JOURNAL OF EXPERIMENTAL ZOOLOGY 307A:471–477 (2007)

Developmental Stage Affects Eggshell-Breaking Strength in Two Ground-Nesting Birds: The Partridge (*Alectoris rufa*) and the Quail (*Coturnix japonica*)

AURORA M. CASTILLA[1*], ANTHONY HERREL[2], GUILLÉM DÍAZ[3], AND AMADEU FRANCESCH[4]

[1]*Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC-MEC), C/José Gutiérrez Abascal 2, Madrid, Spain*
[2]*Department of Biology, University of Antwerp, Universiteitsplein 1, Antwerpen, Belgium*
[3]*Departamento de Medio Ambiente-Federación Catalana de Caza, Granja Cinegética Torreferrusa, Generalitat de Cataluña, Barcelona, Spain*
[4]*Unitat de Genètica Avícola, Centre de Mas Bové, Apartat 415, Reus, Tarragona, Spain*

ABSTRACT We examined the relationship between embryo development and egg hardness in two ground nesting bird species, the red-legged partridge (*Alectoris rufa*; $n = 165$ eggs) and the quail (*Coturnix japonica*; $n = 148$ eggs). For both species, we observed a strong effect of developmental stage on egg hardness. Eggs near hatching were significantly weaker than unincubated eggs (partridge: 18 and 23 N, respectively, and the quail 7 and 10 N, respectively). We additionally explored the effect of incubation on egg hardness in a control sample of non-fertilised quail eggs (i.e., without embryo development). The control eggs maintained in the incubator for the full incubation time (17 days) were significantly harder (7–9 N) than eggs containing fully developed chicks (5–7 N). Thus, the incubation conditions of high temperature and humidity alone seem not to have a significant effect on egg hardness, and support the important effect of calcium uptake. *J. Exp. Zool. 307A:471–477, 2007.* © 2007 Wiley-Liss, Inc.

How to cite this article: Castilla AM, Herrel A, Díaz G, Francesch A. 2007. Developmental stage affects eggshell-breaking strength in two ground-nesting birds: the partridge (*Alectoris rufa*) and the quail (*Coturnix japonica*). J. Exp. Zool. 307A:471–477.

Some bird species, mainly those nesting in hard soils or cavities, likely benefit from stronger eggs as this may provide them with a protection from natural breakage (Mallory and Weatherhead, '90; Brooker and Brooker, '91; Boersma et al., 2004). In some cases, intraspecific egg destruction has led to the evolution of unusually strong eggs (Picman et al., '96; Picman and Honza, 2002). Although eggshell hardness seems to be important for birds, little research has been devoted to this topic for wild species.

In contrast, eggshell strength has been the focus of much research in poultry science because factors affecting egg hardness are of primary concern to commercial egg producers, who may suffer substantial financial losses from cracked or leaking eggs (Hunton, '95; Carnarius et al., '96). Eggshell-breaking strength in farm birds seems to be influenced by many factors including egg colour, size or genetics (Kennedy and Vevers, '73; Francesch et al., '97; Riczu et al., 2004). However, other factors such as the time the eggs spend in the uterus, female condition, age, stress and health status may also affect eggshell-breaking strength (Nys et al., '91; Butcher and Miles, '95). Finally, the diet of the females, either

*Correspondence to: Aurora M. Castilla, Apartado de Correos no. 35, 25280 Solsona, Lleida, Spain. E-mail: aurora@mncn.csic.es
Received 1 February 2007; Revised 17 May 2007; Accepted 24 May 2007
Published online 28 June 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.400

supplemented in farms or acquired directly by the birds in the field seems to affect eggshell-breaking strength (Ciftci et al., 2003; Boersma et al., 2004). Although these studies have been conducted on farm birds, similar factors would likely affect eggshell strength in wild bird species. Despite the considerable body of research devoted to eggshell characteristics, the effect of developmental stage on egg hardness remains unknown for most wild species (but see Bunck et al., '85; Bennett, '95).

It is known that embryonic birds obtain most of their calcium from the eggshell (Sauveur, '88; Packard and Packard, '91; Elaroussi and Deluca, '94; Reynolds, 2004), and that eggshell hardness decrease during development in at least three bird species (e.g., chickens, bobwhite and mallard; Vanderstoep and Richards, '70; Bennett, '95). It is therefore expected that the susceptibility of eggs to breaking or predation could differ during the incubation period, and could in turn influence nest attendance and other aspects of parental care behaviour in adults. Because nest predation is the major cause of mortality in many bird species and is an important source of natural selection (Ricklefs, '69; Martin et al., 2000; Fontaine and Martin, 2006), it is important to understand possible factors that may affect nest predation or egg breakage in wild bird populations, mainly on those experiencing very high rates of nest predation (e.g., partridges; Potts, '80).

In this study, we examined if eggshell hardness decreases during development in two ground nesting bird species (the red-legged partridge, *Alectoris rufa* and the Japanese quail, *Coturnix japonica*). We also compared fertilised eggs with a control sample of non-fertilised eggs to explore the possible effect of incubation conditions (e.g., high temperature and humidity) on eggshell hardness.

MATERIALS AND METHODS

Study area and species

The partridge (*A. rufa*) and the common quail (*Coturnix coturnix*) are sympatric ground nesting birds that have large clutches and long laying and incubation periods (Cramp and Simmons, '80). Partridge clutch size ranges from 10 to 24 eggs. Incubation starts when the clutch is finished and last ca. 24 days. With the common quail, clutch size ranges from 7 to 15 eggs; incubation starts when the clutch is finished and last ca. 17 days. Both species make their nests on soil or hard substrates, which could potentially lead to high rates of egg breakage (e.g., Boersma et al., 2004). However, eggs appear to be strong, as we have observed intact partridge eggs that had been moved out of their nests by sheep and goats (Castilla and Rodríguez, 2002). Also, the common quail rolls eggs up to 60 cm into their nests without breakage (Orcutt and Orcutt, '76), suggesting a considerable eggshell strength. Because quails, and especially partridges, experience high rates of nest predation (Potts, '80; Castilla and Rodríguez, 2002), increased egg hardness could protect them from some predators. Although the principal predators (foxes, corvids, raptors and snakes) can likely consume the eggs of both species without being limited by their hardness, many other small predators (e.g., rodents, lizards) could be limited by eggshell hardness. In fact, the Algerian hedgehog (*Atelerix algirus*) under semi-natural conditions consumed a higher proportion of small and softer eggs (quails) than medium (partridge) or larger and harder (chicken) eggs (Castilla et al., 2007).

Farms and sample

We obtained partridge eggs from two farms, one in central Spain (Burgos) and the other in NE Spain (Barcelona). Because eggs from the wild Spanish common quail (*C. coturnix*) are not commercially available, we used eggs of the Japanese quail (*C. japonica*). The quail eggs were obtained from two farms, one in NE Spain (Catalonia, Lleida) and the other in Mallorca (Balearic Islands). We chose large and well-known farms to be able to select a sample of intact eggs of similar size and colour (brown spotted) to reduce methodological bias. All eggs were from different females to avoid maternal effects. Layers in all farms were fed with a diet rich in calcium and were subjected to relatively low stress levels (e.g., noise, human manipulation). We used only intact eggs for the measurements because, at least in hens, intact eggshells have a significantly higher mean puncture force (35.3 N) than cracked (30.4 N) or leaking (28.4 N) eggshells (Carnarius et al., '96). Eggs were collected in spring of 2005 and 2006.

Partridges from Burgos were 2–4 years old, had started clutches in February 2004 and laid between 10–40 eggs (mean ca. 25 eggs). Partridges from Barcelona were 1–4 years old, had started clutches in March 2004 and laid between 1–42 eggs (mean ca. 17 eggs). Quails from Mallorca were 3–5 months old and laid ca. 50 eggs. Quails from the farm in Lleida were 2–3 months old and laid ca. 60 eggs in 2006. We used eggs of two different races (the common chicks and black ones).

We established developmental categories for eggs as follows: 1 = non-developed (i.e., eggs recently laid that were not put into the incubator), 2 = half developed (eggs after 11 days incubation for the partridge, and 7 for the quail), 3 = eggs of 2–3 days before hatching (eggs after 22 days of incubation for the partridge, and 14 days for the quail). We did remove the eggs from the incubator 2–3 days before hatching to avoid shell breakage.

An additional sample of quail eggs from Lleida was left in the incubator during the total incubation period of 17 days. We selected eggs where chicks did not start pipping and measured egg hardness. Eggs with fully developed chicks were considered stage 4.

Some of the eggs that remained in the incubator for the full incubation period of 17 days were infertile or died during incubation. Of those, we identified the developmental stage using the same stages described above (from 1 to 3). The infertile eggs were used as a control for our study because they were in the incubator during the entire incubation period but lacked an embryo that could have taken up calcium. Thus, a significant decrease of egg hardness in control eggs was not expected.

All eggs were stored in a refrigerator (ca. 6°C) during 1 month before processing at the BioEcological Station of the Museum of Natural Sciences. Other studies have found that cooling does not negatively affect eggshell strength, thus we did not expect this to affect our results (Chen et al., 2002).

Egg size and hardness

Eggs were initially visually selected for similar size (Tables 1 and 2); nonetheless, we measured egg mass, length and width, using an electronic Sartorious AG, balance Goettingen, Germany (to 0.01 g) and digital calipers (to 0.01 mm). To investigate the force needed to break the eggs, we used an isometric Kistler force transducer (type 9203, Kistler Inc., Winterthor, Switzerland) attached to a portable charge amplifier with peak-hold function (type 5995A). A screw with a flat surface (surface area of 3 mm^2) was mounted on the force transducer and pushed onto the egg until the eggshell broke (for a description of the set-up, see Herrel et al., 2001; Aguirre et al., 2003). We measured egg strength at two different locations, near the blunt end and near the centre of the egg (Tables 3 and 4). Because the measurement of egg hardness punctured the eggs, no longitudinal study could be conducted. Thus, different eggs from different females were used for each different stage. Consequently, some variation between stages in egg size unrelated to development might be present. The same worker measured size and force to minimise measurement error.

TABLE 1. Measurements of partridge eggs from different farms

			Partridge eggs		
Farm	Stage	*N*	Length (mm)	Width (mm)	Mass (g)
Barcelona	1	43	39.09 ± 2.11	30.14 ± 1.24	19.40 ± 2.14
Barcelona	2	15	39.89 ± 2.50	30.25 ± 0.62	18.87 ± 0.98
Barcelona	3	14	41.02 ± 1.06	30.41 ± 0.70	18.04 ± 0.83
Burgos	1	66	39.23 ± 1.69	30.11 ± 1.04	19.25 ± 1.65
Burgos	2	13	38.79 ± 1.45	29.78 ± 0.77	17.52 ± 1.41
Burgos	3	14	39.66 ± 2.92	29.96 ± 1.80	17.50 ± 2.58

Indicated are the developmental stage, the means, standard deviations and the sample size (*N*).

Analyses

To examine relationships between egg dimensions and hardness, all data were Log_{10} transformed to ensure normality. First, we tested whether the morphology of the egg affected egg hardness by using multiple regressions with hardness as a dependent factor and egg length, width and mass as independent factors for both species separately. Next, we tested whether quail eggs were different from partridge eggs in size and hardness using a multivariate analysis of variance (MANOVA) coupled to subsequent univariate *F*-tests. As eggs from the two species differed in length, width, mass and hardness, subsequent analyses were conducted separately for each species.

We tested whether eggs from different farms and developmental stages were different in hardness using a two-way MANOVA. Overall, we did not find significant interaction effects between hardness and farm, and farm effects were nonsignificant as well. Following this test, data sets that showed nonsignificance for farm effects were pooled to test for effects of developmental stage. When farm effects were present, the data were separately treated. Differences between eggs from different developmental stages were tested using a univariate F-tests coupled to Bonferroni post hoc tests. All analyses were performed using SPSS V13.

RESULTS

Quail and partridge eggs (unincubated stage 1 eggs) showed significant differences (MANOVA, Wilks' λ: $F_{5,243} = 319{,}73$; $P<0.01$). Eggs from both species differed in all measurements (analyses of variance; all $F_{1,247}>200$; all $P<0.01$). Partridge eggs were larger (length and width), heavier and stronger than quail eggs (Tables 1 and 2). For both species, none of the morphological variables measured (mass, length, width) could explain the variation in egg strength; multiple regressions with egg hardness as the dependent variable and morphological data as independents did not retain significant relationships. For both species and all stages egg hardness was generally higher in the blunt end than in the centre of the egg (see Tables 3 and 4).

For partridges, eggs from different farms had similar hardness (MANOVA, Wilks' λ: $F_{5,155} = 1.55$; $P = 0.18$). The farm × stage interaction effect was not significant (MANOVA, Wilks' λ: $F_{10,310} = 1.21$; $P = 0.28$) indicating that the effect of developmental stage was similar in both partridge farms. We found significant differences in hardness among stages in the centre ($F_{2,159} = 12.51$; $P<0.01$) and the blunt end ($F_{2,159} = 6.19$; $P<0.01$). Post hoc tests showed that egg strength differed significantly between stage 3 and the other two stages, respectively. Stage 3 eggs were significantly weaker than stages 1 and 2 eggs, which were not different from each other. Also, egg mass ($F_{2,159} = 10.35$; $P<0.01$) and egg length ($F_{2,159} = 4.09$; $P = 0.02$) differed between developmental stages. Post hoc tests indicated that stage 1 eggs were heavier than stages 2 and 3 eggs, which were not different from each other. Unexpectedly, we found that stage 3 eggs from both farms were also longer than stage 1 eggs.

For quail eggs from Lleida, we found significant differences between races in egg mass ($F_{1,56} = 11.92$; $P<0.01$) and width ($F_{1,56} = 5.38$; $P = 0.02$) (Table 2). Differences in egg length between the two races were marginally nonsignificant ($F_{1,56} = 3.64$; $P = 0.06$). The eggs of the black race were wider and heavier than those of the common race. The force needed to crack the eggs was also different between races, both in the centre ($F_{1,56} = 13.92$; $P<0.01$) and in the tip ($F_{1,56} = 11.72$; $P<0.01$). Because quail races were different in size, they were treated separately to examine the effect of development stage on egg hardness. For both races, the force needed to crack the eggs in the blunt end did not differ among developmental stages (common race: $F_{2,46} = 0.27$; $P = 0.76$; black race: $F_{2,54} = 2.26$; $P = 0.11$; Table 4).

For both quail races, the force needed to crack the eggs in the centre differed among stages

TABLE 3. Egg hardness of partridge eggs from different farms

Farm	Stage	N	Center (N)	Blunt end (N)
Barcelona	1	43	23.25 ± 5.36	28.68 ± 9.93
Barcelona	2	15	25.39 ± 2.84	34.69 ± 8.97
Barcelona	3	14	18.11 ± 4.34	22.81 ± 6.84
Burgos	1	66	24.13 ± 5.91	28.70 ± 10.84
Burgos	2	13	21.86 ± 5.43	28.81 ± 10.13
Burgos	3	14	18.49 ± 5.29	20.51 ± 8.41

Indicated are the developmental stage, the means, standard deviations and the sample size (N).

TABLE 2. Measurements of quail eggs from different farms

			Quail eggs		
Farm	Stage	N	Length (mm)	Width (mm)	Mass (g)
Mallorca	1	32	33.47 ± 1.15	26.43 ± 0.83	12.38 ± 1.08
Mallorca	2	7	33.56 ± 0.98	26.31 ± 0.61	12.47 ± 0.40
Mallorca	3	5	34.05 ± 1.03	26.32 ± 1.12	11.88 ± 0.78
Lleida—black	1	31	36.10 ± 1.59	27.80 ± 0.87	12.81 ± 1.24
Lleida—black	3	8	36.34 ± 1.59	27.95 ± 0.76	12.82 ± 2.11
Lleida—black	4	18	36.71 ± 1.46	27.83 ± 0.67	12.75 ± 1.35
Lleida—common	1	27	35.19 ± 1.67	27.23 ± 0.95	11.67 ± 1.29
Lleida—common	3	20	35.09 ± 1.00	27.36 ± 0.68	11.81 ± 1.26

Indicated are the developmental stage, the means, standard deviations and the sample size (N).

TABLE 4. Egg hardness of quail eggs from different farms

Farm	Stage	N	Center (N)	Blunt end (N)
Mallorca	1	32	9.83±2.08	12.72±5.03
Mallorca	2	7	9.29±2.52	14.06±6.57
Mallorca	3	5	7.42±2.05	10.14±2.74
Lleida—black	1	18	9.12±2.79	9.03±2.44
Control				
Lleida—black	3	31	7.44±2.57	8.43±2.90
Lleida—black	4	8	6.06±2.38	7.44±1.91
Lleida—common	1	20	6.78±2.06	6.78±2.36
Control				
Lleida—common	3	27	5.13±1.66	6.45±2.30

Control eggs (stage 1) were left in the incubator for 17 days (see Methods). Indicated are the developmental stage, the means, standard deviations and the sample size (N).

(common race: $F_{2,46} = 4.24$; $P = 0.02$; black race: $F_{2,54} = 10.20$; $P < 0.01$). Post hoc tests showed that egg strength in the centre differed significantly between stages 1 and 3 and between 1 and 4. Stages 3 and 4 were similar and significantly weaker than stage 1. Therefore, the control eggs (stage 1) maintained in the incubator during 17 days were significantly harder (7–9 N) than eggs containing fully developed chicks (5–7 N) (Table 4). Thus, the incubation conditions of high temperature and humidity alone seem not to have a significant effect on egg hardness, and support the important effect of calcium uptake by the embryo during development.

For the Mallorca farm, egg size was similar for all stages ($P > 0.05$ in all cases; Table 2). The force needed to crack the eggs did not differ among stages for the blunt end ($F_{2,44} = 0.51$; $P = 0.60$) nor for the centre ($F_{2,44} = 2.90$; $P = 0.07$). Despite the results were not statistically significant, the data show similar trends to those reported for the farm in Lleida and for partridges (Table 4). The absence of significant differences in this case could be attributed to the small sample size obtained for the stages 2 and 3 (most eggs were accidentally broken during transportation). Future studies in this direction should be encouraged.

DISCUSSION

It has been well known for many years that avian embryos draw calcium from the eggshell for development of their own musculoskeletal. However, we find it remarkable that there have been no previous attempts to measure eggshell hardness to determine the degree to which this affects on eggshell strength. This paper addresses this question in an experimental study.

The results of our study using two species of ground nesting birds supported the hypothesis that embryo development results in a reduction in egg hardness of 24% for the partridge (after 22 days of incubation) and of 19–33% for the quail (after 14 and 17 days, respectively). Similar results were found for other bird species *Colinus virginianus* (17% reduction in 20 days) and *Anas platyrhynchos* (15 and 22% reduction in 20 and 25 days, respectively; Bennett, '95). In the common quail *C. coturnix*, to our knowledge there is no existing data on egg hardness; however, eggshell thickness decreased during incubation by 7% (Kreitzer, '72).

The process of calcium mobilisation by the embryo is rather general for birds and reptiles, and the eggshell supplies most of the calcium for embryonic development (Stewart et al., 2004). In our study, the decrease of egg hardness in both species was noticeable only after the second stage. Other authors (Vanderstoep and Richards, '70; Sauveur, '88) also noticed that changes in shell thickness began during the last quarter of the incubation period.

Although the progressive absorption of calcium by the embryo and the decrease of eggshell hardness likely facilitate the breaking of the eggshell by hatchlings, it may also imply that eggs are more vulnerable to breakage and predation at the end of the incubation period. If this is true, it could be compensated by an increase in parental care during that period. It is known that in species with structurally weaker eggs, paternal nest defence is more extensive than in species with harder eggs (Picman and Honza, 2002). At least in partridges, nest attendance indeed seems more reduced during the laying period than during the incubation period (own observations). However, nest attendance during the incubation period of the common quail (*C. coturnix*) seems to be rather constant (Rodríguez-Teijeiro et al., '97; López, 2006). Clearly, more field observations are necessary to better understand the significance of egg hardness in relation to predation in these two species. Although avian life history theory has long assumed that nest predation plays a minor role in shaping reproductive strategies, a recent study has demonstrated that birds can assess nest predation risk and that nest predation plays a key role in the expression of avian reproductive strategies (Fontaine and Martin, 2006).

We found some differences in egg size and hardness between farms that could be attributable to the management of the farms, presence of different races, the type of diet, rates of food ingestion, etc. Also, we did not consider the abundance of spots or their colour intensity when measuring egg hardness and this also could have increased our observed variation. A recent study has shown that variation in pigmentation is associated with variation in shell thickness (Gosler et al., 2005). However, the relationship between eggshell thickness and hardness does not seem to be consistent for different birds (Bunck et al., '85; Bennett, '95). It would be interesting to examine the relationship between both measurements and to explore the effect of pigmentation in the two species examined in this study.

In sum, the effect of developmental stage on egg hardness was similar between farms for both species, and the possible variation in hardness owing to pigmentation did not obscure the expected trend that eggshell hardness decrease during development in the two ground nesting bird species studied here.

We suggest that the values of egg hardness reported here could be used as a reference for comparison of eggs in natural populations. Because some wild birds suffer from calcium deficiency (Graveland and Drent, '97; Cooper et al., 2006), the initial value (stage 1) for eggshell hardness in field populations might be lower. Eggshell thickness of field eggs constitutes a valuable measurement for the quality of the habitat (e.g., calcium availability) or the environment (e.g., presence of persistent organic pollutants) (e.g., Peakall and Lincer, '96; Falk et al., 2006). However, eggshell-breaking strength has been shown to be a more sensitive indicator of contaminant effects than eggshell thickness in some situations (Carlisle et al., '86; Bennett et al., '88). More research is needed to better understand the significance of eggshell hardness and thickness variation in wild bird populations.

Our results also show that partridge eggs are significantly larger and harder than quail eggs. Thus, quail eggs may thus not be good substitutes for partridge eggs in studies trying to quantify nest predation with artificial nests. Similarly, it has been shown that gull eggshells are thinner than those of hens (gull shell = $0.33+0.01$ mm, hen shell = $0.46+0.01$ mm; $P<0.005$; Montevecchi, '76). Because hen and quail eggs are commonly used in studies of avian predation when employing artificial nests (Major, '96), we suggest that researchers should be aware of this aspect and that ''more naturalistic'' alternatives of similar strength are warranted.

ACKNOWLEDGMENTS

We would like to thank the responsibles of the farms in Lleida (Anton Guasch, Miquel Bonet), Burgos (Juan Ramón Arias, Altube S. A.), Mallorca (Juan Miralles) and Barcelona (Generalitat de Cataluña) for allowing us to conduct this study. Further, we would like to thank an anonymous referee, Gunter Wagner, Søren Møller and Manel Puigcerver for constructive comments on the manuscript, and to Evan Marks for improving the English of this manuscript. This is a contribution from the Bio-Ecological Station of the Museum of National Sciences (CSIC)-Ayuntamiento de Sanaüja. This work has been conducted on a contract ''Ramón and Cajal'' from the Spanish National Science Foundation (CSIC, Ministerio de Educación y Ciencia) (to AMC). AH is a postdoctoral fellow of the FWO-Vl.

LITERATURE CITED

Aguirre LF, Herrel A, Van Damme R, Matthysen E. 2003. The implications of food hardness for diet in bats. Func Ecol 17: 201–212.

Bennett JK, Ringer RK, Bennett RS, Williams BA, Humphrey PE. 1988. Comparison of breaking strength and shell thickness as evaluators of eggshell quality. Environ Toxicol Chem 7:351–357.

Bennett RS. 1995. Relative sensitivity of several measures of eggshell quality to the stage of embryonic development. Bull Environ Contam Toxicol 54:428–431.

Boersma PD, Rebstock GA, Stokes DL. 2004. Why penguin eggshells are thick? Auk 121:148–155.

Brooker MG, Brooker LC. 1991. Eggshell strength in cuckoos and cowbirds. Ibis 133:406–413.

Bunck CM, Spann JV, Patte OH, Fleming WJ. 1985. Changes in eggshell thickness during incubation: implications for evaluating the impact of organochlorine contaminants on productivity. Bull Environ Contam Toxicol 35:173–182.

Butcher G, Miles R. 1995. Mala pigmentación de huevos marrones. Industria Avicola 42:11–12.

Carlisle JC, Lamb DW, Toll PA. 1986. Breaking strength: an alternative indicator of toxic effects on avian eggshell quality. Environ Toxicol Chem 5:887–889.

Carnarius KM, Conrad KM, Mast MG, MacNeil JH. 1996. Relationship of eggshell ultrastructure and shell strength to the soundness of shell eggs. Poult Sci 75:656–663.

Castilla AM, Rodríguez C. 2002. Influencia de la depredación y de otros factores sobre la pérdida de nidos de perdiz roja (*Alectoris rufa*) en la Encomienda de Mudela, Castilla La Mancha (Ciudad Real). Serie Técnica. Organismo Autónomo Parques Nacionales, Ministerio de Medio Ambiente, editor. Madrid, Spain. p 134.

Castilla AM, Robles H, Vandongen S, Matthysen E. 2007. El erizo africano (*Atheleris algirus*) prefiere consumir los huevos más pequeños y menos duros de perdiz (*Alectoris rufa*) y codorniz (*Coturnix japonica*). Jara Sedal (in press).

Chen HQ, Anantheswaran RC, Knabel SJ. 2002. Effect of rapid cooling of shell eggs onmicrocrack development, penetration of *Salmonella enteritis*, and egg strength. J Food Process Preservation 26:57–73.

Ciftci I, Yenice E, Eleroglu H. 2003. Use of triticale alone and in combination with wheat or maize: effects of diet type and enzyme supplementation on hen performance, egg quality, organ weights, intestinal viscosity and digestive system characteristics. Anim Feeding Sci Technol 105: 149–161.

Cooper CB, Hochachka WM, Philips TB, Dhondt AA. 2006. Geographic and seasonal gradients in hatching failure in Eastern Bluebirds reinforce clutch size trends. Ibis 148: 221–230.

Cramp S, Simmons K. 1980. *Alectoris rufa*, red-legged partridge. In: Cramp S, Simmons K, editors. Handbook of the birds of Europe, the middle East and north of Africa. The birds of the Western Paleartic. Vol. 2. Oxford: Oxford University Press, UK. p 463–469.

Elaroussi MA, Deluca HF. 1994. Calcium-uptake by chorioallantoic membrana–effects of vitamin D and vitamin K. Am J Physiol Endocrinol Metab 30:837–841.

Falk K, Moller S, Mattox W. 2006. A long-term increase in eggshell thickness of Groeenlandic peregrine falcons *Falco peregrinus* tundrius. Sci Total Environ 355:127–134.

Fontaine JJ, Martin TE. 2006. Parent birds assess nest predation risk and adjust their reproductive strategies. Ecol Lett 9:428–434.

Francesch A, Estany J, Alfonso L, Iglesias M. 1997. Genetic parameters for egg number, egg weight and eggshell color in three Catalan poultry breeds. Poult Sci 76:1627–1631.

Gosler AG, Higham JP, Reynolds J. 2005. Why are birds'eggs speckled? Ecol Lett 8:1105–1113.

Graveland J, Drent RH. 1997. Calcium availability limits breeding success of passerines on poor soils. J Anim Ecol 66:279–288.

Herrel A, Van Damme R, Vanhooydonck B, de Vree F. 2001. The implications of bite performance for diet in two species of lacertid lizards. Can J Zool 79:662–670.

Hunton P. 1995. Understanding the architecture of the eggshell. Worlds Poult Sci J 51:141–147.

Kennedy GY, Vevers HG. 1973. Eggshell pigments of the arauco fowl. Comp Biochem Physiol 44B:11–25.

Kreitzer JF. 1972. The effect of embryonic development on the thickness of the eggshells of *Coturnix* quail. Poult Sci 51:1764–1765.

López S. 2006. Patró d'incubació de la guatlla (*Coturnix coturnix*) en estat salvatge. Tesina de Master en Biologia Experimental. Universitat de Barcelona, Barcelona.

Major RE. 1996. The contribution of artificial nest experiments to understanding avian reproductive success: a review of methods and conclusions. Ibis 138:298–307.

Mallory ML, Weatherhead PJ. 1990. Effects of nest parasitism and nest location on eggshell strength waterfowl. Condor 92: 1031–1039.

Martin TE, Scott J, Menge C. 2000. Nest predation increases with parental activity: separating nest site and parental activity effects. Proc R Soc Lond B 267:2287–2293.

Montevecchi WA. 1976. Field experiments on the adaptive significance of avian eggshell pigmentation. Behaviour 58: 26–39.

Nys Y, Zawadzki J, Gautron J, Mills AD. 1991. Whitening of brown-shelled eggs–mineral-composition of uterine fluid and rate of protoporphyrin deposition. Poult Sci 70:1236–1245.

Orcutt FS, Orcutt AB. 1976. Nesting and parental behavior in domestic common quail. Auk 93:135–141.

Packard MJ, Packard GC. 1991. Patterns of mobilization of calcium, magnesium, and phosphorous by embryonic yellow-headed blackbirds (*Xanthocephalus-Xanthocephalus*). J Comp Physiol B-Biochem Syst Environ Physiol 160:649–654.

Peakall DB, Lincer JL. 1996. Do PCBs cause eggshell thinning? Environ Pollut 91:127–129.

Picman J, Honza M. 2002. Are House Wren *Troglodytes aedon* eggs unusually strong? Test of the predicted effect of intraspecific egg destruction. Ibis 144:57–66.

Picman J, Pribil S, Picman AK. 1996. The effect of intraspecific egg destruction on the strength of Marsh Wren eggs. Auk 113:599–607.

Potts G. 1980. The effects of modern agriculture, nest predation and game management on the population ecology of partridges (*Perdix perdix* and *Alectoris rufa*). Adv Ecol Res 11:1–79.

Reynolds SJ. 2004. Uptake of ingested calcium during egg production in the zebra Finch (*Taeniopygia guttata*). Auk 114:562–569.

Ricklefs RE. 1969. An analysis of nesting mortality in birds. Smithsonian Contrib Zool 9:1–48.

Riczu CM, Saunders-Blades JL, Yngvesson AK, Robinson FE, Korver DR. 2004. End-of-cycle bone quality in white- and brown-egg laying hens. Poult Sci 83:375–383.

Rodríguez-Teijeiro JD, Puigcerver M, Ortiz C, Gallego S, Nadal J. 1997. Incubation behaviour of the common quail (*Coturnix coturnix*) under conditions of food abundance. Historia Animalium 3:45–53.

Sauveur B. 1988. Reproduction des volailles et production d'oeufs. París: INRA.

Stewart JR, Ecay TW, Blackburn DG. 2004. Sources and timing of calcium mobilization during embryonic development of the corn snake, *Pantherophis guttatus*. Comp biochem physiol A-Mol Integr Physiol 139:335–341.

Vanderstoep J, Richards JF. 1970. The changes in eggshell strength during incubation. Poult Sci 49:276–285.