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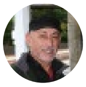

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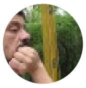

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Spatial variation of mercury levels in nesting Bonelli's eagles from Southwest Portugal: effects of diet composition and prey contamination

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"Capsule": *The effects of diet composition and prey contamination added up to determine the spatial variation of Hg levels in breeding Bonelli's eagles.*

Abstract

Mercury (Hg) was determined in adult Bonelli's eagles (*Hieraaetus fasciatus*) and their avian prey, from samples of feathers collected between 1992 and 2001 at the nesting sites of 21 pairs in Southwest Portugal. Eagle Hg levels showed great variation, reflecting primarily differences in diet composition and food chain biomagnification. Concentrations were positively correlated with the dietary proportion of insectivorous and omnivorous birds (e.g. egrets, corvids and thrushes), with very low levels for pairs feeding mainly on herbivores (e.g. rabbits, pigeons and partridges). Differences in prey contamination among breeding territories added to dietary effects in determining variation of Hg levels in eagles, shaping a spatial pattern that was largely consistent with a source of contamination in a coal-burning power-plant lying upwind of the study area. Despite this presumed contamination, Hg levels seemed to be of little concern to this eagle population, though there might be subtle deleterious effects on the reproductive output of a few pairs. This study emphasizes the need to account for dietary effects when biomonitoring Hg contamination using birds of prey.

Keywords: Biomagnification; Biomonitoring; Birds of prey; Jay; Partridge; Pigeon

1. Introduction

As top predators, birds of prey are exposed to an array of persistent environmental contaminants that biomagnifies through food webs, especially organochlorine pesticides, polychlorinated biphenyls (PCBs), and mercury (Hg). Accumulation of these chemicals has been particularly well documented for aquatic food webs, where species such as sea eagles (*Haliaetus* spp.) and ospreys (*Pandion haliaetus*) have shown poor breeding and enhanced mortality in association with high pollutant burdens (Helander et al., 1982; Wiemeyer et al., 1984, 1988). Although much less documented, population declines attributed to environmental contaminations have also been shown for species feeding on terrestrial food chains such as the sparrowhawk (*Accipiter nisus*) (Newton et al., 1993). Because of this vulnerability to a variety of contaminants, birds of prey have been used extensively as biomonitors of environmental quality (Berg et al., 1966; Lindberg and Odsjö, 1983; DesGranges et al., 1998; Mañosa et al., 2003).

Besides their high trophic status, many birds of prey are territorial, non-migratory and long-lived, and so pollutant burdens recorded in body soft tissues, bones,

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feathers and eggs are likely to reflect chemical contamination within their extended home ranges. This view underlies most biomonitoring programs, which assume, often implicitly, that spatial or temporal variations in pollutant burdens are coupled with comparable spatial or temporal trends in environmental contamination. Although this assumption may sometimes be warranted for birds of prey (e.g. DesGranges et al., 1998), there are at least some circumstances in which it may fall short of reality. A major source of potential shortcomings is related to diet composition, which may elicit variation in pollutant burdens among individuals of the same species collected at different locations or at different times, irrespective of corresponding variation in environmental contamination. For instance, some studies have linked local peak contamination levels in bald eagles (*Haliaetus leucocephalus*), golden eagles (*Aquila chrysaetos*) and peregrine falcons (*Falco peregrinus*) with a high consumption of aquatic birds such as waders and seabirds (Lindberg and Odsjö, 1983; Parrish et al., 1983; Furness et al., 1989; Anthony et al., 1999). Similar effects for species feeding exclusively on terrestrial prey are scarce, though recent evidence suggests that they may also occur (Mañosa et al., 2003). Clearly, there is a need to evaluate in more detail the effects of diet composition on the pollutant burdens of birds of prey feeding on terrestrial food chains, and how these may confound the interpretation of spatial or temporal patterns in environmental contamination.

The present study addresses these issues, by analyzing the relationships between diet composition, prey contamination, and spatial variation of Hg levels in feathers of Bonelli's eagles (*Hieraaetus fasciatus*). These are medium-sized eagles, whose numbers and range have declined markedly in Europe, where they are restricted to the Mediterranean region (Rocamora, 1994). Bonelli's eagles feed primarily on terrestrial birds and mammals, showing significant geographical variation in diet composition depending on local habitat conditions (Real, 1991). The study was carried out in the uplands of southwestern Portugal, where a dense Bonelli's eagle population of great conservation significance lies downwind of a coal-burning power-plant. Because of this, there were concerns that these eagles could be exposed to an important source of Hg contamination, with potential negative repercussions upon their reproductive output and health condition. This justified a closer examination of factors underlying spatial variation in Hg burdens in the eagles and their prey.

2. Materials and methods

2.1. Study area

Data were collected as part of a long-term study on the Bonelli's eagle in the uplands of Algarve and western Alentejo (southern Portugal), from 21 out of 25 eagle territories occupying about 3000 km^2 in a rough triangle linking the mountains of Cercal (341 m), Monchique (902 m) and Caldeirão (589 m) (Fig. 1). The hilly landscape is predominantly covered by cork oak (*Quercus suber*) woods, dense Mediterranean scrub and eucalyptus (*Eucalyptus globulus*) plantations, with sparse human occupation. Bonelli's eagles breed primarily in large cork oaks, eucalyptus and pine trees (*Pinus* spp.), and feed on domestic doves (*Columba labia*), red-legged partridges (*Alectoris rufa*), jays (*Garrulus glandarius*), rabbits (*Oryctolagus cuniculus*), and many other secondary prey (Palma, 1994; L. Palma, unpublished data). The main potential source of Hg contamination is a coal-burning power-plant located at Sines, on the north-west corner of the study area (Freitas et al., 1999). No additional sources of Hg contamination, either telluric or agricultural, were identified within the study area.

2.2. Sampling procedures

2.2.1. Feather samples

From 1992 to 2001, shed feathers of adult Bonelli's eagles and feathers from avian prey remains were collected from nests and neighbouring tree perches to measure Hg levels. Active nests were visited three times during each breeding season, between the end of incubation and shortly after nest abandonment (March–July), and feathers of each species were collected in separate labelled plastic bags and stored in a freezer at −20 °C. Eagle feather samples were obtained on only 2.3 ± 1.1 SD (1–4) years per breeding pair, because the location of some nests was unknown in early years of the study, some pairs did not breed every year and shed feathers were occasionally absent. Most eagle feathers were probably from females, as they tend to spend far more time near nests than males (Blondel et al., 1969; Morvan and Dobchies, 1987; L. Palma, unpublished data), and because the matching between sampling and moulting periods was closer for females than for males (L. Palma, unpublished data). Feathers were used as monitoring units because Hg in feathers reflects body Hg burden (Furness et al., 1986; Thompson et al., 1990) and it is almost entirely in the mono-methylated form (Thompson and Furness, 1989a,b). Furthermore, feathers have been widely used to monitor Hg levels in freshwater, marine and terrestrial bird species (Furness, 1993), including birds of prey (Dauwe et al., 2003). Only body feathers were analysed, since they provide more representative samples for estimating whole-bird Hg content than flight feathers (Furness et al., 1986).

2.2.2. Diet composition

The diet of eagles in each individual breeding territory was analysed from prey remains collected during the visits to active nests and surrounding perches. Although

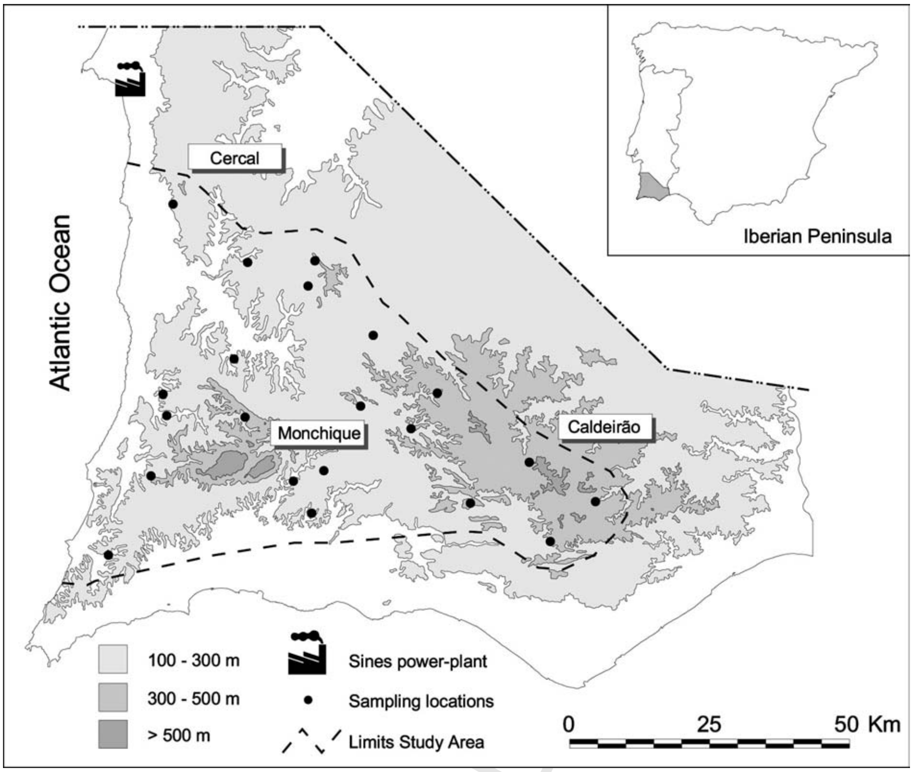

Fig. 1. Locations in Southwest Portugal where feather samples of Bonelli's eagles and their main avian prey were collected for the analysis of Hg contamination (1992–2001). Main mountain ranges are indicated.

remains correspond primarily to prey consumed by nestlings, they likely reflect also the diet of adults, which regularly eat part of the prey delivered to the nests (Blondel et al., 1969; Morvan and Dobchies, 1987; L. Palma, unpublished data). Remains were identified with the help of keys to bird feathers and a reference collection, and the minimum number of individuals in any sample was estimated from the highest number of identical bones of each prey type. This method tends to underestimate the consumption of small prey yielding few remains, while overestimating large prey or prey with a large proportion of rejected parts, such as bird feathers (Real, 1996). However, the method may be considered useful in comparative studies like this one, which aim to detect variation in the relative consumption of different prey, and not estimating the absolute diet composition. Because Hg contamination is strongly dependent on trophic position (Dietz et al., 2000), prey items were categorised according to whether they feed predominantly on plants or animals. Species such as rabbits, partridges, wildfowl and seed-eating passerines were classified as primary consumers, whereas species such as egrets, gulls, birds of prey, corvids and other insectivore passerines were classified as secondary consumers.

2.2.3. Mercury determinations

Feather samples were analysed for total Hg concentration by Cold Vapour Atomic Absorption Spectroscopy (CV-AAS). Samples were digested in a water bath at 70 °C for 6 h by the addition of concentrated H_2SO_4. After this period 5% $KMnO_4$ was added and the solution kept at 70 °C for two more hours. The $KMnO_4$ in excess was reduced with 20% $NH_2OH.HCl$. All reagents used throughout the work were of analytical grade. The glassware was previously decontaminated by immersion in an HNO_3 1:5 solution and then washed with deionized water. Reproducibility was checked by performing successive measurements with the same sample. Relative standard deviations in the range 3–5% were found. Accuracy of the method was within 10% and was monitored analysing reference materials: tuna muscle 350 (International Atomic Energy Agency, Monaco) and RM50 (USA National Bureau of Standards for Biological Material). Minimum detection limits (MDL) of 0.01 μg Hg/g digested sample were quantified using the Kaiser–Currie method (Gibbons and Coleman, 2001). Interferences due to matrix and the pre-treatment were assessed by the method of standard additions before the wet mineralization procedure. Recoveries of added Hg were close to 100%. Hg concentration is given on a wet weight basis.

2.2.4. Statistical analysis

Mean Hg concentrations were computed for feather samples collected from each Bonelli's eagle pair in any given year. The overall Hg concentration corresponding to each breeding pair was then quantified as the mean of concentrations estimated in different years. Samples from avian prey were treated likewise. Before statistical analysis, nondetected measurements were replaced by half the detection limit (Gibbons and Coleman, 2001), and Hg concentrations were log-transformed to approach normality and homogenising variances (Zar, 1996). The arcsine transformation was used likewise for percentage data quantifying diet composition. Differences in Hg levels between species at matching locations were compared using paired-samples *t*-tests (Zar, 1996). Significance levels were corrected for multiple comparisons using the sequential Bonferroni technique (Rice, 1989). Pearson correlations and regression analyses were used to evaluate the relationships between eagle Hg levels, diet composition and avian prey contamination (Zar, 1996).

Spatial patterns in Hg levels for eagles and their main prey were mapped by interpolating to a continuous grid, the concentrations recorded at sampling locations, using inverse-distance weighing (Legendre and Legendre, 1998). Residuals of the regression equation relating eagle Hg levels to diet composition were also mapped, to illustrate the spatial contamination patterns after statistically accounting for dietary effects. In distance weighing, the extinction rule was $1/r^2$ (r is the distance between grid and sampling points), producing a smooth surface and avoiding the need to introduce an artificial cut-off distance (Legendre and Legendre, 1998).

3. Results

3.1. Diet

Eagle diets were described from an average 24.6 ± 15.2 SD (5–64) prey remains identified per eagle pair (Table 1). Almost half the overall remains were pigeons, over 95% of which were identified as domestic pigeons. Other important prey items were red-legged partridges, rabbits and corvids, about 75% of which were jays. Only 17.5% of prey remains corresponded to species categorised as secondary consumers, though their relative importance in the diet varied markedly among breeding pairs, from about 2.1% to 44.4%. Over 95% of individual prey identified corresponded to terrestrial species, with only gulls and mallards (*Anas plathyrhynchos*) feeding regularly on aquatic food chains.

3.2. Eagle and avian prey Hg levels

Bonelli's eagles, red-legged partridges, domestic pigeons and jays showed some marked differences in their Hg concentrations (Table 2). Eagles showed much higher Hg concentrations than both partridges ($t_{18} = 7.513$, $P < 0.001$) and pigeons ($t_{19} = 9.822$, $P < 0.001$), but they had similar levels to those of jays ($t_{10} = 1.630$, $P > 0.1$). Likewise, levels in jays were much higher than in partridges ($t_9 = 7.434$, $P < 0.001$) and pigeons ($t_{10} = 8.195$, $P < 0.001$). Concentrations of Hg in partridges and pigeons were virtually identical ($t_{16} = 0.111$, $P > 0.9$).

Table 1
Composition of Bonelli's eagle diet in Southwest Portugal (1992–2001), as assessed from the remains of 541 identified preys recovered from the nests of 21 breeding pairs

Prey items		*N*	%
Birds			
Cattle egret	*Bubulcus ibis*	11	2.0
Gulls	*Larus* spp.	17	3.1
Red-legged partridge	*Alectoris rufa*	92	17.0
Domestic fowl	*Gallus gallus*	20	3.7
Pigeons	*Columba* spp.	256	47.3
Corvids	Corvidae	43	7.9
Thrushes	Turdidae	12	2.2
Other birds	Mainly Anatidae, Picidae and Strigidae	23	4.3
Mammals			
Rabbit	*Oryctolagus cuniculus*	67	12.4
Hare	*Lepus granatensis*	1	0.2

N = number of individual prey items; % = percentage of total prey recovered.

3.3. Effects of diet and prey contamination

Relationships between eagle Hg levels and diet compositions were assessed using the 17 breeding pairs for which there were more than 10 prey remains. Hg concentrations were negatively correlated with the dietary proportion of pigeons ($r = -0.529$, $P < 0.05$), but not with those of partridges ($r = 0.233$, $P > 0.4$), jays ($r = 0.381$, $P > 0.1$) and rabbits ($r = 0.003$, $P > 0.9$). Analyses for other prey items were not made because they occurred too infrequently in eagle's diet. A strong positive correlation was found for prey categorised as secondary consumers ($r = 0.813$, $P < 0.001$), reflecting the strong influence of prey trophic position on eagle Hg levels (Fig. 2).

Concentrations of Hg in eagles were correlated with those in jays ($r = 0.634$, $P < 0.05$, $n = 11$), but not with

Table 2
Means, standard deviations and ranges of Hg concentrations (μg g^{-1} wet weight) in feather samples of Bonelli's eagles and their main avian prey collected in Southwest Portugal (1992–2001)

Species	*N*	Mean	Standard deviation	Range
Bonelli's eagle	21	1.94	1.54	0.25–5.42
Domestic pigeon	20	0.13	0.17	<MDL–0.70
Red-legged partridge	18	0.11	0.11	<MDL–0.46
Jay	11	1.58	0.71	0.83–3.41

N = number of breeding pairs from which samples were collected; MDL = Minimum Detection Limit.

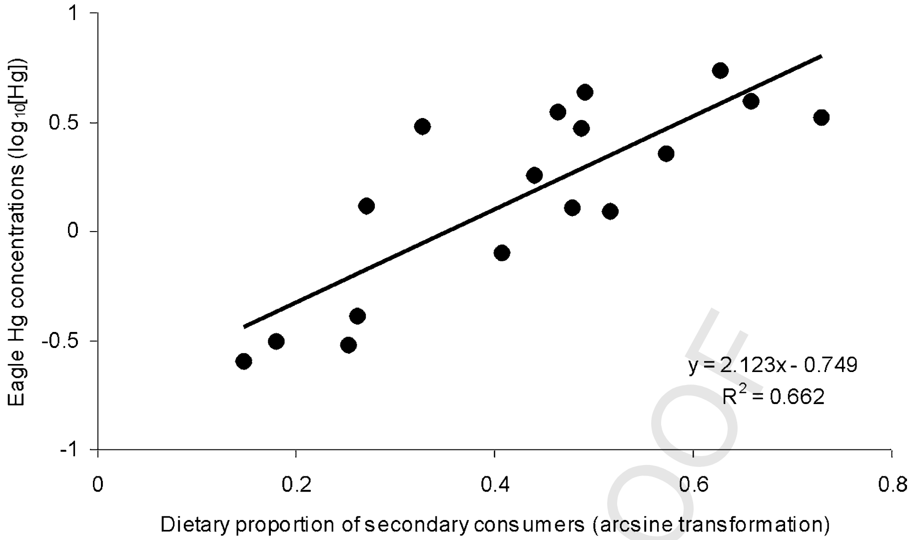

Fig. 2. Mean Hg concentrations (log-transformed; *y*) recorded in 17 Bonelli's eagle pairs breeding in Southwest Portugal (1992–2001), as a function of the dietary proportion (arcsine-transformed; *x*) of secondary consumers.

those in either partridges ($r = -0.064$, $P > 0.7$, $n = 19$) or pigeons ($r = 0.190$, $P > 0.4$, $n = 20$). In a multiple regression accounting for both the effects of diet composition and prey contamination, variation in Hg levels in eagles could be explained to a very large extent by the positive effects of the dietary proportion of secondary consumers and the concentration of Hg in jays (Table 3). Hg concentrations in pigeons and partridges never showed significant effects in similar multiple regressions relating eagle Hg levels with diet composition and prey contamination.

3.4. Spatial patterns

Hg levels in Bonelli's eagles tended to decline eastwards from the relatively high values recorded along the western portion of the coastal mountain ranges of Cercal and Monchique to the low values found in the eastern Caldeirão uplands (Fig. 3). However, high values were also found in one pair breeding along the north-eastern edge of the study area, and in two pairs breeding southeast of Monchique. The residuals of the regression equation between eagle Hg levels and the dietary proportion of secondary consumers (Fig. 2) were used to illustrate the spatial distribution of eagle contamination after correcting for dietary variation (Fig. 3). The emerging spatial pattern underlined the contrast between the western and eastern part of the study area, with the highest Hg levels concentrating around Monchique and the lowest in Caldeirão. High contamination values were also found in two pairs breeding on the north-eastern border of the study area.

For the three avian prey species there were differences in detail for the spatial patterns of Hg contamination, though they all showed a trend for higher values in the western part of the study area (Fig. 3). Furthermore, the highest Hg levels in both pigeons and jays were recorded in the mountain of Cercal, in the sampling site closest to the industrial complex of Sines (Fig. 3). There were, however, exceptions to the west–east gradient of declining Hg levels, with some high values also recorded at the eastern end of Caldeirão for both pigeons and partridges.

4. Discussion

The Hg levels found in feathers of Bonelli's eagles breeding in the uplands of south-western Portugal showed great variation, which seemed to reflect primarily differences in diet composition and food chain biomagnification. The highest concentrations were recorded in pairs incorporating a high proportion of secondary consumers in their diet, whereas much lower values were found for eagles feeding almost exclusively on herbivores such as rabbits, pigeons and partridges. Comparable effects of trophic chain length have been

Table 3
Multiple linear regression relating Hg concentrations in 10 Bonelli's eagle pairs breeding in Southwest Portugal, to prey contamination and diet composition ($R^2 = 0.954$, $F_{2,7} = 35.676$, $P < 0.001$)

Variables	Regression coefficients	*t*	*P*
Intercept	−0.678	−4.181	<0.01
Concentration of Hg in jays (log-transformed)	1.716	4.577	<0.01
Dietary proportion of secondary consumers (arcsine-transformed)	1.461	4.302	<0.01

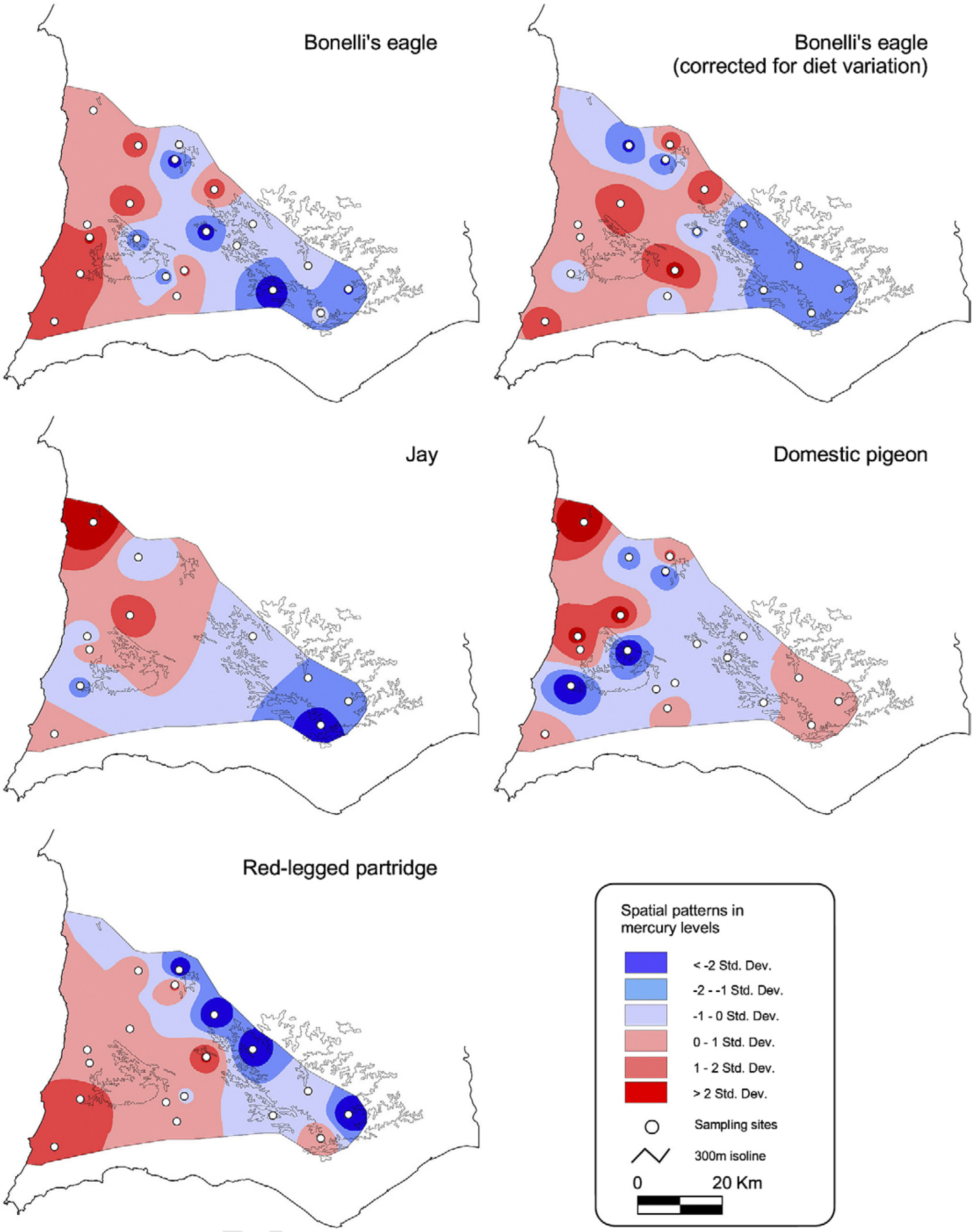

Fig. 3. Spatial distribution of Hg concentrations measured in Bonelli's eagles and their main avian prey. Values are given as standard deviations from the mean, to increase comparability among maps. Eagle data corrected for diet composition are the residuals of the linear regression depicted in Fig. 2, between eagle Hg levels and the dietary proportion of secondary consumers.

noted mainly in marine and freshwater systems (e.g. Elliot et al., 1996; Anthony et al., 1999), with comparable data generally lacking for terrestrial food webs. In a study involving organochlorine contaminants in goshawk (*Accipiter gentilis*) eggs, however, Mañosa et al. (2003) also documented the highest concentrations in association with a higher consumption of passerine birds relative to that of rabbits. The paucity of data for terrestrial chains is probably related to their shorter length in relation to that of aquatic ones, which lessens the potential for Hg biomagnification along the food web (Dietz et al., 2000). Nevertheless, this study strongly suggests that food web length in terrestrial systems may also be a major source of variation in Hg contamination for top predators such as the Bonelli's eagle, which can feed at multiple trophic levels.

After statistically accounting for dietary effects, Hg concentrations in eagles also reflected the contamination level in some of their prey. Indeed, there was a strong correlation between Hg concentrations in feathers of eagles and jays, though no such relationship was apparent for pigeons and partridges. The general pattern for other secondary consumers might have been similar to that recorded for jays, though the shortage of feather samples precluded the testing of this hypothesis. Lack of relationship between eagle and pigeon contamination was unexpected, as these are the staple food of eagles. However, Hg levels were very low in herbivorous prey, which suggests that eagles acquire most of their burden through the intake of secondary consumers. Additional information on the sources of variability in prey contamination is needed to gain a better understanding of the mechanisms leading to Hg accumulation in the eagles.

The strong relationship between eagle Hg levels, diet composition and contamination of prey collected from nests, suggests that concentrations found in shed body feathers probably resulted primarily from exposure during the breeding season. In the study area, adult eagles are largely resident within the breeding territories, starting to visit the nests in November, long before the shed feathers could be found, and remaining in the surroundings at least until juvenile emancipation in August–September (L. Palma, unpublished data). The shed body feathers analysed were generally collected in the late nestling and early fledging periods ($>85\%$ in April–June), corresponding to the post-nuptial moult, which may extend until early autumn (Parellada, 1984; L. Palma, unpublished data). These shed feathers grew during the previous moulting season, thus receiving Hg that had been stored in body tissues over the preceding months (Furness et al., 1986; Furness, 1993; Dauwe et al., 2003). Hg probably accumulated in the adult eagles mainly while foraging within their extended breeding ranges, thus integrating contamination from areas lying in general within 10 km from the nests (L. Palma, unpublished data). This supports the assumption that variation among pairs in the concentrations recorded in shed feathers should reflect at least partly the broad scale spatial trends in environmental contamination, once the dietary effects are accounted for.

Regional Hg concentration trends in prey species and in Bonelli's eagles, after correcting for dietary variation, broadly agree with the hypothesis of a contamination source in the industrial complex of Sines, presumably associated with the coal-burning power-plant, though there were differences among species. Reasons for these differences are unclear, but they may probably be attributed to local factors and sampling variability, which likely added to the large scale contamination trend in influencing the spatial patterns observed. These local factors are impossible to assess with the data collected, but they may result from variation among eagle breeding territories concerning the diets or feeding habitats of the prey species captured. Despite these confounding factors, there was a general trend for higher Hg levels in the western uplands of Cercal and Monchique, which lie immediately downwind of the industrial complex and are thus probably more likely to be contaminated from airborne pollutants than the eastern Caldeirão mountains. Furthermore, precipitation along the coastal uplands, particularly in Monchique, is in general much higher than further inland, which may favour the removal from the atmosphere and local wet deposition of Hg emitted in combustion facilities (Carpi, 1997). This view is also supported by the distribution in lichens of pollutants presumably originating from the Sines coal-powered electric plant, namely Hg, sulphur and selenium, which tended to show higher concentrations in the western uplands than in the east (Freitas et al., 1999). Although comparable patterns were not readily apparent in a similar study using mosses (Figueira et al., 2002), these results call for a more detailed examination of the distribution and biological effects of contaminants emitted from Sines up to several tens of kilometres from the source. This is particularly important in the case of Hg, which biomagnify through food chains and may negatively affect endangered top predators such as the Bonelli's eagle.

Although the mean Hg contamination recorded in eagles can be considered generally low, the highest levels detected might be of concern regarding eventual adverse impacts on the breeding productivity of some individual pairs (Berg et al., 1966; Lindberg and Odsjö, 1983; Parrish et al., 1983; Movalli, 2000). Establishing a benchmark for critical Hg concentrations in feathers is difficult, however, because Hg bonded to keratin and sequestered in feathers no longer represents a risk to the bird (Furness, 1993), and its levels may be uncorrelated with concentrations in eggs (e.g. DesGranges et al., 1998). Nevertheless, Hg concentrations in eagle feathers reported in this study, were correlated with those found in a small sample of addled eggs ($n = 13$) collected from 10 breeding territories in a concurrent study (Blanco, 2001). There was a strong linear relationship between Hg levels in feathers and eggs from individual pairs ($R^2 = 0.772$, $F_{1,8} = 27.078$, $P < 0.001$), with feather levels of 4.1 $\mu g\,g^{-1}$ corresponding to eggs containing the benchmark of 1.0 $\mu g\,g^{-1}$ (wet weight). This concentration may be the lowest associated with deformities of particularly sensitive embryos, though it is unlikely to affect more than a small percentage of eggs (Heinz and Hoffman, 2003). In this study, only two out of 21 Bonelli's eagle pairs (9.5%) showed feather levels in excess of this threshold (4.3–5.4 $\mu g\,g^{-1}$), and may thus be considered moderately susceptible to reproduction impairment due to Hg contamination. For the overall breeding population, however, it is unlikely that Hg contamination can negatively affect the reproductive output.

Results from this study add to a body of evidence derived primarily from aquatic food webs, suggesting that diet variation may have major confounding effects in studies biomonitoring environmental contamination using birds of prey (Anthony et al., 1999; Mañosa et al., 2003). To overcome potential shortcomings, some authors recommended that bird species with narrow and inflexible diets should be used in contamination studies, rather than generalist feeders (Monteiro and Furness, 1995). However, true dietary specialists are probably hard to find, and so the critical assumption of constant diets across space and time may frequently be unwarranted. A detailed knowledge of diet variation and the statistical control of dietary influences, as in this study, may thus be generally required to derive meaningful trends in Hg environmental contamination from the corresponding spatial or temporal variation in concentrations recorded in birds of prey.

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