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Grit selection in waterfowl and how it determines exposure to ingested lead shot in Mediterranean wetlands

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SUMMARY

Waterfowl ingest lead shot because they confuse it with grit, but there has been limited study of differences among species and locations. The spatial and interspecific variation in the quantity and size composition of ingested grit and in the ingestion of lead shot by eight waterfowl species in the three main wintering areas in the western Mediterranean (Doñana, Ebro Delta and Camargue) was investigated. Variation in the mass of grit in the gizzard was related to bird species, whereas size composition of ingested grit was more closely related to locality and less to species. Birds with a large proportion of vegetation in their diets had more grit in the gizzard. Grit size composition was related to prevalence of lead shot ingestion. Thus, the quantity of grit in the gizzard is an attribute of species, and grit size composition (which largely determines the risk of ingestion of lead shot) is more affected by local conditions. This conclusion is supported by a meta-analysis of previous studies of the incidence of lead shot ingestion in 51 locations and 27 waterfowl species in North America and Europe. The prevalence of lead shot ingestion in a given waterbird species was highly variable between localities, and was not consistently different between dabbling, grazing and diving species.

Keywords: Anatidae, geographical variation, lead poisoning, phenotypic plasticity, Rallidae

INTRODUCTION

Lead shot is used for hunting purposes and each cartridge fired results in the deposition of approximately 35 g of lead in the environment. Field and experimental studies suggest that waterfowl (Anatidae and Rallidae) ingest shot because they confuse it with grit (Trost 1981; Moore *et al.* 1998; Mateo & Guitart 2000). Intoxication due to the ingestion of lead shot is an important cause of mortality in waterfowl, especially in Europe where the use of lead shot for hunting is widespread (Beintema 2001) and affects globally-threatened species (Mateo *et al.* 2001). Approximately 2.5% of the autumn duck population in North America formerly died annually owing to lead poisoning (Bellrose 1959). The relative survival of mallards *Anas platyrhynchos* in the Camargue was 19% lower in individuals that had ingested lead shot (Tavecchia *et al.* 2001). Although the use of lead shot cartridges has been banned in some countries or areas important for waterfowl (AEWA [African-Eurasian Waterbird Agreement] 2002), large quantities of lead shot remain in sediments and can be consumed by waterfowl many years after release of lead shot into the field (Pain 1992).

The characteristics of the grit ingested by waterfowl in a particular locality differ between species (Mateo *et al.* 2000). It has been suggested that differences in grit characteristics are related to differences in the structure of the bill (Nudds 1992; Nudds & Wickett 1994), although recent field data do not support this hypothesis (Mateo *et al.* 2000). Granivorous birds may consume more grit than insectivorous species (Gionfriddo & Best 1996). Whatever the reasons for interspecific differences in characteristics of ingested grit, ingestion of lead shot seems to be greater in species that consume grit > 2 mm in diameter, which is similar to the size of shot (Hall & Fisher 1985; Pain 1990*a*; Mateo *et al.* 2000). It also seems likely that exposure to ingested lead shot and other sources of contamination will be higher in species consuming a greater quantity of grit.

In this paper we quantify interspecific and spatial variation in the amount and size of grit ingested by waterfowl, and compare these patterns with those of ingested lead shot. We address two main questions using our data. First, we test whether grit mass and size are determined by bird species, morphology, diet or by locality. Second, we assess whether certain species are particularly susceptible to lead shot ingestion, itself dependent on grit characteristics, across their range, or whether shot ingestion affects different species at different localities.

To test the validity of our conclusions, we review published studies on European and North American waterfowl to estimate the magnitude of interspecific and local effects in explaining the frequency of lead shot ingestion by waterfowl in the Northern hemisphere. We test previous suggestions that diving ducks are most susceptible and grazing ducks are least susceptible to lead shot ingestion (Bellrose 1959; Pain 1992).

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Table 1 Presence of lead pellets in the gizzards of waterbirds sampled at Doñana, the Ebro delta and the Camargue. For each locality, the number of gizzards examined for pellets and for grit characteristics, respectively, is given (separated by a slash). Prevalence corresponds to the percentage of gizzards with pellets. Diet corresponds to the percentage of vegetation as estimated from 136 gizzards in Doñana and from Tamisier & Dehorter (1999). No comparable estimates of diet are available for the Ebro delta.

Species	*Doñana*			*Ebro*		*Camargue*			
	Sample size	*Prevalence*	*Diet*	*Sample size*	*Prevalence*	*Sample size*	*Prevalence*	*Diet*	*Feeding method*
Anas acuta	5/5	20.0	30.0	123/123	76.4	5/1	0.0	0.0	Dabbling
Anas clypeata	61/62	1.6	10.9	25/25	32.0	109/116	14.7	0.0	Dabbling
Anas crecca	45/45	0.0	11.4	27/27	29.6	281/276	13.2	0.0	Dabbling
Anas platyrhynchos	56/56	7.1	21.0	50/50	46.0	172/159	36.0	0.0	Dabbling
Anas strepera	15/15	0.0	12.9	23/23	8.7	73/102	5.5	90.0	Dabbling
Aythya ferina	7/7	0.0	15.0	25/25	72.0	23/2	8.7	0.0	Diving
Fulica atra	63/64	1.6	39.2	30/30	3.3	74/13	13.5	82.0	Grazing
Netta rufina	4/4	0.0	50.0	20/20	20.0	5/1	0.0	55.0	Diving

METHODS

Waterfowl were obtained from the Camargue, Ebro delta and Doñana (Table 1), which are deltaic wetlands showing similarities in the sediment composition because grit of natural origin is very scarce in the lower stretches of the Rhone, Ebro and Guadalquivir Rivers, respectively. Particles of > 0.5 mm only represent 0.2–4.5% of weight in sediments of the Ebro delta and Doñana (Mateo *et al.* 1997, 1998) and grit > 1 mm is practically absent in the Camargue (Pain 1990*a*). Lead shot densities are high in several parts of these wetlands (> 200 shot pellets m^{-2}), especially in the Camargue and the Ebro delta (Pain 1991; Mateo *et al.* 1997). Clinical cases of lead poisoning have been diagnosed in several waterfowl species in these wetlands (Hovette 1974; Mateo *et al.* 1998).

In the Ebro Delta, 262 birds were obtained from hunters during the 1991–1996 hunting seasons (Mateo *et al.* 2000), and 61 dead birds found in the field. In the Camargue, 670 gizzards were obtained from hunters shooting on marshes on and around the Tour du Valat Estate from 1995–2001 (J.-Y. Mondain-Monval *et al.*, unpublished data 1995–2001; Mondain-Monval *et al.* 2002). In Doñana, 258 gizzards were obtained between 1998 and 2000, mainly from birds arriving dead at wildlife rehabilitation centres and individuals that died following a toxic spill in Doñana, together with a few illegally shot birds confiscated by police (Figuerola *et al.* 2002). All samples were collected between September and February inclusive. Sample size changed between analyses because grit was not stored for all gizzards examined for lead shot presence, and the presence or absence of lead was not recorded in some grit samples (Table 1).

The gizzard of each bird was opened and its contents washed into a plastic cup. Vegetation was removed by decantation, and the precipitate was dried at 60°C until a constant weight was reached. Lead shot presence was determined with X-rays, identified with binoculars and removed from grit. Grit was sieved (sieve sizes: 4, 3, 2, 1.5, 1 and 0.5 mm) and each size class of particles was weighed to the nearest 0.001 g.

Data on bill lamellar density for each species was taken from Mateo *et al.* (2000). Lamellar density was expressed as lamellae cm^{-1} in the upper mandible and measured as the distance between the first 20 lamellae to the front of the nostril. The body mass of each species was taken from Figuerola and Green (2000) and expressed as the mean of male and female measurements. The coot *Fulica atra* was excluded from the analyses testing the relationship between bird morphology and grit characteristics because coots lack bill lamellae.

It has been suggested that diet affects grit selection by birds (Barnes & Thomas 1986; Mateo *et al.* 2000). To test the possible role of diet in explaining interspecific and interlocality differences in grit characteristics, we used a gross estimate of diet based on the per cent volume of food in the gizzard consisting of leaves and tubers. This variable allowed us to differentiate birds that primarily feed on vegetation from those with seed and invertebrate based diets. Dietary vegetation data for Camargue samples were taken from Tamisier and Dehorter (1999) and for Doñana from 136 individuals used in our grit analyses. Despite using comparable methods, no relationship was found between the proportion of vegetation in the diet of the same species in the Camargue and Doñana (Spearman rank correlation, $n = 8$, $\rho = 0.35$, $p = 0.39$), indicating that individuals of a given species consume different food types in each locality. No information was available on diet at the Ebro Delta, and data from this locality were excluded from analyses including diet.

Literature review

For published reports of the presence of ingested lead shot in waterfowl in North America and Europe, we only considered studies that reported the number of individuals examined and number (or proportion) with shot in the gizzard, and that included at least two species with at least 10 individuals sampled each. The complete data set includes 246 805 individuals and 27 species, and is available from the authors on request.

Statistical analyses

Grit size composition was characterized via a compositional analysis of the fractions as percentages (Aitchison 1986) using a principal component analysis (PCA) on the correlation matrix of the log ratios of each fraction (divided by grit ≤ 0.5 mm).

Table 2 Eigenvectors for the first principal component (PC1) calculated over the correlation matrix. The log ratio of the proportion of grit in each grit size fraction was calculated (see Methods) and used to characterize differences in grit size.

Grit size fraction	*PCl*
Size > 4.0 mm	0.11245
3.0 < size ≤ 4.0 mm	0.38304
2.0 < size ≤ 3.0 mm	0.47803
1.5 < size ≤ 2.0 mm	0.51768
1.0 < size ≤ 1.5 mm	0.48861
0.5 < size ≤ 1.0 mm	0.32464

The use of log ratios was necessary because the sum of all the fractions equals one, so separate analyses for each fraction were not independent (Aitchison 1986). The first axis obtained from the PCA summarized 52.7% of total variance (Table 2). PC1 was larger for individuals with predominantly medium-sized grit (between 1 and 3 mm).

The relative contribution of species and locality in explaining differences in the quantity and size composition of grit was tested using an analysis of variance model including species, locality and their interaction, the magnitude of the effects of each factor being estimated following Graham (2001), based on expected mean squares with the program JMP v4.0.1 (SAS Institute 2000*a*). Species and locality were considered random factors because only a fraction of the species in the waterbird community was sampled for this study. When the interaction between two factors was significant we used test 'slices' to identify the levels between a factor where the interaction occurred (see SAS Institute 2000*b*).

Both univariate and multivariate tests were performed to explore variation in grit characteristics and ingested lead shot prevalence in relation to bird morphology (body mass, lamellar density and their quadratic terms to allow for curvilinear relationships) and locality (as a fixed factor). When lead shot ingestion presence was the dependent variable, grit abundance and size composition were also included as independent variables in the analyses. Preliminary univariate analyses identified the fraction of 3–4 mm grit as that most strongly related to interspecific variation in lead shot prevalence in two of the three localities (results not shown). Thus, we included this variable as a predictor in the models testing the relationship with lead shot prevalence. For multivariate tests, we followed a forward stepwise multiple regression model selection procedure including the variable which made the strongest contribution to explaining variation (deviance in the models with binomial error) in the dependent variable, then fitting the model again until no other variable increased the fit of the model with a $p < 0.05$. Model construction was repeated including diet as a predictor, excluding data from the Ebro delta. Complete results for these models are only presented when a significant effect of diet was recorded. Analyses were done with JMP when the dependent variable was continuous (grit abundance and PC1) and with the GENMOD procedure in SAS 8.2 (SAS Institute 2000*b*) with a binomial error distribution for presence/absence of lead shot data.

Sex was not recorded for many birds in Camargue and only for some in Doñana. Thus we tested for sexual differences in grit characteristics in a reduced sample of 420 individuals of four species (*Anas clypeata*, *A. crecca*, *A. platyrhynchos* and *A. strepera*). Neither sex, nor its interactions with locality or species were significantly related to variation in grit quantity ($p \geq 0.31$) or grit size composition (PC1: $p \geq 0.08$). Consequently, sex was excluded from further analyses so as to incorporate the maximum number of species in the study.

The possible biases due to the pooling of hunted birds and birds found dead in the field in our samples were analysed for data from the Ebro delta. The quantity and size composition of grit found in hunted and collected birds were compared with a mixed model ANOVA, including the fixed factor origin (hunted or found dead), the random factor species (only species with at least three individuals in each origin category were considered) and the interaction between both factors. No significant effect of sampling method on grit characteristics was found ($p > 0.09$ in all cases), but a significant interaction between species and sampling method on grit quantity was found $F_{2,197} = 27.24$, $p < 0.0001$), because hunted birds had more grit than birds found dead for two species (*A. acuta* and *F. atra*) but not in the other (*A. platyrhynchos*). Thus we repeated key analyses for grit quantity and lead shot ingestion while excluding birds found dead, without finding any qualitative change in the results.

The effect of species and locality in explaining presence/absence of lead shot in the gizzards of sampled birds was tested by fitting a GLM model with species, locality and their interaction as random effects (macro GLIMMIX for SAS 8.2; Littell *et al.* 1996). A binomial error and logit link was used to model presence (1) or absence (0) response data (see Littell *et al.* 1996). The significance of random factors was determined using likelihood ratio tests (LRTs) comparing models with and without the variable to test (Littell *et al.* 1996). To keep constant the variance absorbed by random factors among comparisons we used the Parms Statement to fix the value of the covariance and dispersal parameters as estimated by the simplest model (the one with less variables, see SAS Institute 2000*b*). However, the results of these LRTs did not differ qualitatively from those obtained when none of the parameters were fixed. The amount of variance explained by each random factor in the final models was calculated according to the model linearization method (Goldstein *et al.* 2002). For all the models with binomial distributed error we calculated the model dispersion parameter (ϕ) that was used to correct all the statistical tests for data dispersion. Ideally ϕ should be equal to 1, and large departures from this value indicate that data are overdispersed. Only one of the analyses suffered from severe overdispersion, the reasons for this being explained below.

For the analyses of data obtained from the literature review of geographical variation in lead ingestion two broad categories of data were considered, namely data collected from a single wetland complex, and summary reports of information collected across a country or state (regional studies hereafter). Although both types of data have usually been

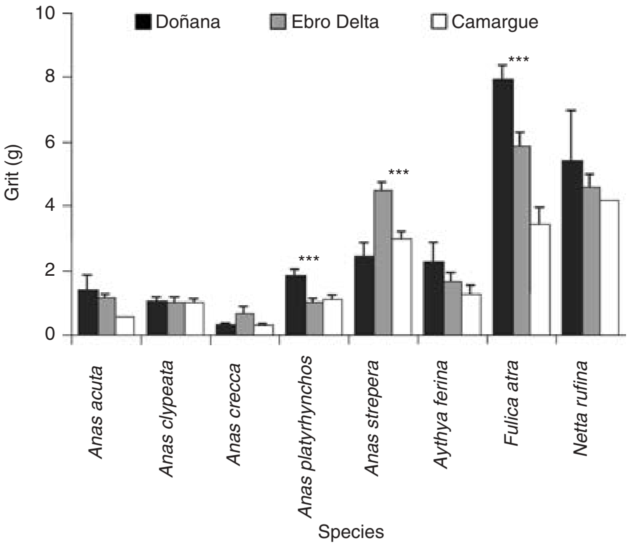

Figure 1 Abundance of grit in the gizzards of waterfowl at three localities in the west Mediterranean. Species and locality were considered random factors fitted in a type 3 analysis. For each species the significance of interlocality difference was analysed with test slices. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

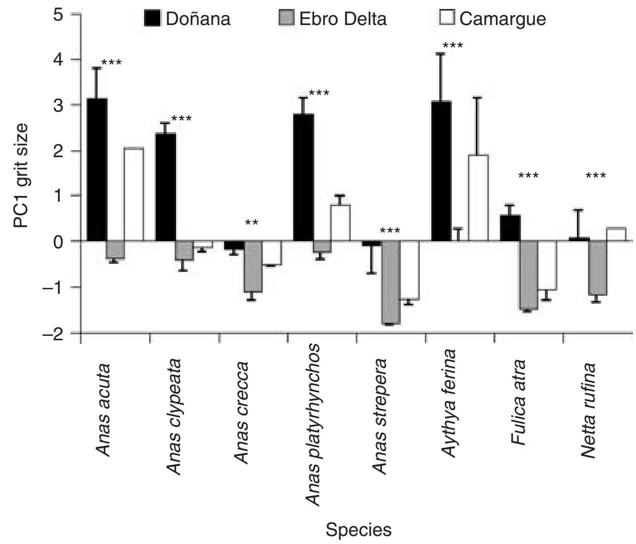

Figure 2 Local and interspecific variation in grit size estimated from the first axis of a principal component analysis on the log ratio transformed fractions of grit. Species and locality were considered random factors fitted in a type 3 analysis. The significance of differences between localities was tested for each species using test slices. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

pooled for analyses (Bellrose 1959; Pain 1990*b*), preliminary analyses identified type of study as an important source of heterogeneity in the estimates of lead shot prevalence. This suggests that estimates obtained from pooling data from different places can be strongly influenced by the tendency to collect different species in places with different levels of lead shot contamination. Consequently, type of study was included as a fixed factor in the analyses. The number of gizzards with lead shot was modelled as a binomially distributed variable with number of gizzards examined as the denominator using the macro GLIMMIX for SAS 8.2.

RESULTS

Interspecific and spatial variability in the characteristics of ingested grit

Grit quantity differed greatly between species ($F_{7,14} = 12.00$, $p < 0.0001$; 60.2% of variance explained) (Fig. 1). Locality did not explain a significant amount of variance in grit quantity ($F_{2,14} = 1.07$, $p = 0.37$). However, there was a significant interaction between these two factors, ($F_{14,1227} = 12.86$, $p < 0.0001$, 13.0% of variance explained), owing to significant differences between localities for some species (Fig. 1). Given that birds hunted and birds found dead differed in grit quantity for some species, we repeated the analyses removing data from Doñana and the 61 birds found dead from Ebro delta, with no qualitative change in the results (results not shown).

Grit size composition estimated as PC1 (Fig. 2) differed between species ($F_{7,14} = 7.76$, $p = 0.0006$, 18.8% of variance explained). In contrast to grit quantity, locality had a major role in explaining variability in PC1 ($F_{2,14} = 34.38$, $p < 0.0001$, 30.4%). A significant interaction between species and locality occurred ($F_{14,1227} = 3.72$, $p < 0.0001$), but it explained little variance (4.7%) compared to the main effects.

The overall variance explained by the models was much larger for grit quantity (variance attributed to error = 24.7%) than for grit size composition (46.1%), suggesting that much of the variation in grit size was due to factors other than bird species and locality.

Bird morphology, diet and grit characteristics

The variable best explaining variation in the quantity of grit was species body mass (Table 3). The relationship between grit quantity and (body mass)^2, lamellar density and (lamellar density)^2, although significant in univariate analyses, were not significant after controlling for body mass in a multivariate analysis (Table 3). The proportion of vegetation in the diet was positively related to grit quantity ($F_{1,12} = 13.52$, $p = 0.003$), and the relationship with body mass was no longer significant after controlling for diet ($F_{1,11} = 3.05$, $p = 0.11$).

Locality was the only variable explaining variation in grit size composition, with neither morphology (Table 3) nor diet being important ($F_{1,12} = 1.62$, $p = 0.23$).

Prevalence of lead shot ingestion in Camargue, Doñana and Ebro delta

Both locality and species explained a significant and independent amount of variance in lead shot ingestion prevalence (LRT model species versus species + locality, $\chi^2 = 88.85$, $df = 1$, $p < 0.0001$; model locality versus species + locality, $\chi^2 = 137.29$, $df = 1$, $p < 0.0001$). Furthermore, a significant

Table 3 ANOVA of mass and size of grit in waterfowl gizzards, size composition of grit estimated from the first component (PC1) of a PCA on the log ratios of grit size fractions. For significant variables model parameter estimates are given. Locality was entered in the model as a fixed factor. For variables not included in the final model, the significance when added to the final model is given.

	Grit mass estimate	*F*	*df*	*p*	*PC1 estimate*	*F*	*df*	*p*
Univariate								
Locality		0.21	2,18	0.82		6.36	2,18	0.008
Camargue					0.01			
Doñana					0.97			
Ebro					−0.98			
Grit mass						1.56	1,19	0.23
Body mass	0.00	6.57	1,19	0.02		0.86	1,19	0.37
(Body mass)^2	0.00	5.56	1,19	0.03		0.65	1,19	0.43
Lamellar density	−0.17	6.28	1,19	0.02		0.10	1,19	0.76
(Lamellar density)^2	−0.01	4.24	1,19	0.05		0.06	1,19	0.82
Multivariate								
Locality		0.26	2,17	0.77		6.36	2,18	0.008
Camargue					0.01			
Doñana					0.97			
Ebro					−0.98			
Grit mass						2.67	1,17	
Body mass	0.00	6.57	1,19	0.02		1.36	1,17	0.26
(Body mass)^2		0.52	1,18	0.48		1.01	1,17	0.33
Lamellar density		0.92	1,18	0.35		0.15	1,17	0.70
(Lamellar density)^2		0.41	1,18	0.53		0.08	1,17	0.77

interaction between both factors occurred (LRT model species + locality versus species × locality, $\chi^2 = 26.03$, $df = 1$, $p < 0.0001$). Only 3.8% of the variance was explained by species, while 15.0% was related to locality and 4.1% to the interaction of species and locality factors ($\phi = 0.93$). The proportion of individuals of each species with shot pellets in their gizzards was not repeatable between localities (as estimated by the intra-class correlation, $r_i = 0$, $F_{7,16} = 0.72$, $p = 0.66$; see Lessells & Boag 1987). The ranking of species in each locality according to their prevalence of ingested lead shot in the gizzard ($r_i = 0.17$, $F_{7,16} = 1.60$, $p = 0.21$) was also not repeatable and this was also the case when using centred and standardized data for each locality ($r_i = 0.28$, $F_{7,16} = 2.17$, $p = 0.09$).

Among bird morphology, grit characteristics and diet, locality was the most important variable explaining the presence of lead shot in the gizzard (Table 4). Prevalence of lead shot ingestion also increased with the relative presence of grit of 3–4 mm of diameter (Table 4). Neither the proportion of vegetation in the diet ($F_{1,9} = 0.03$, $p = 0.87$), nor other variables were related to the presence of lead in the gizzard (Table 4).

Functional groups and lead shot ingestion

No differences in lead shot prevalence were detected between functional groups (dabbling, diving and grazing species) in data from Doñana, the Camargue and the Ebro delta

Table 4 GLM model analysing variations in the presence of lead shot in waterfowl gizzards, using a forward regression procedure using type III tests. For variables in the final model, estimates are given. For variables not included in the final model, the significance when added to the final model is given. The deviance for the null model was 333.77, the final multivariate model explained 84.5% of the original deviance and had a model dispersion (ϕ) = 2.96.

	Univariate tests					*Multivariate tests*			
	Deviance	*Estimate*	*F*	*df*	*p*	*Estimate*	*F*	*df*	*p*
Locality	136.11		13.07	2,18	0.0003		27.31	2,17	<0.0001
Camargue		−1.65				−1.48			
Doñana		−3.58				−3.54			
Ebro		0.00				0.00			
% Grit 3–4 mm	217.66	0.32	9.52	1,19	0.006	0.30	27.79	1,17	<0.0001
Grit mass	332.22		0.09	1,19	0.77		4.22	1,16	0.06
Body mass	262.75	0.00	5.04	1,19	0.04		0.20	1,16	0.66
(Body mass)^2	267.92	0.00	4.63	1,19	0.04		0.09	1,16	0.76
Lamellar density	272.68		3.76	1,19	0.07		0.00	1,16	0.95
(Lamellar density)^2	278.10		3.14	1,19	0.09		0.00	1,16	0.94
PC1	326.81		0.39	1,19	0.54		0.43	1,16	0.52

($F_{2,14} = 0.34$, $p = 0.72$, $\phi = 1.98$). Analyses of a larger dataset extracted from the literature review, including data from 27 species and 51 areas, showed a significant effect of functional group ($F_{2,385} = 3.71$, $p = 0.03$) and lead shot prevalence was higher in studies restricted to one place ($F_{1,385} = 7.54$, $p = 0.006$). There was also a significant interaction between these two factors ($F_{2,385} = 6.12$, $p = 0.002$). Test slices determined that this interaction was due to a lack of differences between functional groups among studies from one place ($F_{2,385} = 0.45$, $p = 0.64$), but a very significant difference in the regional studies ($F_{2,385} = 8.80$, $p = 0.0002$). A post-hoc contrast determined that, in regional studies, diving species had higher prevalences than both dabbling ($t_{385} = 3.60$, $p = 0.0004$) and grazing species ($t_{385} = 3.22$, $p = 0.001$), but no differences between dabbling and grazing species were detected ($t_{385} = 0.75$, $p = 0.46$) (Fig. 3). None of these contrasts was significant for studies done in only one place ($p \geq 0.35$), however, there was high dispersion of the model ($\phi = 9.97$), indicating a bad fit of the data to the binomial distribution. To help explain this overdispersion of the data, separate models were calculated for single wetland and regional studies. Model dispersion was much lower for single wetland studies ($\phi = 2.39$) but much higher for the regional model ($\phi = 12.79$), probably due to the inclusion of data derived for different species from different places.

Overall, bird species explained 4.3% and locality 3.7% of variance in the prevalence of lead ingestion. However, these results differed greatly between single wetland and regional studies. While species explained a significant amount of variance both in single wetland and regional studies (5.8% and 3.6% of variance respectively), the amount of variance explained by locality was much larger for the single wetland (11.4%) than for the regional studies (0.4%).

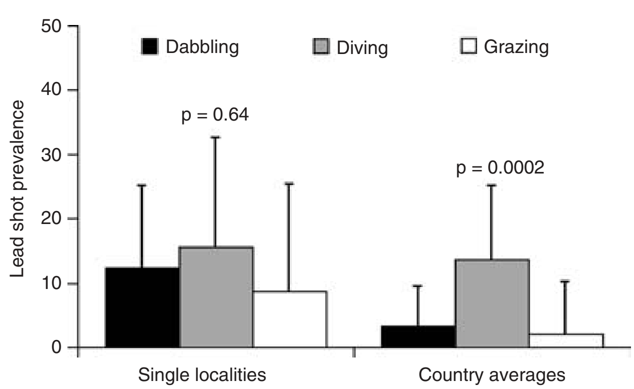

Figure 3 Mean least squares lead shot prevalences for species pertaining to different foraging functional groups. Separate estimates are reported for studies made in one wetland complex (16 studies) and those reporting averages over a country or state (35 studies). Bars correspond to standard error and significance values to slices testing the significance of feeding method in each group of studies. Species and locality were considered random factors, and wetland complex and feeding method fixed factors. The model was fitted in a type 3 analysis.

DISCUSSION

Our results suggest that grit quantity is an extension of a species' phenotype, given the strong effect of species on grit quantity in comparison to differences between localities. But a completely different pattern occurs for grit size composition, with locality having a more important role than species in explaining variability. In a previous study of two other duck species in Spain, Mateo *et al.* (2001) also recorded major differences in grit size composition between localities. The strong impact of environment on the characteristics of ingested grit does not support a role for bill morphology in grit selection, in contrast to Nudds (1992). We found no relationship between lamellar density and grit characteristics. Our results suggest that selection of grit size is not via the straining of sediments and retention of particles larger than the interlamellar distance, thus no support was found for the hypothesis of Nudds (1992) and Nudds and Wickett (1994) that species with higher lamellar density select finer grit.

There are at least three possible explanations for the important variation in grit size composition between localities. Firstly, the size of grit available for consumption by birds varies between sites with different geology. The variation in grit size may also reflect spatial variation in the diet of a given species. We have no data on the characteristics of grit available in our study sites, which are huge areas in which it is extremely difficult to know where grit consumption takes place. Secondly, grit size may vary owing to variation in diet. A given species consumes different food items in the Camargue and Doñana, as shown by differences in the proportion of vegetative parts consumed in each locality. Waterfowl show great spatial and temporal variation in diet (Krapu & Reinecke 1992; Green *et al.* 2002) which can not be readily controlled for at the large temporal and spatial scale of our study. The third possibility is related to the heterogeneity in years and methods of sampling in the present study, factors that could affect lead shot prevalence (Heitmeyer *et al.* 1993) and grit characteristics. However, all our results remained the same when non-hunted birds were excluded from the analyses, and the characteristics of the available grit in the three study areas are unlikely to have changed during the time frame of this study (1991–2000).

Can diet explain some of the differences in grit characteristics?

Differences in the diets of birds among localities are likely to influence grit characteristics. Although the proportion of vegetative parts in the diet was related to the quantity of grit, no relationship with grit size was found. However, food contents in the gizzards were not examined in detail, and proportion of leaves and tubers give only a very general characterization of diet. Additionally, gizzard contents at the time of sampling do not necessarily reflect diets on a wide temporal scale, given that birds hunted were often attracted to the hunting area through bait (with rice, corn or other commercial seeds) that is not necessarily main food items. More detailed analyses of the relationship between grit size composition and diet in waterfowl are necessary, using finer estimates of diet composition than in our study (see Player 1971; Mateo *et al.* 2000).

In our study, no relationship between grit size and body mass was found in eight species with different degrees of herbivory, contrasting with the data from 90 species of birds in North America, where mean grit size was positively related to body mass, but not to diet (Best & Gionfriddo 1991; Gionfriddo & Best 1996). However, relationship between bird and grit size can greatly depend on diet, body mass and mean grit size being positively related in granivorous species but not in non-granivorous species (de Leeuw *et al.* 1995).

Spatial and interspecific variation in occurrence of lead ingestion

We found a strong effect of locality on the risk of lead shot ingestion and a strong interaction between locality and species, indicating that the ranking of species according to their exposure to lead intoxication changes between localities. This was confirmed by analyses of a larger data-set from 51 study areas (16 local and 35 country or state wide studies) and 27 Anatidae and Rallidae species. Consequently, within the range of species included in our study, intoxication via lead shot ingestion should not be considered as a conservation problem for individual species more prone to ingestion, but rather as a threat to all waterfowl species that may affect any one of them in some wetlands but not in others. The impact on a given species at a particular locality will be largely determined by the interaction of different factors affecting diet and grit size selection. It is important to bear in mind that the prevalence of ingested lead shot is a conservative measure of the prevalence of lead intoxication in a population (Anderson & Havera 1985).

Previous analyses have suggested that feeding method influences the risk of ingestion of lead shot, with the prevalence being higher for diving ducks, intermediate for dabbling species and lower for grazing species (Bellrose 1959). Pain (1990*b*, 1992) reviewed the prevalence of lead ingestion in different countries and concluded that interspecific differences remained constant between species and sampling areas, although no statistical test was provided. However, by lumping data from different places and studies within a country, variation at a local scale was underestimated in that study (as recognized by Pain 1990*b*, 1992), a problem also illustrated by the large overdispersion of data from regional studies in relation to single wetland studies and expected according to a binomial distribution. Data for different species or functional groups may come from places with major differences in the density of lead shot in the sediments. Our reanalysis of data from previous studies suggested that the relationship between feeding functional group and lead shot ingestion is more complex than previously thought. Firstly, none of our analyses supported a higher prevalence of lead shot ingestion in dabbling than in grazing species (contrary to Bellrose 1959 and Pain 1990*b*, 1992). Secondly, both dabbling and grazing species had a lower prevalence of lead shot than diving species only when analysing regional studies.

We believe that our results are due to lower densities of lead shot in places where dabblers and grazers were sampled than those where divers were sampled. At nationwide, state or flyway scale, diving ducks may make more use of intensively hunted wetlands with high shot densities than dabblers or grazers. In the Mediterranean region at least, divers tend to concentrate in deeper, more permanent wetlands where hunter activity tends to be more intensive and consistent between years, leading to the accumulation of high shot densities in the sediments. This would explain why, when species from different feeding groups were sampled in the same places, no differences among functional groups were detectable. The higher prevalence of ingested shot observed in single wetland studies probably reflects a tendency to perform these studies where lead shot ingestion was known to be a risk for waterfowl, for example as a consequence of intensive hunting activities, or mortality episodes caused by lead poisoning. On the other hand, regional studies tend to be country/estate wide surveys, with less bias towards sites with particularly acute lead poisoning problems.

In conclusion, ecological factors operating at the species scale are more likely to be related to interspecific variation in grit abundance, whereas differences in grit size composition are more likely to be explained by factors operating at a local scale. Grit size is a good predictor of lead shot ingestion. We have not found evidence that shot ingestion is influenced by total grit mass, although this has implications for the ingestion of metal contaminants found naturally in grit (Bendell-Young & Bendell 1999; Mateo & Guitart 2003). We have found that intoxication by lead shot ingestion is a general problem for waterfowl conservation, with the impact on a given species largely determined by factors operating at a local scale.

The lack of clear patterns of interspecific variation in prevalence of ingested lead shot is explained by the lack of clear interspecific differences in grit size. However, it remains possible that, at the level of whole biogeographical populations, diving ducks are more exposed to shot ingestion because they concentrate more in wetlands where ducks have been intensely hunted and consequently a high abundance of lead pellets occurs in the sediments. In other words, lead poisoning may play more of a role in explaining ongoing population declines (Wetlands International 2002) for diving ducks than for other waterfowl. More research is required to establish whether or not this is the case, and to increase understanding of how diet and other factors determine grit size selection (and thus tendency to ingest lead shot) by waterfowl.

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