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Use of grit supplements by waterbirds: an experimental assessment of strategies to reduce lead poisoning

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Abstract The ingestion of spent Pb shot due to confusion with grit or inadvertently with food particles causes Pb poisoning in a large number of waterbirds, this being one of the main causes of mortality for some species. Lead ammunition for hunting is being progressively banned in more countries, while grit supplementation has been proposed as a management measure to reduce the ingestion of deposited Pb shot. Studies of grit selection with waterfowl in semi-captivity and in the wild were undertaken to evaluate preferences in the colour and geochemical composition of grit, whether it was available dry or in water, its position within the wetland and the relationship between grit ingestion and feeding behaviour. Grit ingestion was higher when food was included in the treatments. In the absence of food, red grit was taken in higher amounts than grey in semi-captivity but not in the wild. Siliceous grit was taken in a higher amount than calcareous when offered dry, but not in water. No differences in the amount of ingested grit were found among different positions within the wetland. The number of feeding attempts in plots supplemented with grit was higher than in those without grit, although the highest numbers of feeding birds were found in plots supplemented with food. Grit ingestion in waterfowl is intimately associated with feeding behaviour. To optimize the effectiveness of grit supplementation to reduce the risk of Pb poisoning in waterfowl, calcareous and siliceous grit may be combined and applied in feeding sites or mixed with bait to attract birds.

Keywords Gastrolith · Hunting · Lead poisoning · Wildlife conservation · Wildlife management

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Introduction

Gastroliths, more commonly known as grit, are used by birds to grind food in the gizzard and in some species also as a dietary supplement (Gionfriddo and Best 1999). However, this implies a risk of exposure to toxic metals depending on the geochemical composition of grit (Bendell-Young and Bendell 1999). Moreover, many species of aquatic and terrestrial birds ingest Pb shot pellets as grit or inadvertently with food particles and thus suffer from Pb poisoning (Trost 1981; Mateo and Guitart 2000). Lead shot ingestion is therefore a threat for birds inhabiting intensively hunted environments with low availability of grit, e.g. coastal wetlands (Pain 1990; Mateo et al. 1997), uplands or shooting ranges (Ferrandis et al. 2008; Thomas et al. 2009).

Heavily hunted wetlands worldwide have accumulated spent Pb ammunition above 100 shot per square metre in the upper 20 cm of sediments (Pain 1992; Mateo 2009).

Annual mortality due to Pb poisoning in the USA before the ban of Pb shot use for waterfowl hunting was estimated as 2% to 3% (1.6–2.4 million birds) of the population of waterfowl (Bellrose 1959; Friend 1987). In Europe, Pb poisoning is currently estimated to cause an annual mortality of 8.7% of the wintering populations of more than 11 million waterfowl from 17 species, i.e. a loss of almost one million waterfowl per winter (Mateo 2009). In addition, an inverse correlation has been shown between Pb shot ingestion rates and population trends of waterfowl in Europe (Mateo 2009), and Pb poisoning is suggested as a major cause of mortality of several endangered species (Fisher et al. 2006; Taggart et al. 2009).

Despite efforts to reduce or eliminate the input of Pb shot in most wetlands (Mateo 2009), the problem often persists because spent Pb shot remains in soil for 100–300 years (Rooney et al. 2007). Thus, in addition to the ban of Pb ammunition, management practices such as land cultivation, regulation of water levels, and grit supplementation have been proposed to reduce the risk of Pb poisoning (Godin 1967; Sanderson and Bellrose 1986; Pain 1991; Thomas et al. 2001). Grit supplementation has the advantages that it may be used in different types of habitats with low availability of grit, the environmental impact is relatively low, and it can be inexpensive in terms of material and labour cost. Grit supplementation has also been successfully applied for the control of parasitic infestations in red grouse (*Lagopus lagopus scoticus*; Newborn and Foster 2002) and for a controlled gizzard release of contraceptives for the control of pest waterfowl (Hurley and Johnston 2002).

Grit supplementation to reduce Pb poisoning in waterfowl was applied experimentally in 1958 in the Camargue (River Rhône delta, France; Hoffmann 1960). One year after the supplementation, a decrease in Pb shot ingestion from 39.4% to 8.3% was observed in tufted ducks (*Aythya fuligula*) but not in common teal (*Anas crecca*) or mallards (*Anas platyrhynchos*; Hoffmann 1960). More recently, grit supplementation helped reduce mortality by Pb poisoning due to the ingestion of Pb weights in mute swans (*Cypus olor*) at Cork Lough (Ireland; O'Halloran et al. 2002; O'Connell et al. 2009). Experiments with captive mallards showed that grit availability reduced both Pb shot ingestion (Mateo and Guitart 2000) and the effects of Pb poisoning (Godin 1967; Longcore et al. 1974).

Several characteristics of grit may influence its use by birds, such as composition, particle size, colour or shape (Gionfriddo and Best 1999). Moreover, the chemical composition of grit may help reduce Pb absorption due to the presence of calcium or phosphates (Heinz et al. 2004; Dauwe et al. 2006; Martinez-Haro et al. 2009). Grit composition has also been related to the retention time in the gizzard, calcareous grit (marble) having a shorter half-life than siliceous grit (quartzite; Trost 1981; Mateo and Guitart 2000). In addition, the size of grit particles selected is an important characteristic for each species that is influenced by diet, being larger in granivorous species and more fine and abundant in herbivorous species (Pain 1990; Mateo et al. 2000a; Luttik and de Snoo 2004). However, grit size composition for a given species varies greatly between localities, whereas total grit quantity in the gizzard is a species attribute (Figuerola et al. 2005).

Grit ingestion has been identified as independent of feeding behaviour for some species of geese (*Anser* spp.), which move toward certain areas, such as dunes or sandbars, to ingest grit (Mateo et al. 2000b; Lee et al. 2004). However, there are few data about the gritting behaviour in most waterfowl species. In the present work, studies of grit selection with waterfowl in semi-captivity and the wild were undertaken to evaluate the relationship between grit ingestion and feeding behaviour, preferences in grit colour (red or grey), geochemical composition of grit (siliceous or calcareous), whether it is dry or in water, the position of grit within the wetland (shore, open water or on artificial platforms) and the use of areas supplemented with grit by different waterfowl species under field conditions. This information is essential to develop strategies of grit supplementation in wetlands contaminated with Pb shot.

Material and methods

The study was conducted in semi-captivity and in the wild in 2007 and 2008. Semi-captive experiments were conducted in a Concerted Nature Reserve (CNR) for birds (Cañada de los Pájaros) in Andalusia near to Doñana Natural and National Park (south Spain; Fig. 1). This reserve contains a gravel pit with semi-natural management where food is supplied daily for captive waterfowl and where free-ranging waterfowl also come to feed from the surrounding wetlands. The most common species present at CNR were common coot (*Fulica atra*), common pochard (*Aythya ferina*), geese, mallard, red-knobbed coot (*Fulica cristata*) and shoveler (*Anas clypeata*).

Experiments in the wild were performed at Medina Lagoon (ML), a 120-ha Natural Reserve in Andalusia (Fig. 1). Owing to its importance for waterbirds (Amat 1984; Martí and del Moral 2002), it was protected under the Ramsar convention in 1989. It is particularly important for red-knobbed coot and the globally threatened white-headed duck (*Oxyura leucocephala*). Further, ML has the highest density of Pb shot pellets in sediments, resulting from hunting activity, recorded for any European natural wetland, with 399 shot per square metre in the upper 30 cm (Mateo et al. 2007).

The experiments were conducted with grit of a fixed density 2.6 g/ml, siliceous grit (crushed quartzite), pur-

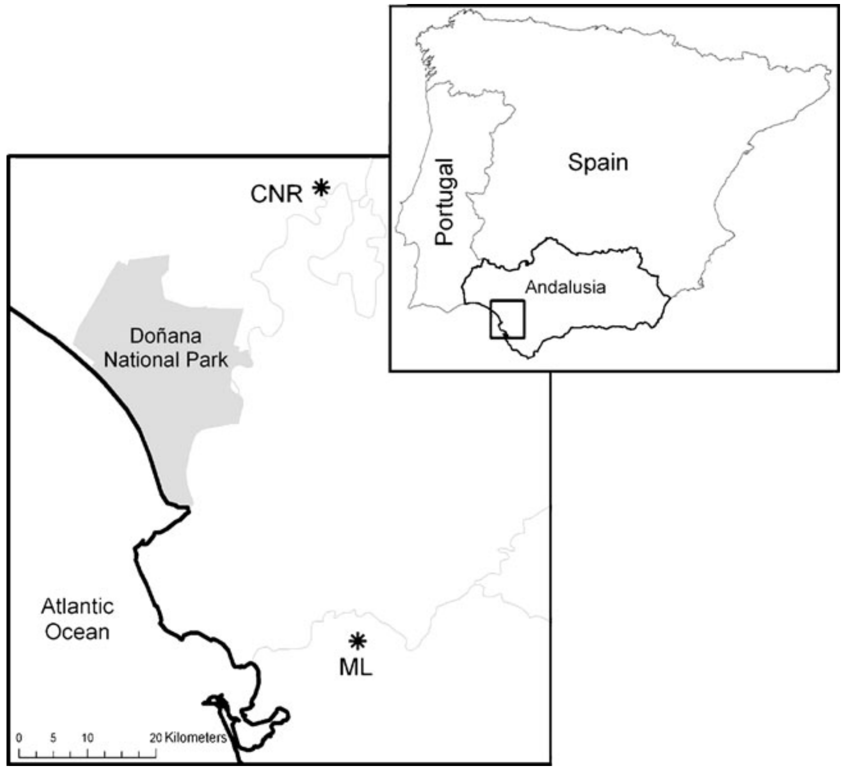

Fig. 1 Geographic location of the study areas in Andalusia (southern Spain). Study areas, Concerted Nature Reserve (*CNR*) of Cañada de los Pájaros and Medina Lagoon (*ML*), are represented by *asterisks*. Rivers (*grey lines*) and the perimeter of Doñana National Park (*grey area*) are also displayed

chased from a pet shop (aquarium fine gravel) and calcareous grit (crushed marble) from a local floor tile factory. Before undertaking experiments, grit was thoroughly washed with tap water and dried. Finally, grit was sieved with mesh sizes of 1 and 4 mm to obtain particles within this range, in accordance with that most commonly found in gizzards of waterfowl species most affected by Pb poisoning (Pain 1990; Mateo et al. 2000a). Two different approaches were used to monitor grit ingestion in the experiments. The first one used in semi-captivity and the wild (September 2007 to March 2008) involved the monitoring of amounts of grit taken by birds from PVC watertight gutters (containers) of 50 cm long×20 cm wide× 12 cm high. After all the experiments carried out with this approach, remaining grit was sieved to separate and weigh the fraction of 1–4 mm. The second one, designed on the basis of results from the other experiments, was to record the number of waterbirds feeding in the wild in plots of 15×15 m seeded with different treatments (September 2008).

Experiments on grit ingestion

Experiment 1—September–October 2007 Grit colour is important for grit selection in several bird species (Gionfriddo and Best 1999). Two types of calcareous grit (red or grey) were offered in containers with or without wheat grain in a 1:4 ratio (grit/grain, *v/v*). The mass of grit taken from each one of the four treatments (three containers per treatment) was recorded and replaced daily during 7 days at CNR and every 4 days during 3 weeks at ML. Water was not added to the containers so as to facilitate the visual selection of grit by birds.

Experiment 2—February 2008 Differences in grit ingestion may depend on the flooding level and the presence of food at sites where waterfowl search for food and/or grit (Lee et al. 2004). To test these effects, red calcareous grit was offered in 12 containers located on the shore of the lake at CNR. Grit was offered alone or mixed with wheat in a 1:4 ratio (grit/grain, *v/v*) and with or without tap water at a depth of 5 cm. The mass of grit taken from each one of the four treatments (three containers per treatment) was recorded and replaced daily during 5 days at CNR. Three additional groups of two fenced containers, with henhouse mesh of 41 mm, were placed alongside experimental containers and used as controls to check for grit removal by non-waterfowl (e.g. passerines or rats (*Rattus norvegicus*)) during the experiments.

Experiment 3—March 2008 The geochemical composition of grit may influence the visual and tactile selection of grit by birds. Calcareous or siliceous grit was offered in containers with or without wheat grain in the same proportion as in the previous experiments. The mass of grit taken from each of the four treatments (three containers per treatment) was recorded and replaced daily during

5 days at CNR. As geochemical composition may affect tactile selection in water because of differences in the shape and surface texture of grit particles, this experiment was also repeated with containers with 5 cm depth of water. The calcareous grit used in our experiments was generally more flattened, and the siliceous grit was more rounded in shape. Since the shape of grit is largely affected by its geochemical nature, we cannot rule out an effect of shape on grit selection in our experiments. As in experiment 2, six additional fenced containers were used as controls.

Experiment 4—September 2008 Some waterfowl species show preferences for specific environments to pick up grit (e.g. greylag geese (*Anser anser*), Mateo et al. 2000b; purple gallinule (*Porphyrio porphyrio*), see Renner 2010). This experiment was performed at ML to study differences in grit ingestion from 18 containers located (1) on the shore of the lagoon, (2) on the water surface at 40 cm from the bottom and 1 m from the shore or (3) on wooden platforms located 4 to 5 m from the shore and 1 m from the bottom. A mixture of calcareous and siliceous grit (1:1, *v*/*v*) was offered with or without wheat at a ratio of 1:1 (grit/grain, *v*/*v*). To limit the consumption of grain by rats, all the containers were filled with lagoon water. The mass of grit taken from each one of the six treatments (three containers per treatment) was recorded and replaced every 2 days for 2 weeks. Six additional fenced containers surrounded by henhouse mesh of 41 mm, two in each position, were used as controls.

Experiment 5—September 2008 Finally, the effect of grit and food supplementation on the feeding behaviour of waterbirds in the wild was studied at ML. In this experiment, the interaction between grit and food was studied, as in previous experiments, but was evaluated under more natural conditions as a pilot study of grit supplementation in the field. In this case, it was not possible to measure the exact amount of grit ingested, but we evaluated the use by birds of experimental plots with different supplies of grit and grain. Treatments were carried out in 5×5 m square plots in the lagoon, separated from each other by 15 m. Corners of plots were marked with four iron fence posts of 2 m height. The mean depth (SD) of the plots was 32 (±2)cm, within a range of 28–35 cm, so that the lagoon bottom was available for coots and ducks through diving, neck dipping and upending (Thomas 1982; Pöysä 1983a; Guillemain et al. 2000). Plots were located parallel to the shoreline in an area with a level bottom of soft sediment with macrophytes of 5 cm height above the bottom. Four different treatments with four replicates were tested with grey calcareous grit (1 kg/m^2) and grain (0.5 kg/m^2), which corresponds to a volumetric ratio of 1:1 (*v*/*v*). The treatments were either grit or grain, a mixture of the two, or neither. Plots were installed 1 week before the treatments were started. Observations were made by telescope from a distance of 400 m from the experimental area. The observation period of 3 h started at 8.00 a.m. Every minute, one plot was observed, and the number of birds of each species eating in the plots was recorded, identifying three methods: neck dipping (for ducks only), upending or diving. Before plots were supplemented, birds were observed during 3 days to identify differences in the use of plots by birds. These results were used to balance the distribution of plots among the four different treatments according to their use before supplementation. Grit and food were supplemented, late in the afternoon on day 0, and then birds were monitored daily during 8 days. After each daily observation, plots were checked by touching the bottom manually because the water was too turbid to see the bottom, and grit (0–12 kg) or grain (0–6 kg) was added in order to maintain the initial proportions.

Statistical analyses

The mass of grit taken from containers was normalized with square root transformation, except for experiment 4 in which log (+0.1) was applied. Data were analysed using general linear models (GLM). The factors included in the models were grit colour and grain presence (experiment 1), water and grain presence (experiment 2), grit composition and grain presence with and without water (experiment 3) and location and grain presence (experiment 4). Replicate and day were included in the models as random effects to control for spatial and temporal variations. All models were calculated including the results of the fenced containers as controls (factor fenced/open). A backward stepwise procedure was used to select the final models, excluding the interactions when they had non-significant ($p>0.05$) effects.

In experiment 5, species were analysed individually and also combined into three groups: dabbling ducks, diving ducks and coots. Statistical analyses were performed with data recorded after the lagoon plots were supplemented. The number of birds of each species feeding, or the number of attempts per bird feeding on the plots per minute, was used as dependent variables. In the same way, analyses were performed with the groups of dabbling ducks, diving ducks and coots. Generalized estimated equations (GEE) were used to fit a repeated measurements logistic regression with a negative binomial error structure, with a scale parameter calculated for each dependent variable as $K=x^2/(s^2-x)$, with x and s^2 as the mean and variance of the distribution data (Fowler et al. 1998). A log-link function and the best assumption for a working correlation matrix (using the quasi-likelihood under independence model criterion goodness-of-fit coefficient, which helps to choose between two correlation structures) were selected for each model. The models were implemented using plot as subject variable and minute and day as within-subject variables. Models were constructed using presence of grit, grain and their interaction as factors. In those models in which the numbers of attempts per bird were considered as a dependent variable, the number of birds feeding was included in the model as a scale weight variable.

Spearman rank correlations between the number of birds of each group feeding in the plots and the day of treatment were calculated to test for temporal trends due to learning of the locations of treated plots by birds. All tests were performed using SPSS 17.0, and the level of statistical significance used was $p \leq 0.05$.

Results

In experiments 1 to 4, the mass of grit taken from the fenced containers used as controls (Table S1) was lower than that taken from containers used for treatments (GLM, all p values <0.008), and the amount of grit spilled around treatment and control containers was negligible (<1%). Grain was completely consumed in all the non-fenced containers in experiments performed in semi-captivity and was almost completely consumed in those performed in the wild.

In experiment 1, the amount of grit taken was much higher in containers with grain in both the semi-captive and wild situations (Table 1). Red grit was consumed more than grey grit in semi-captivity when offered without grain, but this difference was not observed when grain was mixed with grit as shown by the interaction between grit colour and food (Table 1). No differences in colour selection were found in the wild (Table 1).

In experiment 2, the amount of grit taken by waterbirds was relatively low in containers filled with water and relatively high when grain was mixed with grit (Table 2).

In experiment 3 carried out without water in the containers, the amount of grit taken was much higher for siliceous than for calcareous grit ($F_{1,\ 82}=6.469$, $p=0.013$; Fig. 2). However, no statistically significant differences were found in grit consumption related to its composition when the containers were filled with water ($F_{1,\ 84}=0.261$, $p=0.611$; Fig. 2). A significant interaction was found between grit composition and food in water ($F_{1,\ 84}=9.162$, $p=0.003$), with a greater selection of siliceous grit when mixed with food and a greater selection of calcareous grit when offered alone (Fig. 2).

In experiment 4, the amount of grit taken was not affected ($p=0.379$) by the position of the containers in the lagoon, although a significant ($p<0.001$) interaction was found between location and presence of grain (Table 3). The presence of grain in the containers significantly ($p<0.001$) increased the consumption of grit (Table 3).

The species observed in the experimental plots prior to starting experiment 5 were common coot, gadwall (*Anas strepera*), mallard and red-crested pochard (*Netta rufina*), and the list increased with common pochard and white-headed duck after supplementation. The numbers of waterbirds feeding in experimental plots supplemented with grain were higher than in non-supplemented plots for all avian species (GEE, all p values<0.03; not tested for red-crested pochard due to too few observations recorded). Similar results were found when grouping species into dabbling ducks (gadwall, mallard and unidentified ducks), diving ducks (common pochard, red-crested pochard and white-headed duck) and coots ($\chi_1^2=19.841$, $p<0.001$, $\chi_1^2=52.230$, $p<0.001$; $\chi_1^2=98.923$, $p<0.001$, respectively; Fig. 3a). The counts of feeding birds were not affected by supplementation with grit (GEE, all p values>0.05). However, a positive effect of the presence of grit was found in the number of feeding attempts by foraging birds for mallards ($\chi_1^2=244.189$, $p<0.001$) and diving ducks ($\chi_1^2=6.137$, $p=0.013$; Fig. 3b).

The number of diving ducks and coots feeding in plots supplemented with grit and grain increased significantly over time ($r_s=0.976$, $n=8$, $p<0.001$; $r_s=0.952$, $n=8$, $p<0.001$; respectively; Fig. 4), but this was not observed for dabbling ducks ($r_s=0.371$, $n=8$, $p=0.365$). In the plots

Table 1 Mass of grit taken when the effect of grit colour and presence of food were tested in semi-captive waterfowl in the Concerted Nature Reserve of Cañada de los Pájaros and wild waterfowl in Medina Lagoon (Andalusia, southern Spain)

Experiment 1	Treatment	Number	Grit taken (g)		Statistics
			Mean	SE	
Semi-captive	Grey grit	21	8.5	1.9	Colour: $F_{1,\ 78}=4.049$, $p=0.048$
	Red grit	21	19.0	4.0	Food: $F_{1,\ 78}=125.842$, $p<0.001$
	Grey grit–food	21	43.8	2.6	Colour×Food: $F_{1,\ 78}=7.981$, $p<0.001$
	Red grit–food	21	42.0	4.4	
Wild	Grey grit	9	6.6	2.3	Colour: $F_{1,\ 43}=0.232$, $p=0.633$
	Red grit	9	7.2	3.1	Food: $F_{1,\ 43}=217.046$, $p<0.001$
	Grey grit–food	14	80.0	7.7	
	Red grit–food	14	86.6	6.7	

Table 2 Mass of grit taken when the effect of the presence of water and food were tested in semi-captive waterfowl in the Concerted Nature Reserve of Cañada de los Pájaros (Andalusia, southern Spain)

Experiment 2	Treatment	Number	Grit taken (g)		Statistics
			Mean	SE	
Semi-captive	Grit–water	15	1.3	0.5	Water: $F_{1,\ 85}$=95.540, p<0.001
	Grit–dry	15	20.1	3.3	Food: $F_{1,\ 85}$=26.318, p<0.001
	Grit–water–food	15	5.9	1.0	
	Grit–dry–food	15	49.0	4.1	

supplemented only with grain, the positive trend over time was only significant for diving ducks (r_s=0.714, n=8, p=0.047; Fig. 4b). No significant trends were found in plots supplemented only with grit or in controls (r_s all p values>0.05; Fig. 4).

Discussion

Grit ingestion by ducks and coots may be intimately associated with foraging behaviour. In all of our experiments, the amount of grit taken from containers or the number of birds attracted to supplemented plots was higher when grain was also supplied. However, some other waterbirds, such as greylag geese wintering in Doñana National Park, have been observed repeatedly visiting specific sites to get grit (Mateo et al. 2000b). Geese fly regularly from the Doñana marshes to neighbouring sand dunes, a predictable behaviour that was exploited historically by hunters to shoot geese early in the morning (Mateo et al. 2000b). Tide-dependent movement to sandbars to ingest grit has also been reported for black brant (*Branta bernicla nigricans*) in South Humboldt Bay, California (Lee et al. 2004). This preference for specific grit sites is not limited to geese. In the Ebro delta (northeastern Spain), purple gallinules were seen in groups taking grit from trails in places where food was not present (Renner 2010). Thus, some waterbird populations or species appear to have developed specific behaviours to search for grit.

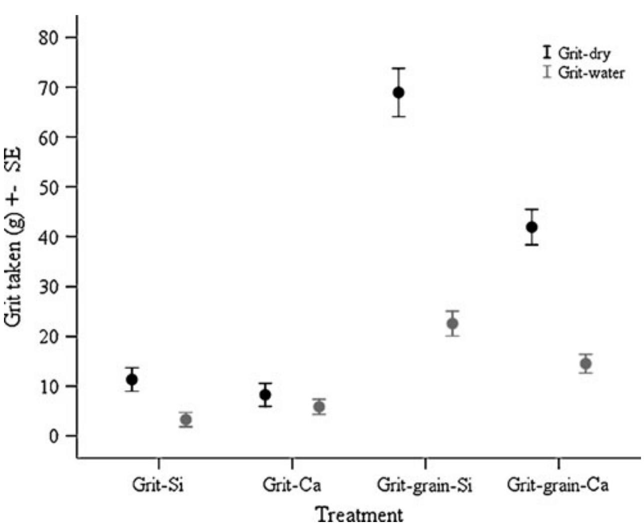

Fig. 2 Mean mass of siliceous (*Si*) and calcareous (*Ca*) grit taken (± SE) by semi-captive waterfowl in the Concerted Nature Reserve of Cañada de los Pájaros when the effect of grit composition and presence of food were tested in dry versus underwater contexts (experiment 3)

The mechanisms of grit handling and ingestion vary among avian taxa. In Rallidae, grit must be picked up and directly swallowed, in a similar way as described for food items (Pöysä 1983b; Irwin and O'Halloran 1997). But ducks and geese have different feeding mechanisms, including pumping through the lamellae to filter out particles of larger size than the interlamellar gap (Kooloos et al. 1989; Gurd 2006). In Anatidae, the presence of water at feeding or gritting sites may affect grit ingestion. In experiments 2 and 3, the amount of grit taken by the waterbirds from the containers was significantly lower in those with water. This result is probably explained because birds could wash the mixture of grit and grain in their mouths and selectively swallow the grain because of its lower density than grit. In fact, ducks feeding from the containers without water were seen going back and forth to the water, perhaps to facilitate the swallowing of grain and grit (authors, personal observation; this has been also described by Thomas (1982) for wigeon). The presence of water allows waterfowl to eliminate undesired material such as soil or mud in food (Mateo et al. 2006). In some situations, however, the presence of water may help birds take grit. Lee et al. (2004) found that grit-ingestion behaviour in black brant was more prevalent on sandbars that were still submerged, with water depth less than 0.5 m. There is a cost to ingesting too much grit as it reduces the intake rate of food, so birds may sometimes wish to ingest food without grit (Amat and Varo 2008).

Birds are able to distinguish food among other particles, such as grit, by sight and smell (Martin 2007; Steiger et al. 2008). During visual selection of grit, birds may be influenced by several grit characteristics such as size, colour, shape, surface texture and geochemical composition (Gionfriddo and Best 1999). Most of the grit found in waterfowl ranged from 0.5 to 3.0 mm in size, which was the size used in our experiments (Thomas et al. 1977; Pain 1990; Mateo et al. 2000a). The size of grit selected by birds has been related to diet (Thomas et al. 1977; Pain 1990; Mateo et al. 2000a; Luttik and de Snoo 2004) and also to

Table 3 Mass of grit taken when the location in the wetland and presence of food were tested on wild waterfowl in Medina Lagoon (Andalusia, southern Spain)

Experiment 4	Treatment	Number	Grit taken (g)		Statistics
			Mean	SE	
Wild	Shore	21	0.1	0.1	Location: $F_{2,\ 155}$=0.975, p=0.379
	Lagoon	21	1.7	1.1	Food: $F_{1,\ 155}$=199.300, p<0.001
	Platform	18	0.3	0.1	Location×Food: $F_{2,\ 155}$=7.357, p<0.001
	Shore–food	21	7.2	3.0	
	Lagoon–food	21	9.4	2.5	
	Platform–food	20	4.9	1.3	

local availability (Luttik and de Snoo 2004; Figuerola et al. 2005). As for colour, we found that red grit was only consumed more than grey grit when it was not mixed with grain in the experiment in semi-captivity, although this difference was not observed in wild. There are no previous studies testing the effect of grit colour on grit selection by waterfowl, but several studies have described the colour of grit found in gizzards (Thomas et al. 1977; Gionfriddo and Best 1999; Lee et al. 2004; Luttik and de Snoo 2004). Just as with grit size, the colour and composition of grit ingested

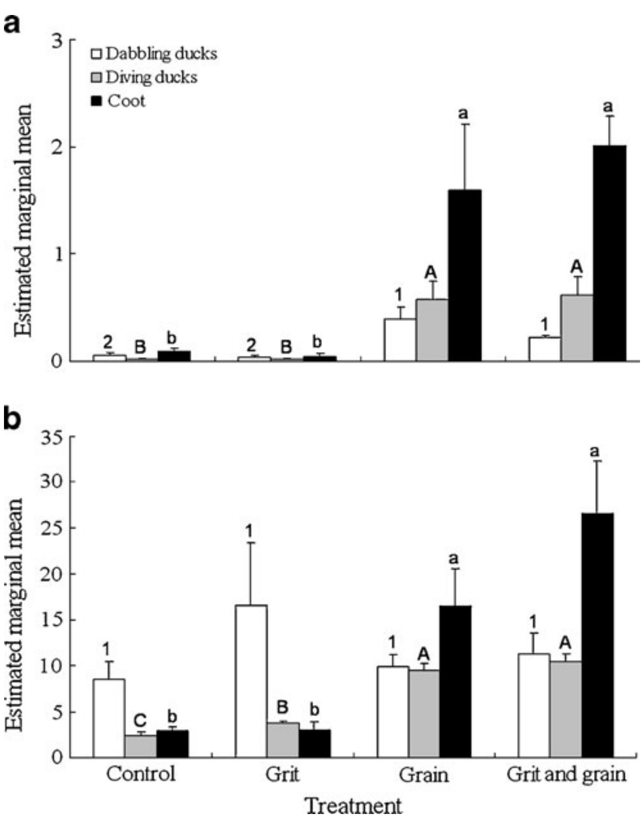

Fig. 3 Estimated marginal means (± SE) obtained in GEE models of **a** the number of wild birds feeding and **b** the number of attempts per minute, adjusted for the number of wild birds feeding (experiment 5) in Medina Lagoon. Means for dabbling ducks, diving ducks and coots sharing a number, *capital letter* or *lowercase letter*, respectively, did not differ significantly (p>0.05)

by waterfowl depends partly on the availability in the environment (Gionfriddo and Best 1999; Lee et al. 2004).

The geochemical composition of grit is important because it affects the hardness, shape and surface texture of the particle, its ability to grind food and the presence of essential elements such as calcium (Gionfriddo and Best 1999). We observed a higher selection of siliceous than calcareous grit when it was offered to waterbirds mixed with grain, especially when it was not in water. Siliceous grit has been commonly found in gizzards, and this may be related to its abundance in some environments and to its hardness and thus resilience in the gizzard (Gionfriddo and Best 1999). Silicon dioxide is one of the most abundant components of the continental crust (66%), whereas calcium oxide only represents 4.2% (Rudnick and Fountain 1995). The half-life of grit in mallard gizzards was 3.1 days for siliceous grit (calculated according to the results and equations given by Trost 1981 in his experiment #6) and 1.4 days for calcareous grit (Mateo and Guitart 2000). The higher ingestion of siliceous grit observed in the present study may also be due to its more rounded shape than calcareous grit, so the effect of shape in grit ingestion by waterbirds should be studied in the future. Differences in the type of grit ingested were related to sex in experimental studies with captive mallards (Trost 1981). When offered a choice among commercial calcium, granite, oyster shell, quartzite and sandstone, female mallards selected oyster shell and sandstone whereas males selected sandstone (Trost 1981), although the author suggested that the basis for grit selection was not purely mineral composition. However, calcium supplements for females during the egg-laying period play an important role. This has been well documented in poultry production where limestone and oyster shell are commonly used to improve yolk pigmentation and increase resistance of eggshells and tibia bones (Koreleski and Światkiewicz 2004; Lichovnikova 2007; Safaa et al. 2008). Furthermore, several studies have addressed calcium requirements and the influence of this on the distribution and breeding success of pheasants and passerines (McCann 1939, 1961; Harper and Labisky 1964; Graveland and Drent 1997; Reynolds et al. 2004; Wilkin et al. 2009; Jones et al. 2010). Despite this, little attention has

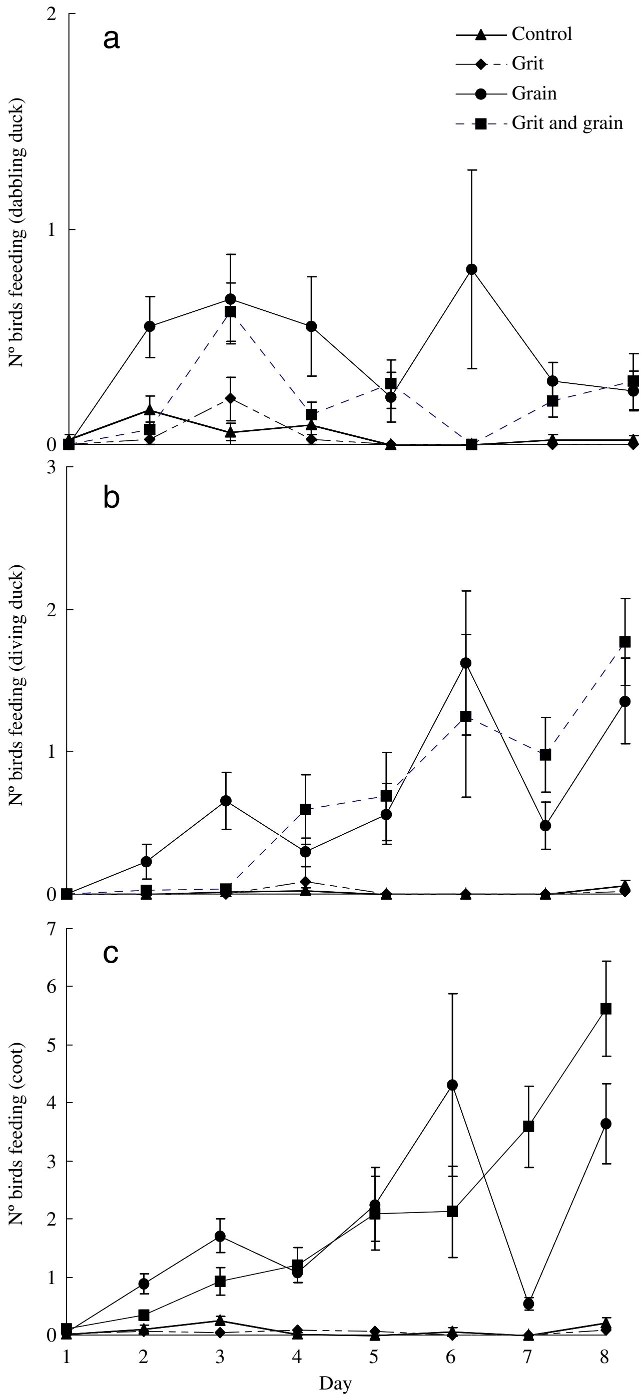

Fig. 4 Temporal evolution of the number of wild birds feeding per minute on plots with different treatments after supplementation in experiment 5 for **a** dabbling ducks, **b** diving ducks and **c** coots in Medina Lagoon

been paid to the study of calcium use and requirements for waterfowl. Flint et al. (1998) found that, in spring on the Yukon–Kuskokwim Delta (Alaska), geese of several species, especially females, were foraging on empty clam shells, supposedly to meet calcium requirements.

Very little information exists about the sites selected by waterbirds to seek for grit, but preferences have been recorded in a few species (Mateo et al. 2000b; Lee et al. 2004). In our experiment 4, we could not detect significant differences in grit consumption from containers located at different positions in a lagoon (shore, bottom, platform), but in all of these locations, grit consumption was enhanced by the inclusion of grain.

Grit per se seemed to be little attractive for waterbirds in the containers in the wild, probably because it was presented in an artificial context that permitted us to quantify the amounts consumed by birds. However, grit supplementation in a more natural way, as in experiment 5, showed again that the attraction of large numbers of birds to specific sites was only accomplished with grain supplementation. Up to 50 of the estimated 7,700 coots at ML were observed feeding at the same time in the 200-m^2 area that we supplemented with grain. Food supplementation in the experimental plots of the present study (500 g/m^2) supplied a higher grain density than commonly observed in natural wetlands (50–70 g/m^2; Baldassarre and Bolen 1984; Arzel et al. 2007). The greater attractiveness of grain compared with grit was not an unexpected result because grit, despite being necessary for birds, is required in smaller quantities than food. Grit is also retained in the gizzard for relatively long periods. However, the possible usefulness of supplementing grit alone is supported by the observation in experiment 5 that, for mallards and diving ducks, a higher number of feeding attempts were seen in plots supplemented with grit than in control plots.

Management implications

Grit ingestion is mostly associated with foraging behaviour in ducks and coots. Therefore, the first recommendation for grit supplementation strategies to reduce Pb poisoning would be to distribute the grit at natural feeding sites in the wetlands. The artificial supply of grain may not be desirable because of the risk of eutrophication and the proliferation of rats. As these natural feeding sites are usually flooded and thus visibility is reduced, the colour of grit may be of little importance in grit selection. Our recommendation would be to use a mixture of both calcareous and siliceous grit for several reasons: (1) calcareous grit may decrease the solubility of Pb in the digestive tract of birds because it tends to increase gizzard pH and helps to precipitate insoluble Pb carbonate (Martinez-Haro et al. 2009); (2) calcareous grit produces a higher calcium concentration in the intestine than siliceous grit (Martinez-Haro et al. 2009). Calcium is a natural antagonist of Pb absorption in the intestine and thus alleviates the toxic effect of Pb (Longcore et al. 1974; Mykkanen and Wasserman 1981; Scheuhammer 1996); and

(3) siliceous grit is harder and therefore has a longer half-life in the gizzard (Mateo and Guitart 2000). Thus, siliceous grit may reduce the need to replace grit in the gizzard and thus the probability to ingest Pb shot.

Few differences in grit consumption were observed among those locations tested in the present study. However, we propose grit supplementation in shallow waters, close to the shore. The ideal depth range is up to 30–45 cm, to allow both diving and dabbling ducks to reach the sediments. Some species, such as white-headed duck, are very reluctant to leave the water and are unlikely to use grit placed on the shore.

Until now, only two applications of grit supplementation to reduce the ingestion of Pb shot or Pb fishing weights have been attempted under field conditions (Hoffmann 1960; O'Halloran et al. 2002). The first effort was in 1958, when three tonnes of grit were supplemented in a heavily hunted 12-ha area of the Camargue, where grit was absent. One year after supplementation, Pb shot prevalence was reduced significantly in tufted ducks but not in mallards or common teal (Hoffmann 1960). The second application was carried out at Cork Lough, an urban site of 6 ha and 1.5 m maximum water depth where coarse fishing was popular. In this case, 40 tonnes of sandstone <10 mm was first spread in 1986, and 50 tonnes was added annually from 1997 to 2000 (O'Halloran et al. 2002). Later in the same area, 150 tonnes of grit was supplemented from 2001 to 2005 (O'Connell et al. 2009). After grit supplementation, declines in the median blood lead level and in the proportion of swans suffering from acute lead poisoning were observed (O'Halloran et al. 2002; O'Connell et al. 2009).

In Spain, calcareous grit can be purchased at 21 €/tonne and siliceous grit at 100 €/tonne. The transportation cost from local quarries may be 4 €/tonne. So we estimate that the cost of incorporating calcareous and siliceous grit (1:1, *w*/*w*, with 65 particles per gram of mix) to achieve a grit density in sediments ten times higher than 400 Pb shot per square metre would be about 40 €/ha. Depending on the input rate of additional spent shot, burial rates and rates of grit ingestion and retention by waterbirds, grit should be supplemented regularly to maintain a higher proportion of grit than shot pellets in sediments.

In some wetlands where hunters regularly bait with grain around the hunting blinds, grit could be mixed with the grain with a minor labour cost. Grit supplementation as defined here could also be applied in uplands or shooting ranges that have a shortage of grit where Pb shot ingestion by wild birds is documented (Ferrandis et al. 2008; Thomas et al. 2009). In conclusion, the present paper provides new information to understand grit selection and ingestion by waterbirds as well as the economic cost per hectare for grit supplementation by wetland managers.

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