# **PRODUCTION, MODELING, AND EDUCATION**

# Age affects the laying performance and egg hatchability of red-legged partridges (*Alectoris rufa*) in captivity

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**ABSTRACT** Red-legged partridge breeders are frequently reared in captivity with the aim of producing fertile eggs and chicks. However, little is known regarding the role of breeder age on fertility and egg production performance. Therefore, we investigated the effects of breeder age on egg size and shape, flock reproductive performance, fertility, hatchability, and embryonic mortality. In experiment 1, the effects of breeders' age on fertility, hatchability of eggs, and embryo mortality were evaluated. We found that partridge breeder age significantly affected the onset of egg laying, egg production, number and proportion of settable eggs, hatchability, and fertility. Specifically, 1-yr-old females laid their first egg 1 wk later than 2, 3, or 4-yr-old birds. Furthermore, 2-yr-old females produced a significantly higher number of settable and fertile eggs than other females (53.0 vs. 46.4 to 48.5) and had a lower incidence of embryo mortality. In experiment 2 we examined the effects of breeder age, egg size, and egg shape on egg hatchability. We found that small eggs (<17.6 g) had a significantly lower hatchability than medium (17.6 to 18.9 g) or large eggs (>18.9 g). However, we did not observe any significant correlation between egg shape and hatchability. These results suggest that red-legged partridge breeders attain maximum reproductive capacity at 2 yr of age and produce fewer eggs with lower weight and hatchability thereafter.

Key words: red-legged partridge, age, egg weight, egg shape, hatchability

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# INTRODUCTION

The red-legged partridge (Alectoris rufa) is a native species of Southwestern Europe, occurring naturally in Portugal, Spain, France, and Northwestern and Central Italy. It has also been successfully introduced into other European countries, including the United Kingdom (Aebischer and Lucio, 1997). Wild populations of redlegged partridges have been decreasing due to a variety of factors, including the deterioration of their natural habitat and increased hunting pressure. Therefore, redlegged partridge breeders are now farm-raised to produce fertile eggs and chicks. According to Portuguese law, these chicks can then be used to bolster the wild population wherever necessary. However, little is known regarding red-legged partridge egg production, fertility, and hatchability.

Under natural conditions, the reproductive season of the red-legged partridge lasts 14 to 16 wk (Gaudioso et al., 2002) and is restricted to the spring season. Most birds lay only until a clutch (7 to 20 eggs) is complete (Harrison, 1991), and only then will the incubation period begin. Tavares et al. (2001) observed wild clutches with a mean dimension of 13.4 eggs and a hatchability of 93% in central Portugal. However, farmraised red-legged partridge breeders without brood can exhibit higher productivity when supplied with abundant feed. For instance, González-Redondo (2004a) described clutches of up to 30, 35, or 40 eggs in the first, second, and third reproductive seasons, respectively, when farm-raised partridges are housed with natural light. Furthermore, 95% of eggs laid under these conditions are fertile, and approximately 80% are hatch chicks (González-Redondo, 2004b). After the third reproductive season, however, egg production and reproductive performance declines, as observed in other avian species (Sauveur, 1988). Consequently, red-legged partridge breeders are generally used for 3 to 4 reproductive seasons.

In chickens (*Gallus domesticus*) and other avian species, egg weight and shape index may have an important influence on overall hatchability (Whiting and Pesti, 1983; Narushin and Romanov, 2002). For instance, eggs with parameters outside the average range have lower hatchability, especially when the eggs are more pointed

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than round (Narushin and Romanov, 2002). Furthermore, there is a positive correlation between Japanese quail (*Coturnix japonica*) egg weight and hatchability (Senapati et al., 1996). However, information regarding the parameters affecting egg quality in red-legged partridges is limited.

The important role of red-legged partridges as game birds and the problems associated with hatchability, which may be due to bird age or egg quality, underscore the necessity for more detailed research in this area. Therefore, the aim of the present work was to study the effects of breeder age on egg production, fertility, and hatchability as well as the effects of egg shape and weight on hatchability.

# MATERIALS AND METHODS

#### Experiment 1

The study was conducted at a farm located in North Portugal (41°36'N). Red-legged partridge breeder pairs aged 1 (110 pairs), 2 (110 pairs), 3 (110 pairs), or 4 (110 pairs) yr were lodged in outdoor flat-deck cages  $(50 \times 110 \times 40 \text{ cm})$ . Birds were fed a commercial cornsoy-based red-legged partridge breeder diet (2,750 kcal of AME/kg, 16.5% of CP, and 2.5% of calcium) ad libitum and were subjected to natural lighting. Laying performance was controlled during a reproductive season lasting from early March through late August. Eggs were collected and identified twice daily (11 and 17 h). Small eggs or those with thin, broken, dirty, or absent shells were culled. Collected eggs were fumigated and stored at  $12^{\circ}$ C and 80% RH for 1 to 7 d. The incubator was loaded weekly. After storage, eggs were moved to an incubation room maintained at 22 to 24°C to warm up for 12 h before being set in the incubator. The incubator was set at  $37.8^{\circ}$ C with a RH of 55% for 21 d. Egg were turned  $90^{\circ}$  hourly up to d 15. After 21 d of incubation, eggs were transferred to the hatcher and set at  $37.5^{\circ}$ C with a RH of 75%.

The length of the reproductive season (d) was determined as the period between the first and last egg laid. Hen-day production (%) was estimated as the ratio between the number of eggs laid and the length of the reproductive season. Proportion of settable eggs out of total eggs laid was determined, and hatchability (%) was determined as the ratio of hatched chicks to settable eggs. Out of 21,294 eggs produced, 20,472 settable eggs and 16,802 hatched eggs were analyzed. All of the unhatched eggs (1,819 eggs) of 220 pairs (55pairs of each age) were opened at the end of incubation and examined macroscopically to determine fertility. For this purpose, all clear eggs (1,102 eggs) were assumed to be infertile. Fertility was determined as the ratio of hatched chicks plus unhatched fertile eggs (total fertile eggs) to settable eggs, and embryo mortality was determined as the ratio of unhatched fertile eggs to total fertile eggs.

#### **Experiment 2**

A total of 864 eggs were randomly obtained from 36 pairs of red-legged partridges during the first 11 wk of the laying period. Eggs were numbered, individually weighed, and width at equator and length were measured. Egg shape index (**ESI**) was determined as the ratio of egg width to egg length. Eggs were placed in groups of six depending on the female age (1, 2, 3, and 4 yr), egg weight (small weight =  $16.7 \pm 0.04$ ; medium weight =  $18.4 \pm 0.04$ ; large weight  $19.7 \pm 0.04$  g), and ESI (ovoid ESI < 0.78; round ESI > 0.78). A total of 24 treatments (4 ages × 3 sizes × 2 ESI) with 6 replicates per treatment were obtained. Eggs were incubated and hatchability was determined as described in experiment 1.

#### Statistical Analysis

Data obtained in experiment 1 and the physical parameters of eggs measured in experiment 2 were analyzed with a 1-way ANOVA, using age as a factor. Each pair of red-legged partridges was considered a replicate. In experiment 2, effects of age, egg weight, and egg shape on hatchability were analyzed using a 3-way ANOVA. Because there was no significant interaction between factors, only effects of factor levels were analyzed. Differences among means were partitioned by the Tukey test. Statements of statistical significance were based upon P < 0.05. An ANOVA was performed using the GLM procedures of SAS software (SAS Institute, 2003). All values are expressed as mean  $\pm$  SE.

# RESULTS

#### Experiment 1

The first and last eggs were laid March 30 and July 23, respectively. We found that the day of the first egg laid, egg production, hen-day production, number and proportion of settable eggs, number of chicks, hatchability, fertility, and embryo mortality were all affected (P < 0.05) by the age of the partridge pair (Table 1). One-year-old females laid their first egg 7 and 5 d later than 2- and 3-yr-old partridges (P < 0.05), respectively. However, no effects were observed on the end of laying activity. Two-year-old females had a longer (P <(0.05) laying period (113 d) than 1-yr-old females (105) d). Furthermore, 2-yr-old females produced more eggs than did their 1-yr-old counterparts (53.0 vs. 46.4 to)48.5; P < 0.05). Two-year-old birds laid at a higher rate and produced more settable eggs (51.3 vs. 43.8to 45.6) and chicks (44.1 vs. 34.9 to 38.8) than 3- and 4-yr-old birds (P < 0.05). In addition, the proportion of settable eggs was higher in 1- and 2-yr-old females (97.0 and 97.3%, respectively) than in 4-yr-old females (93.9%; P < 0.05). Overall, 2-yr-old females showed the best hatchability and the highest fertility (P < 0.05),

Table 1. Effect of pair age on date of first egg, egg production, settable eggs, fertility, embryo mortality, and hatchability

	Age (yr)					
Item	1	2	3	4	P(F)	n
Date of first egg	Apr. $7 \pm 1.3^{a}$	Mar. $30 \pm 1.0^{c}$	Apr. $2 \pm 1.1^{bc}$	Apr. $3 \pm 0.8^{ab}$	< 0.001	440
Date of last egg	Jul. $21 \pm 1.5$	Jul. 21 $\pm$ 1.4	Jul. $17 \pm 1.8$	Jul. $23 \pm 1.3$	0.087	440
Laying period (d)	$105 \pm 2.1^{\rm a}$	$113 \pm 1.7^{\rm b}$	$106 \pm 2.3^{\rm ab}$	$111 \pm 1.7^{ab}$	0.012	440
Egg production (no.)	$48.5 \pm 1.5^{\rm ab}$	$53.0 \pm 1.4^{\rm a}$	$46.4 \pm 1.5^{b}$	$46.5 \pm 1.2^{b}$	0.003	440
Hen-day production (%)	$45.6 \pm 1.0^{\rm ab}$	$46.4 \pm 0.8^{\rm a}$	$42.9 \pm 0.8^{\rm bc}$	$41.6 \pm 0.8^{\circ}$	< 0.001	440
Settable eggs (no.)	$46.5 \pm 1.4^{\rm ab}$	$51.3 \pm 1.4^{\rm a}$	$44.6 \pm 1.5^{b}$	$43.8 \pm 1.3^{\rm b}$	0.001	440
Chicks (no.)	$38.8 \pm 1.3^{\rm b}$	$44.1 \pm 1.2^{a}$	$34.9 \pm 1.2^{c}$	$35.0 \pm 1.1^{c}$	< 0.001	440
Settable eggs (%)	$97.0 \pm 0.6^{\rm a}$	$97.3 \pm 0.4^{\mathrm{a}}$	$95.8 \pm 0.5^{\mathrm{ab}}$	$93.9 \pm 1.1^{\rm b}$	0.004	440
Hatchability (%)	$83.3 \pm 1.0^{\rm b}$	$86.4 \pm 0.8^{\rm a}$	$78.9 \pm 1.2^{c}$	$80.2 \pm 1.0^{\circ}$	< 0.001	440
Fertility (%)	$89.3\pm0.9^{\rm ab}$	$89.7\pm0.9^{\rm a}$	$86.4 \pm 1.1^{c}$	$86.7 \pm 1.3^{bc}$	0.042	220
Embryo mortality (%)	$7.3\pm0.8^{\rm a}$	$4.7 \pm 0.8^{\mathrm{b}}$	$8.6\pm0.8^{\rm a}$	$8.0\pm0.8^{\rm a}$	0.002	220

<sup>a-c</sup>Means within a row that lack a common superscript letter differ significantly (P < 0.05).

and hatchability and fertility were negatively affected when breeders were older than 2 yr. Moreover, embryo mortality values were lowest in eggs produced by 2-yrold females.

The effects of partridge age on factors determining egg culling are shown in Table 2. Age had no significant effect on any of the selected egg-culling variables, such as shell-less, thin-shelled, broken-shelled, small eggs, deformed eggs, or dirty eggs, when comparing birds aged 1 to 3 yr. However, our results indicate that 4-yr-old females produced a higher proportion of culled eggs (6.1 vs. 3.0% or 2.7; P < 0.05). This effect could be explained by the tendency of eggs with thin shells, broken shells, no shells, or small eggs to increase with breeder age.

# Experiment 2

Egg weight, width, length, and ESI were dependent on the age of the females (Table 3). Specifically, 1-yrold birds produced eggs with the highest weight (19.0 g), width (30.0 mm), and length (38.7 mm), as well as those with the lowest ESI (0.776). These differences were significant (P < 0.05) when compared with eggs laid by 4-yr-old females (Table 3).

Increased female age had a negative effect on egg hatchability (P < 0.05), with the lowest values being obtained in eggs produced by 4-yr-old females (Table 4). In addition, egg size significantly affected hatchability (P < 0.05) because smaller eggs exhibited lower hatchability than medium and large eggs (70.0 vs. 84.6 and 88.9, respectively). Neither effects of egg shape on hatchability nor interaction between female age, egg size, and egg shape were observed.

# DISCUSSION

In this study, red-legged partridges reared in captivity in North Portugal (41°36'N) began the reproductive season in the end of March, 1.5 mo later than previously observed by González-Redondo (2006). This discrepancy might be the result of the use of artificial light and a lower latitude and warmer winter in the previous study. For instance, it has previously been shown that young females just reaching sexual maturity have a low responsiveness to natural photoperiodic stimulation (Creighton, 1988; Cabezas-Díaz et al., 2005), which can delay the beginning of the reproductive season. Similar to the timing observed by González-Redondo et al. (2003), we found that at the end of July, after 1 mo of decreasing natural photoperiods, females finished their reproductive season.

Female partridges reared in captivity produced 46.4 (3 yr of age) to 53 eggs (2 yr of age) in our study, values close to those previously reported by Béjar (1991) and greater than the 35 to 45 eggs reported by González-Redondo (2004a). Therefore, captive females produce more eggs than wild partridges (12 to 18 eggs; Green, 1984; Nadal, 1995). This could be due to the longer laying period in captivity (105 to 111 d vs. 28 to 35 d; Green, 1984; Nadal, 1995) as well as the lack of laying cessation for brooding (Gaudioso et al., 2002). In

**Table 2.** Effect of female age on culled eggs (% total eggs; n = 440)

	Age (yr)				
Item	1	2	3	4	P(F)
Culled egg (%)	$3.0 \pm 0.63^{a}$	$2.7 \pm 0.44^{\mathrm{a}}$	$4.2 \pm 0.54^{\mathrm{ab}}$	$6.1 \pm 1.06^{\rm b}$	0.004
Shell-less or thin shells (%)	$0.60 \pm 0.15$	$0.77 \pm 0.28$	$1.26 \pm 0.34$	$2.12 \pm 0.76$	0.072
Broken shell (%)	$1.29 \pm 0.24$	$1.03 \pm 0.26$	$1.33 \pm 0.22$	$1.92 \pm 0.52$	0.287
Dirty eggs (%)	$0.08 \pm 0.05$	$0.01 \pm 0.01$	$0.00 \pm 0.00$	$0.02 \pm 0.02$	0.137
Small size (%)	$0.99 \pm 0.54$	$0.88 \pm 0.25$	$1.62 \pm 0.33$	$2.00 \pm 0.55$	0.220
Deformed eggs (%)	$0.05\pm0.03$	$0.02\pm0.02$	$0.00\pm0.00$	$0.02\pm0.02$	0.413

<sup>a,b</sup>Means within a row that lack a common superscript letter differ significantly (P < 0.05).

**Table 3.** Effect of female age on egg characteristics (n = 864)

	Age (yr)				
Item	1	2	3	4	P(F)
Weight (g) Egg width (mm) Egg length (mm) Egg shape index	$\begin{array}{c} 19.0\pm0.1^{\rm a}\\ 30.1\pm0.1^{\rm a}\\ 38.7\pm0.1^{\rm a}\\ 0.776\pm0.002^{\rm b} \end{array}$	$\begin{array}{c} 18.1\pm0.1^{\rm b}\\ 29.5\pm0.1^{\rm c}\\ 38.4\pm0.1^{\rm b}\\ 0.769\pm0.002^{\rm b} \end{array}$	$\begin{array}{c} 18.3 \pm 0.1^{\rm b} \\ 29.6 \pm 0.1^{\rm bc} \\ 38.3 \pm 0.1^{\rm b} \\ 0.773 \pm 0.002^{\rm b} \end{array}$	$\begin{array}{c} 18.1\pm0.1^{\rm b}\\ 29.9\pm0.1^{\rm ab}\\ 37.6\pm0.1^{\rm c}\\ 0.795\pm0.007^{\rm a}\end{array}$	<0.001 <0.001 <0.001 <0.001

<sup>a-c</sup>Means within a row that lack a common superscript letter differ significantly (P < 0.05).

addition, the more abundant food available to farmed partridges is likely to increase egg production.

Egg production increased from the first to the second reproductive season, decreasing thereafter. Notably, a similar age effect on egg production was observed previously by other groups (Martin, 1995; Gaudioso et al., 2002; González-Redondo, 2004a; Cabezas-Díaz et al., 2005). Specifically, we found that in the first and second reproductive seasons, 97% of eggs were settable, with the proportion of settable eggs decreasing thereafter. However, we observed no effects of age on the proportion of shell-less, thin-shelled, broken-shelled, small, deformed, or dirty eggs when these variables were isolated and analyzed. The fertility of the eggs ranged between 89.7% in 2-yr-old females and 86.4% in 3-yrold females. These values were higher than those found by Paci et al. (1992) in red-legged partridges kept in colonies (73.5 to 80.1%). There is no clear explanation for this difference, but it could result from differences in bird genotypes, management, or environmental conditions.

Pair age negatively affected egg fertility and hatchability, reflecting decreased reproductive capacity similar to that observed in other avian species. This effect

Table 4. Effect of female age, egg size, and egg shape on hatchability (n = 144)

Factor	Hatchability
Age (yr)	
1	$81.0 \pm 3.0^{\rm ab}$
2	$87.1 \pm 2.4^{\rm a}$
3	$79.1 \pm 3.1^{b}$
4	$77.4 \pm 2.9^{b}$
Egg size	
Large	$88.9 \pm 2.3^{\rm a}$
Medium	$84.6 \pm 2.4^{\rm a}$
Small	$70.0 \pm 2.6^{\rm b}$
Egg shape	
Ovoid	$81.3 \pm 2.1$
Round	$81.0 \pm 1.9$
Effect tests	P(F)
Age	0.049
Egg size	< 0.001
Egg shape	0.916
$Age \times egg size$	0.380
$Age \times egg shape$	0.392
$Egg size \times egg shape$	0.303
Age $\times$ egg size $\times$ egg shape	0.397

 $^{\rm a,b} \rm Means$  within a row that lack a common superscript letter differ significantly (P < 0.05).

could result from a decline in the ability of older females to retain sperm in oviductal sperm storage tubules (Brillard, 1992). Alternatively, the decline could be due to a reduction of male fertility (Saul et al., 2001), which is likely caused by decreases in the number of spermatozoa in the ejaculate, reduced volume of semen (Kotłowska et al., 2005), and mating behavior (Appleby et al., 2004). In addition, captive red-legged partridges are not allowed to choose their own pairs, and pairs are maintained over the reproductive seasons, which is not the case in wild birds. This breeding system could result in physiological and ethological modifications with negative effects on reproductive success (Bottoni et al., 1993; Gaudioso et al., 2002), which would be more evident in later reproductive seasons.

The hatchability of the settable eggs ranged between 78.9% (3-yr-old females) and 86.4% (2-yr-old females). This hatchability was higher than that observed in red-legged partridges kept in cages (67.5%; González-Redondo, 2006) or in colonies (53 to 59%; Paci et al., 1992). These differences might be due to different bird management practices. As the age of breeders increases, hatchability declines as a consequence of decreases in egg fertility and increases in embryo mortality. Few studies on the effects of reproductive season number on hatchability and embryo mortality have been conducted; however, Yilmaz and Tepeli (2009) observed lower hatchability of chukar partridge eggs in the second reproductive season compared with the first. In addition, in other avian species, hatchability decreases with bird age (Fasenko et al., 1992; Elibol et al., 2002).

Lighter red-legged partridge eggs have low hatchability, a finding similar to that observed in Japanese quail (Narahari et al., 1988; Senapati et al., 1996; Contreras et al., 2008) and chicken eggs (Whiting and Pesti, 1983; Pedroso et al., 2005). However, no negative effects on hatchability were observed for heavy eggs in our study, which differs from the findings of Narushin and Romanov (2002). Furthermore, older partridges produced lighter eggs, confirming a previous study by Cabezas-Díaz et al. (2005). Therefore, the negative effects of age on egg weight and hatchability could be correlated.

Egg shape index did not affect hatchability, corroborating the results obtained for Japanese quail eggs (Contreras et al., 2008) and contrasting with the finding that eggs of avian species with normal shape hatch more successfully (Whiting and Pesti, 1983; Narushin and Romanov, 2002).

In conclusion, we find that the reproductive capacity of red-legged partridges reared in captivity is significantly affected by age, with 2-yr-old pairs producing the highest number of fertile and hatchable eggs. Therefore, it may be disadvantageous to maintain birds older than 3 yr old as breeders. In addition, we find that eggs with weight lower than 16.7 g have a lower overall hatchability and should not be used for incubation when the number of eggs produced exceeds incubator capacity.

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