag is a strictly piscivorous species whose main prey is well known (Álvarez 1998, Velando & Freire 1999); (3) the breeding area that we studied can be clearly divided into 2 sectors that differ in productivity and isotopic baseline values (Fraga 1981, Figueiras et al. 2002, Bode & Álvarez-Ossorio 2004, Bode et al. 2007); (4) geomorphological and hydrodynamic differences between these 2 sectors are known to result in differences in food web length and structure (Bode et al. 2003, 2004, Signa et al. 2008); (5) the stable isotope diet-tissue fractionation for the European shag has been previously studied in a fish-feeding experiment (Bearhop et al. 1999); (6) chick feathers of this species are a suitable sampling unit because they represent a delimited spatial and temporal frame (Becker et al. 1993, Sanpera et al. 2007), and because, in contrast to adult feathers, interpretation problems caused by mobility, Hg bioaccumulation and differences between Hg and isotopic turnovers are avoided (Bond 2010). In order to be able to compare and appropriately interpret the $\delta^{15}N$ of shags and prey from the 2 sectors, we calculated the shag's and prey's trophic level, which essentially normalized the $\delta^{15}N$ of shags and prey, relating it to the $\delta^{15}N$ of the bases of their respective food webs. For this purpose, the mussel *Mytilus galloprovincialis*, collected from both sectors, was also analyzed and used as an isotopic baseline indicator.

MATERIALS AND METHODS

The study area, located along the northwest coast of Spain, is part of the Iberian Coastal Large Marine Ecosystem, a continental shelf region of the Eastern Atlantic Ocean (see Fig. 1). Topographical features and circulation patterns play a crucial role in the dynamic of the coastal marine ecosystem of this region, with a coastline indented with rías, drowned river valleys that remain open to the sea. On the basis of oceanographic and biological features, we divided the study area into 2 sectors: the Atlantic sector (from the Miño River [41° 52′ N, 8° 52′ W] northward to Cape Finisterre [43° 00′ N, 9° 18′ W]), and the Cantabrian sector (to the west of Cape Estaca de Bares [43° 47′ N, 7° 40′ W]) (Fig. 1). The Atlantic sector is directly influenced by a coastal upwelling, which occurs from March to October, and as a result has higher productivity than the Cantabrian region, which is less influenced by upwelling dynamics (Fraga 1981, Botas et al. 1990, Figueiras et al. 2002). Moreover, such factors as river discharge and other oceanographic characteristics of the Atlantic coast maintain higher levels of productivity in the Atlantic sector. An increase of planktonic $\delta^{13}C$ and $\delta^{15}N$ has been correlated with higher levels of productivity (Bode & Álvarez-Ossorio 2004), and plankton sampled in the Atlantic sector has shown higher isotopic values than plankton sampled in more northern zones (Bode et al. 2007).

During the breeding season of 2004, we sampled fresh adult pellets (remains of indigestible, regurgitated prey), as well as down and definitive feathers (both of which reflect isotopic signatures of prey supplied by the parents) of European shag chicks at 6 insular colonies located within the 2 sectors. These

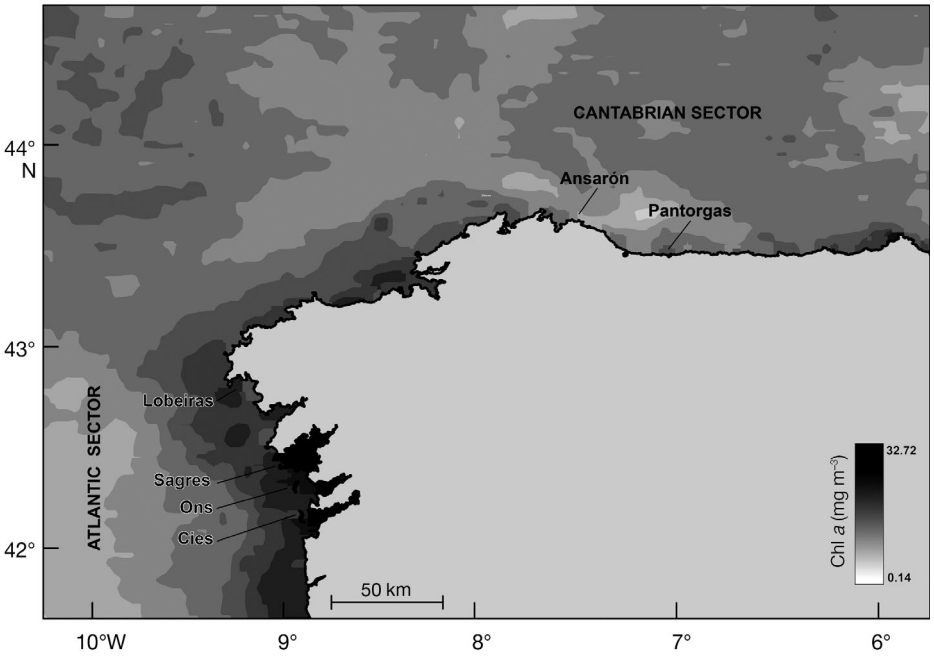

Fig. 1. *Phalacrocorax aristotelis*. Location of colonies sampled and chlorophyll *a* (chl *a*) concentrations of area during chick-rearing period in Atlantic and Cantabrian sectors of northwest Spain

colonies were: Cíes, Ons, Sagres and Lobeiras in the Atlantic sector, and Ansarón and Pantorgas in the Cantabrian sector (Fig. 1). Since the feeding range of breeding European shags is typically <4 km from their colonies (Velando & Munilla 2011), pellet and feather analysis provided closely localized information on the colonies and their surroundings. Local inshore fishermen supplied samples of the most relevant prey of European shag diet, as inferred from pellets and from the literature (Álvarez 1998, Velando & Freire 1999). Because bivalve $\delta^{13}C$ and $\delta^{15}N$ values have been showed to correlate, respectively, with ambient particulate organic matter and with values of water-dissolved $\delta^{15}N\text{-}NO_3$ (Gustafson et al. 2007), they have been widely regarded as suitable time-integrated isotopic baselines for community studies (Hobson & Welch 1992, Cabana & Rasmussen 1996, McKinney et al. 2001, Jennings & Warr 2003, Gustafson et al. 2007). Since isotopic turnover for mussels has been found to be only 113 d, and small sample sizes generate means representative of a larger population, we also collected mussels *Mytilus galloprovincialis* from the 2 study sectors as isotopic baseline indicators. A further reason for this is that isotopic turnover for mussels, in particular, has been found to be only 113 d, and small sample sizes generate means representative of a larger population. Mussels have, in fact, been largely regarded as suitable time-integrated isotopic baselines for community studies (Hobson & Welch 1992, Cabana & Rasmussen 1996, McKinney et al. 2001, Jennings & Warr 2003, Gustafson et al. 2007).

Pellet analysis

We identified prey taxa in 289 freshly regurgitated pellets produced by adults and collected in the breeding colonies during the chick-rearing period. In total, 4538 prey items were identified by means of characteristic hard remains, mostly sagittal otoliths and pharyngeal dental plates. The number of body parts was used to estimate the number of individuals (i.e. prey items) in a pellet. In the case of otoliths, this was achieved by simply dividing the number of otoliths by 2 (rounding up to the nearest whole number). Otolith pairing, the standard method of estimating prey number from otolith counts, was not feasible, as most otoliths were too eroded by the birds' digestive action (see Harris & Wanless 1993). Diet description was based on the numerical frequency of prey items (Table 1).

Stable isotope analysis (C, N, and S)

Feathers were cleaned in a solution of 0.25 M NaOH, oven-dried at 60°C and stored in polyethylene bags until analysis. In the case of mussels, the whole soft bodies were extracted. For fish prey types, muscle samples were processed. To homogenize samples for stable isotope analysis, we ground feathers and freeze-dried mussel and fish muscle samples to an extremely fine powder using a 6750 Freezer/Mill cryogenic grinder (Spex CertiPrep), operating at liquid nitrogen temperature. Since an enrichment in

Table 1. *Phalacrocorax aristotelis*. Relative frequencies (%) of prey fishes found in fresh pellets from adult shags collected during chick-rearing period in Atlantic and Cantabrian colonies. n = sample size

Prey taxon	Atlantic sector				Cantabrian sector	
	Cíes (n = 587)	Ons (n = 754)	Sagres (n = 951)	Lobeiras (n = 881)	Ansarón (n = 625)	Pantorgas (n = 776)
Atherina presbyter	13.3	4.8	7.8	14.6	7.2	16.9
Gymnammodytes semisquamatus	55.3	63.7	36.7	25.8	31.8	7.9
Trisopterus spp.	1.0	4.6	9.3	3.6	0.2	0.9
Gobiidae	11.8	2.5	3.8	1.6	10.7	0.9
Labridae	12.1	18.6	38.2	52.2	44.8	68.2
Others	6.5	5.8	4.2	2.2	5.3	5.2

C, N and S signature after lipid extraction has been described (Hobson & Clark 1992, Pinnegar & Polunin 1999, Sotiropoulos et al. 2004, Oppel et al. 2010), the $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ of prey species with different lipid content (*Gymnammodytes semisquamatus, Trisopterus luscus* and *Labrus bergylta*) were analyzed. Analysis was carried out before and after lipid extraction using several chloroform-methanol (2:1) rinses (Folch et al. 1957) to check the effect on isotopic signatures. No significant differences in tissue $\delta^{15}N$ and $\delta^{34}S$ were found after performing lipid extraction. However, $\delta^{13}C$ varied depending on the species after lipid extraction process (from –1.29‰ for *L. bergylta* to –2.47‰ for *G. semisquamatus*). Thus, stable isotopes were determined on lipid-extracted samples. Weighed sub-samples of the powdered feathers, prey and mussels (~0.36 mg each for $\delta^{13}C$ and $\delta^{15}N$, and 3.6 mg for $\delta^{34}S$) were placed into tin buckets and crimped for combustion. Isotopic analyses were carried out by elemental analysis-isotope ratio mass spectrometry using a ThermoFinnigan EA 1112 Flash elemental analyzer for N and C, and an EA 1108 for S, coupled to a Delta V Isotope Ratio Mass Spectrometer (Thermo Scientific) via a CONFLOIII interface.

Stable isotope ratios were expressed in conventional notation as parts per thousand (‰), using:

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

where X is ^{15}N, ^{13}C or ^{34}S and R is the corresponding ratio ^{15}N:^{14}N, ^{13}C:^{12}C or ^{34}S:^{32}S.

The standards used for ^{15}N, ^{13}C and ^{34}S were atmospheric nitrogen (VAIR), Pee Dee Belemnite (VPDB), and Canyon Diablo Troilite (VCDT), respectively. International standards (IAEA) were inserted every 12 samples to calibrate the system and compensate for any drift over time. Precision and accuracy for $\delta^{13}C$ measurement was ≤0.1‰, for $\delta^{15}N$ ≤–0.3‰ and for $\delta^{34}S$ ≤0.3‰.

To compare the $\delta^{15}N$ signature of chick feathers and prey from the 2 sectors, we computed their trophic position using the following equation:

$$\text{Trophic position}_{consumer} = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta\delta^{15}N$$

where λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$ (e.g. λ = 2 for primary consumers such as mussels; Post 2002), $\delta^{15}N_{consumer}$ is measured directly, and $\Delta\delta^{15}N$ is the trophic fractionation, the enrichment in $\delta^{15}N$ per trophic level. (We used a mean fractionation of 3‰ derived from Vander Zanden & Rasmusssen 2001).

Mercury analysis

Determination of Hg level was carried out using a PerkinElmer ELAN 6000 inductively coupled plasma optical emission spectrometer. We digested feather and prey samples (~100 mg) using 1 to 2 ml HNO_3 and 0.5 to 1 ml H_2O_2 in Teflon containers for 14 h at 90°C.

The accuracy of the analysis was checked by measuring certified reference tissue (Human Hair, BCR 397). Mean recoveries ranged from 80 to 92% and no corrections were made.

Statistical analysis

We routinely checked the values of stable isotope ratios and Hg concentrations for normality using Q-Q plots. Hg concentrations showed skewed distributions and were normalized applying a logarithmic transformation.

Mussel data from 4 localities were compared using 1-way ANOVA. We used 2-way ANOVA to compare mean values among the 4 prey species sampled in

the 2 sectors. Comparisons between sectors (Atlantic and Cantabrian) and among colonies within each sector were made using a nested ANOVA model for log Hg and isotope values in chick feathers. We used Levene's test to check for homoscedasticity. To test for *a posteriori* pairwise differences, we used Tukey's procedure or a sequential Sidak adjustment for nested analysis. Statistical analysis was carried out using SPSS 15.0 (IBM).

RESULTS

Pellet data from adults (Table 1) show that, while *Gymnammodytes semisquamatus* and Labridae were the first and second most important diet items in the Atlantic sector, the most important prey in the Cantabrian colonies were from the Labridae, with *G. semisquamatus* in second place. In both sectors, *Atherina presbyter* was the third most important prey item.

We found significant differences in isotopic signatures between the 2 sectors for mussels, chick feathers and fish prey. Cantabrian mussels showed lower $\delta^{13}C$ (Fig. 2a; $F_{3,26} = 145.9$, $p < 0.001$) and $\delta^{15}N$ signatures (Table 2; $F_{3,26} = 37.9$, $p < 0.001$) and higher ^{34}S values (Fig. 2a; $F_{3,26} = 19.7$, $p < 0.001$) than Atlantic ones. Differences in prey fishes found in both sectors were not constant (e.g. *Atherina presbyter*, *Gobius* spp., *Symphodus melops* and *Trisopterus luscus*), and therefore there was a significant interaction between sector and species for $\delta^{15}N$ ($F_{3,31} = 6.79$, $p = 0.001$) and $\delta^{34}S$ ($F_{3,31} = 3.15$, $p = 0.04$), but not for $\delta^{13}C$ ($F_{3,31} = 0,97$, $p = 0.42$). However, for the 3 isotopes, general trends were the same as those described for mussels: Cantabrian prey showed lower $\delta^{13}C$ (Fig. 2b) and $\delta^{15}N$ signatures (Table 2) and higher $\delta^{34}S$ values (Fig. 2b) than Atlantic prey. In contrast, when computing trophic level, Cantabrian prey showed a higher trophic level (from 0.2 to 0.4) than Atlantic prey, except for *A. presbyter,* which occupied the same low trophic level in both sectors (Fig. 3). Although neither Cantabrian nor Atlantic prey showed differences for $\delta^{13}C$ and $\delta^{34}S$, in the case of both sectors we found significant differences in both $\delta^{15}N$ and trophic level between the 2 main prey species consumed. In the Atlantic sector, *Gymnammodytes semisquamatus* and *A. presbyter* showed lower $\delta^{15}N$ values (Table 2; $F_{\text{Welch } 4,11} = 93.80$, $p < 0.001$) and lower trophic level (Fig. 3; $F_{4,29} = 46.23$, $p < 0.001$) than *T. luscus*, *Gobius* spp. or *S. melops*. Also, in the Cantabrian sector, *A. presbyter* showed lower $\delta^{15}N$ (Table 2; $F_{4,20} = 115.07$, $p < 0.001$) and lower trophic level (Fig. 3; $F_{4,20} = 111.6$ $p < 0.001$) than *T. luscus*, *Gobius* spp., *S. melops* or *Labrus bergylta*. Unfortunately we could not obtain *G. semisquamatus* in the Cantabrian sector from fishermen or local markets. If, however, we were to estimate its trophic level based on isotopic differences between same species in both sectors, its values would be similar to the values of *A. presbyter*. Consequently, in-

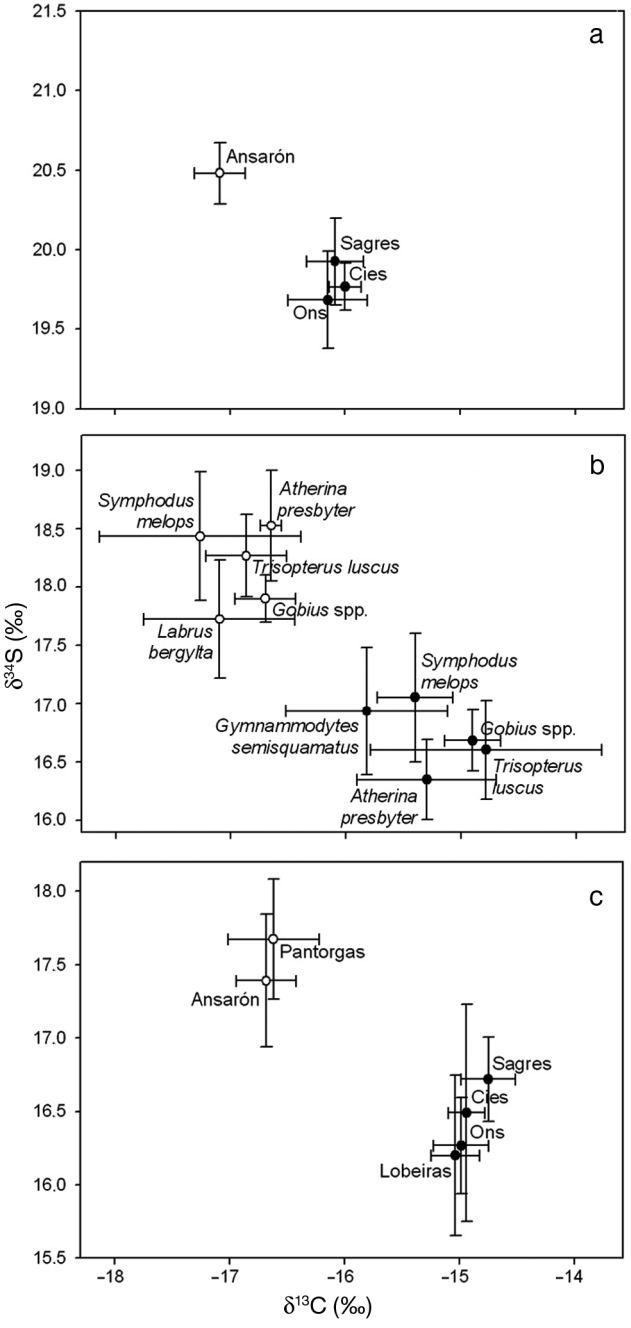

Fig. 2. *Phalacrocorax aristotelis*. Dual stable isotope plots of sulphur-carbon showing isotopic signatures (mean ± SD) of (a) mussels, (b) potential prey, and (c) shag colonies sampled in the Atlantic (●) and Cantabrian sectors (○)

Table 2. *Phalacrocorax aristotelis*. Nitrogen isotopic signatures (‰) and Hg concentrations (ng g^{-1}) (mean ± SD) in mussels, shag chick feathers and potential shag prey sampled in the Atlantic and Cantabrian sectors

	n	$\delta^{15}N$	Hg	Median
Baseline				
Atlantic sector				
Mytilus galloprovincialis	20	7.80 ± 0.22	–	–
Cantabrian sector				
Mytilus galloprovincialis	10	5.82 ± 0.25	–	–
Potential prey				
Atlantic sector				
Ammodytidae				
Gymnammodytes semisquamatus	8	9.71 ± 0.25	119.59 ± 17.14	119.04
Atherinidae				
Atherina presbyter	8	10.23 ± 0.42	105.98 ± 15.01	107.18
Gadidae				
Trisopterus luscus	5	12.10 ± 0.76	212.08 ± 26.47	209.72
Gobidae				
Gobius spp.	6	11.78 ± 0.24	175.81 ± 38.95	160.42
Labridae				
Symphodus melops	3	11.77 ± 0.12	190.21 ± 24.73	199.52
Cantabrian sector				
Atherinidae				
Atherina presbyter	8	8.21 ± 0.25	245.76 ± 23.66	245.53
Gadidae				
Trisopterus luscus	3	11.53 ± 0.67	762.11 ± 141.20	812.83
Gobidae				
Gobius spp.	3	11.03 ± 0.15	460.94 ± 70.88	489.77
Labridae				
Symphodus melops	3	10.43 ± 0.38	537.18 ± 120.29	467.73
Labrus bergylta	4	11.53 ± 0.22	872.72 ± 164.84	867.9
***P. aristotelis* chick feathers**				
Atlantic sector				
Cíes	20	13.13 ± 0.30	542.97 ± 193.61	505.18
Ons	15	13.45 ± 0.26	771.42 ± 496.68	754.14
Sagres	12	13.56 ± 0.22	1071.59 ± 384.91	1086.05
Lobeiras	13	13.29 ± 0.34	886.57 ± 419.79	822.34
Cantabrian sector				
Ansarón	10	13.71 ± 0.48	5094.45 ± 1824.99	4385.67
Pantorga	15	13.84 ± 0.27	3087.50 ± 1399.43	3451.33

terpretations of shag feeding ecology that take into account such estimation would not vary from those made based only upon the prey species included in this study.

Similarly to the geographic trend reflected by mussels and prey items, chick feathers sampled at Cantabrian colonies presented lower $\delta^{13}C$ (Fig. 2c; $F_{1,79}$ = 697.4, $p < 0.001$) and higher $\delta^{34}S$ (Fig. 2c; $F_{1,79}$ = 76.7 $p < 0.001$) values than those sampled among Atlantic colonies. In contrast to the difference of 2‰ found for $\delta^{15}N$ in mussels, chick feathers from Cantabrian colonies showed slightly higher $\delta^{15}N$ ($F_{1,79}$ = 34.5, $p < 0.001$, 95% CI of the difference between 0.29 and 0.59 ‰) than chick feathers from Atlantic colonies and, as reflected by SE (Table 2), an important overlap occurred between values from both sectors. Only chick feathers from Cíes showed a lower $\delta^{15}N$ than those from Sagres (Fig. 2c; $F_{4,79}$ = 3.6, p = 0.01). However, once their trophic position was calculated, Cantabrian chicks shower a higher trophic level (Fig. 3; $F_{1,79}$ = 908.1, $p < 0.001$, 95% CI of the difference between 0.73 and 0.84 trophic level) than those from the Atlantic sector.

Log Hg concentrations for prey were explained by trophic position ($F_{1,49}$ = 191.7, $p < 0.001$) and by sector ($F_{1,49}$ = 172.8, $p < 0.001$), and were higher in the Cantabrian sector (Table 2, Fig. 4a). However, in the case of chick feathers, sector and trophic position were strongly correlated, so when trying to model its log Hg concentrations (Fig. 4b), the trophic position (with a high covariance by its continuous nature) stands out as the only significant effect ($F_{1,78}$ = 9.4,

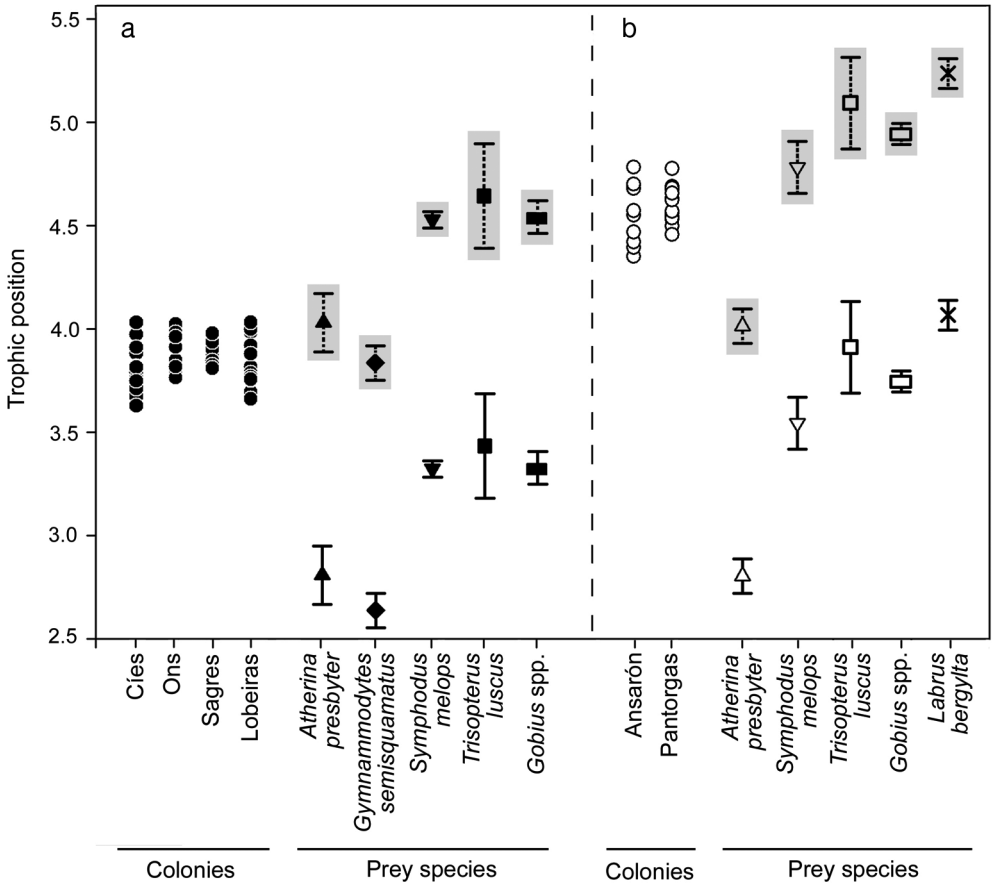

Fig. 3. *Phalacrocorax aristotelis*. Trophic position of shag chick feathers (circles) and potential prey species (triangle, diamond, inverted triangle, square, brick and cross, all mean ± SD) sampled in (a) Atlantic (filled symbols) and (b) Cantabrian (open symbols) sectors. Shaded boxes indicate predicted trophic position of shags on basis of single-species fish diet considering fractionation factor of 3.6‰ described in a feeding experiment with captive shags (Bearhop et al. 1999)

p = 0.003). The estimated slope for chick feathers overlaps with that found in prey data (95% CI of estimated slopes for trophic position are 0.25 to 1.2 in chick feathers and 0.31 to 0.42 in prey). Although the difference between sectors was not significant, it is consistent with that found in prey data, and thus the possibility of a sector effect cannot be discarded (Table 2, Fig. 4).

DISCUSSION

Our study demonstrates that the $\delta^{15}N$ signature of seabirds is strongly influenced by feeding ecology and spatial variation. In contrast, the $\delta^{13}C$ and $\delta^{34}S$ signatures are affected mainly by spatial differences. Although stable isotope analysis is frequently used to infer diet and migration movements of seabirds, our results highlight the difficulties inherent in studies that do not take into account the isotopic signatures of prey and baseline signatures or the interpretation of isotopic data based solely upon foraging and geographic patterns available in the literature. In our case, omission of the values of potential prey and baselines, or application of only a single baseline to the food webs of the 2 sectors (assuming isotopic homogeneity because of geographical closeness), would have led to significantly distorted interpretations of feeding ecology.

The $\delta^{15}N$ signatures did not differ by more than 0.5‰ between Atlantic and Cantabrian shags (Table 2). Without considering the baselines of both sectors, this difference could have been interpreted as occupancy of a similar trophic level. Also, in the case of $\delta^{13}C$ and based on bibliographic evidence of benthic or pelagic gradients (France 1995), the higher values of Atlantic colonies could be attributed to greater reliance on resources from benthic habitats. Nevertheless, when we contextualized the isotopic values by taking into account values of local baselines and those of potential prey, an opposite view of the same landscape emerged.

In the case of $\delta^{15}N$, mussels reflected significant differences (2‰) in the spatial variation between the isotopic baselines of the 2 sectors (Table 2). Moreover, pelagic and semi-pelagic fish species feeding on plankton (*Atherina presbyter* and *Gymnammodytes semisquamatus*) showed lower $\delta^{15}N$ values (Table 2), and, in the case of both areas, lower trophic level (Fig. 3) than omnivorous benthic and demersal

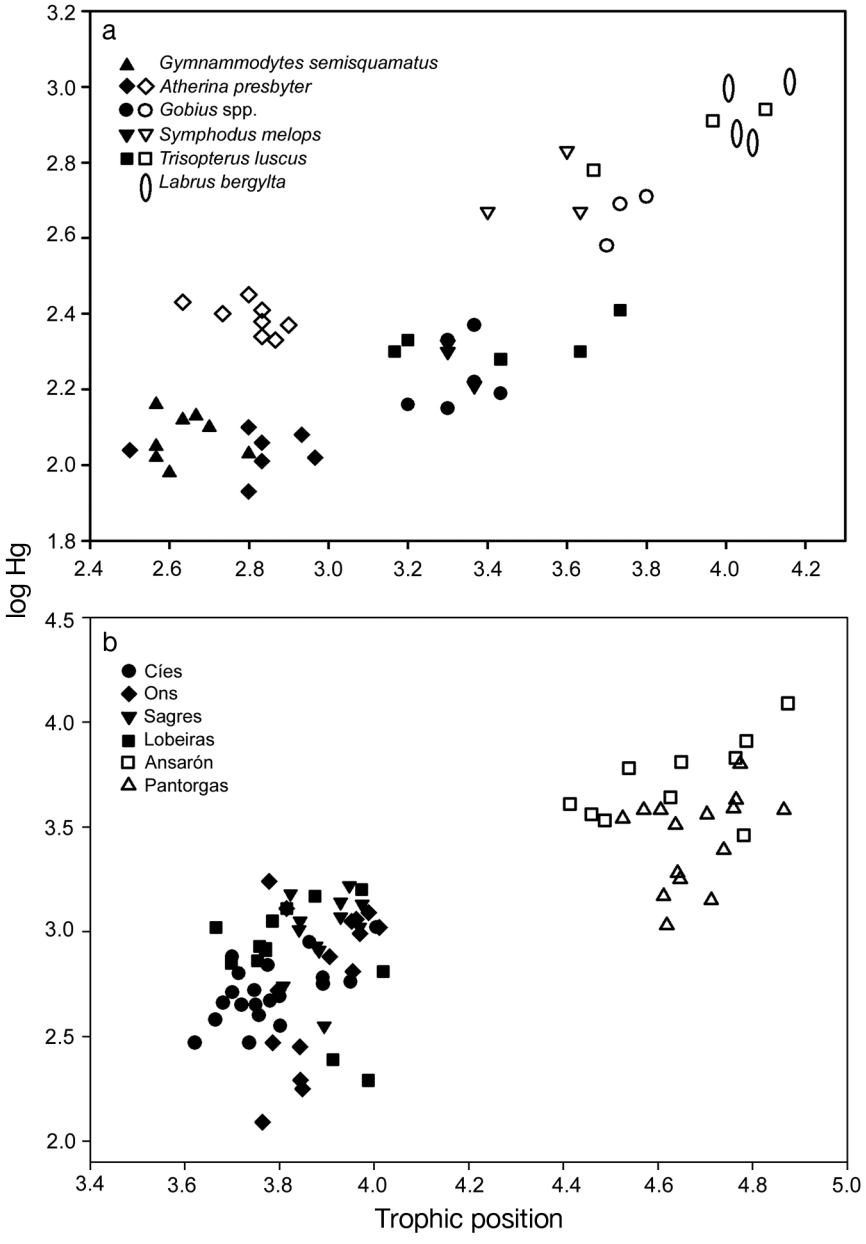

Fig. 4. *Phalacrocorax aristotelis*. Relationship between trophic position and Hg concentrations (log scale) in (a) potential prey and (b) chick feathers sampled in Atlantic (filled symbols) and Cantabrian sectors (open symbols)

species from sandy and rocky bottoms (*Trisopterus* spp., *Symphodus melops*, *Labrus bergylta* and *Gobius* spp.). Consequently, when the $\delta^{15}N$ signatures of shags are corrected by their respective baselines, the resultant trophic positions inform us that Atlantic chicks occupy a lower trophic level than Cantabrian chicks (3.9 vs. 4.7; Fig. 3). While shag chicks from Atlantic colonies were fed more on pelagic or semi-pelagic species (Fig. 3a), those from the Cantabrian colonies were fed more on benthic species (Fig. 3b). This dietary description also matches the variability in the feeding habitats reflected in adult pellet composition (Table 1) and what is described in the literature (Álvarez 1998, Velando & Freire 1999). Differences in prey availability caused by geomorphological and hydrodynamic differences between sectors has been associated with a higher adult biomass consumption of *G. semisquamatus* in Cíes and Ons (Velando & Freire 1999) and with a diet based mainly on *L. bergylta* and *S. melops* at La Caladoria, another European shag breeding colony on the Cantabrian coast (Álvarez 1998).

On the basis of our results, the $\delta^{15}N$ signature, as previously suggested (Bearhop et al. 2001, Cherel & Hobson 2007, Phillips et al. 2009), is not only affected by diet but is also largely influenced by oceanic processes. Our findings imply that, since seabirds tend to concentrate and move between habitats of enhanced and changing productivity, such as shelf edges, frontal zones and upwellings (Weimerskirch 2007), the simultaneous influence of feeding ecology and baseline spatial variation as a result of oceanic processes on their $\delta^{15}N$ signatures may be frequent. Such influence should therefore be carefully considered when interpreting isotopic variability among samples from different localities.

With regard to $\delta^{13}C$, Atlantic mussels, prey and chick feathers showed higher signatures than Cantabrian samples, reflecting the same geographic tendency from baseline to consumers (Fig. 2). However, pelagic or semi-pelagic and benthic prey species in the 2 sectors were not statistically different (Fig. 2b). Thus, in this study, the differences in $\delta^{13}C$ signature between sectors appeared to be influenced mainly by geography-related isotopic variation rather than by differences in diet composition. Finally, although inter-sector differences between $\delta^{34}S$ signatures were smaller than for $\delta^{13}C$, mussels, prey and chick feathers also showed the same geographic trend, with Atlantic samples showing lower values than Cantabrian sam-

ples (Fig. 2). Given that we found no differences among $\delta^{34}S$ of prey species in either of the 2 sectors (Fig. 2b), our results emphasize the usefulness of $\delta^{34}S$, in addition to $\delta^{13}C$, as reliable geographic markers. Regional differences in $\delta^{34}S$ and $\delta^{13}C$ baseline can be ascribed to the varying influence of natural sources. In the case of our study area, an increase of planktonic $\delta^{13}C$ has been related with levels of productivity (Bode & Álvarez-Ossorio 2004). Moreover, the rías also support large populations of macroalgae and seagrasses that are a significant source of organic matter for littoral food webs and that have been shown to have higher $\delta^{13}C$ and lower $\delta^{34}S$ than plankton (Kharlamenko et al. 2001, Bode et al. 2006). Thus, both higher productivity and a greater contribution of macroalgae and seagrasses to organic matter may explain the higher $\delta^{13}C$ values and lower $\delta^{34}S$ of mussels, prey and shag chicks from the Atlantic sector.

This study also indicates that the overlapping effects of the factors that simultaneously shape the isotopic signatures of seabirds may have been underestimated in previous research. Spatial and seasonal changes in the diet of the same species commonly occur in response to fluctuations in food availability, energy requirements or reproductive constraints (Knoff et al. 2002, Soto et al. 2006, Drago et al. 2009); they may result in a change in the trophic level, such as that observed in this spatial study. Such a change would involve a difference of approximately 2 to 3‰ in the $\delta^{15}N$ of consumer tissue, the same isotopic variation interpreted as related to geography between areas thousands of km apart (Wallace et al. 2006, Gómez-Díaz & González-Solís 2007) in studies in which it was not possible to discriminate between the influence of geographic variation in baseline isotopic levels and the influence of diet.

The differences in $\delta^{13}C$ between sites may also be underestimated if baseline signatures are not investigated. Without previous information, the 2 sectors sampled in the present study might have been considered as belonging to the same isotopic province within the Atlantic Ocean because they are separated by only 100 km. However, $\delta^{13}C$ signatures of chick feathers differed by 2‰, the same isotopic variation associated with migration between regions separated by 7000 km within the Atlantic (Caut et. al. 2008, Navarro et al. 2009, Ramos et al. 2009b). Cherel & Hobson (2007) pointed out that latitudinal and inshore/offshore and benthic/pelagic gradients induce an overlap in $\delta^{13}C$ values of consumers, which can lead to strong misinterpretations of foraging origins. Our results illustrate how baseline isotopic signatures reflect marine physical processes and different sources of organic matter that result in spatial changes of $\delta^{13}C$ and $\delta^{34}S$ and thus can also lead to distorted interpretations.

Hg concentrations also seemed to be influenced by both feeding ecology and baseline levels characteristic of each area. Since log Hg concentrations of potential prey from both sectors were positively correlated with their trophic position (Fig. 4a), the higher Hg concentrations found in Cantabrian than in Atlantic chick feathers (Fig. 4b) seemed to be related to a greater consumption of higher-trophic-level prey. Nevertheless, regional differences between sectors clearly had a stronger influence than trophic level on Hg concentrations of prey and shag chicks. In this regard, a previous monitoring program carried out in our study area reflected that mussels sampled along the Cantabrian coast showed higher Hg concentrations than those sampled along the Atlantic coast (Besada et al. 2011). In accordance with this result, Cantabrian prey and chick feathers in the present study were seen to contain between 2 and 5 times more Hg than Atlantic prey and chick feathers (Table 2). This study thus also demonstrates that, without interfering factors such as bioaccumulation related to age or sex, Hg analysis of chick feathers can be very helpful when exploring geographical patterns of pollution, and in contaminant-monitoring programs. Once again, however, absolute comparison across systems can be confounded by the simultaneous effect of trophic ecology and regional variation on Hg levels. Consequently, both factors should be considered in order to gain a fuller understanding of the mechanisms of contaminant distribution.

In summary, this study shows the importance of assessing spatial variation in baseline isotopic signatures and their progressive integration through the food web to make appropriate and conclusive interpretations of marine consumer isotopic signatures. In this regard, the use of complementary methods such as Hg analysis may permit more detailed interpretation of feeding ecology or geographic distribution.

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