

EFFECT OF INTENSIVE REARING ON FUNCTIONAL MORPHOLOGY: A CASE STUDY WITH THE ROCK PARTRIDGE (*ALECTORIS GRAECA GRAECA*)

Paolo Viola¹, Riccardo Primi¹, Federica Gabbianelli², Pier Paolo Danieli¹,
Livia Lucentini³, and Andrea Amici^{1*}

¹Department of Agriculture and Forest Science, DAFNE. University of Tuscia, Viterbo, Italy.
*E-mail: amici@unitus.it

²Department for Innovation in Biological, Agro-food and Forest systems, DIBAF, University of Tuscia, Viterbo, Italy.

³Department of Biotechnology, University of Perugia, Italy.

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Abstract

Rock partridge (*Alectoris graeca*) is included in Annex I of the EU Birds Directive as Species of Conservation Concern (SPEC 1), because of the continuing decline in the area of occupancy. In this context, *ex situ* conservation programs and production systems of captive Rock partridges suited to the wild are necessary to ensure that insurance, reintroduction or restocking goals are met. Anyway, morphological changes occurring in captive-reared animals indicate the loss of animal's ability to survive in natural environment. In this study, we used the offspring of wild Apennine rock partridges (*Alectoris graeca graeca*). The young birds were divided into two groups, the first (G1) was housed in enriched and wild-like environment, the second (G2) was subjected to intensive-rearing conditions. Starting at 14 days post-hatching (DPH) and continuing up to 98 DPH, body mass (BM), tarsus length (TL), tarsus depth (TD), tarsus width (TW), head width (HW), head length (HL), wing length (WL) and beak length (BL), were measured fortnightly. Birds in G1 showed shorter heads ($F = 10.50$, $p = 0.000$) and thinner tarsi ($F = 9.80$, $p = 0.000$) at 84 DPH. At 98 DPH also the TD measurement ($F = 8.71$, $p = 0.001$) became significantly lower in G1 than in G2. Birds in G1 showed morphological disadvantages (shorter heads and less developed limbs) as a possible result of limited physical and cognitive exercise in simple artificial environment. Our results suggest that intensive rearing does not contribute to relevant morphological disadvantages within 70 DPH. Accordingly, birds for restocking or reintroduction should be translocated within this time limit into enriched and wild-like environment where they can exercise before the release into the wild. A first figure of morphological measurements for Apennine Rock partridge is reported.

Key words: captive morphological changes, environmental enrichment, morphometry, rearing systems, sexual dimorphism in size.

Introduction

Rock partridge (*Alectoris graeca* Meisner, 1804) has been recently declared

as a near-threatened species in Europe (Birdlife International 2018) and vulnerable at national scale, in Italy, by Peronace et al. (2012) and Rondinini et al. (2013). The

species is included in Annex I of the EU Birds Directive and has been recently classified into Species of Conservation Concern (SPEC 1), because of the continuing decline in the area of occupancy (Amici et al. 2009, Staneva and Burfield 2017).

Captures of wild birds for translocations, which are needed to reinforce wild populations and restore metapopulation functionality, are ill-timed (Ellis et al. 1978), because the small isolated populations are at a critical state (Cattadori et al. 2003, Amici et al. 2013, Trocchi et al. 2016). Therefore, *ex situ* conservation programs and production systems of captive Rock partridges suited to the wild are necessary to ensure that insurance, reintroduction or restocking goals are met (Armstrong and Seddon 2008).

In *ex situ* conservation programs, efforts commonly concentrate on preserving the genetic variability of the captive populations (Frankham et al. 1986). Although the preservation of genetic variability is clearly a principal objective, the behavioural, physiological and morphological changes require attention because potential alterations caused by captive breeding could affect the animal's ability to survive in natural environment (Liukkonen-Anttila et al. 1999, 2000; Millán et al. 2003; Price 2002; Alonso et al. 2005; O'Regan and Kitchener 2005). Such changes have commonly been detected in multigenerational captive animals and have often been attributed to the 'passive selection for animals that are behaviourally more suited to captivity' (O'Regan and Kitchener 2005). Morphology and behaviour have also been reported to respond and exhibit plasticity in adaptability to early environmental stimuli (Whiteside et al. 2016, Santilli and Bagliacca 2017).

Natural or semi-natural rearing systems can mitigate the negative effects of intensive rearing, allowing for better survival rate after release (Buner and Aebischer 2008, Pérez et al. 2015, Mihajlov et al. 2018). However, breeders rarely exploit this type of complex and expensive rearing strategy.

The effect of hand-rearing systems on the behaviour, physiology, soft tissues (gut, heart, liver, and gizzard) and muscles development have been studied in wild galliform species (Paganin and Meneguz 1992, Bergero et al. 1995, Putaala et al. 1997, Liukkonen-Anttila et al. 2000, Tobalske and Dial 2000, Millán et al. 2003, Hess et al. 2005, Gaudioso et al. 2011a). Environmental enrichment protocols (Whiteside et al. 2016), antipredator training (Gaudioso et al. 2011b, Sánchez-García et al. 2016), complex diets (Whiteside et al. 2015) and pre-release acclimatization of young birds in protected wild-like environment (Cocchi et al. 1998, Buner and Aebischer 2008), have been proposed to mitigate the negative effects of intensive hand-rearing systems.

However, captive morphological changes and associated potential disadvantages affecting viability of captive-bred Rock partridges when released into the wild need more attention and insights to understand if, how early and how strongly the effect of strict captivity could affect the functional morphology of the bird.

The aims of this study were to assess at which stage of growth and to what extent intensive rearing affects the external morphology of Rock partridge providing a first morphological description of the Apennine Rock partridge (*Alectoris graeca graeca*) and evaluating the magnitude of sexual dimorphism in size.

Material and Methods

Assumptions

According to available knowledge, we developed our study on the premise that studying multigenerational captive birds, which are available in large quantities, would generate biased results because long periods of captivity can alter animal morphology (Price 2002, O'Regan and Kitchener 2005). Therefore, we decided that working with small samples representative of an uncorrupted wild phenotype was better than a large sample of multigenerational captive birds. We based our study on four basic assumptions:

1. the wild phenotype, including the morphology, is the most functional in the wild;
2. offspring of wild parents have a genotype not corrupted by conscious or inadvertent artificial selection;
3. when subjected to stimuli like those in the wild, offspring of wild parents can adequately represent the wild morphology;
4. morphological changes recorded in offspring produced by wild parents and reared under intensive conditions are the result of plasticity in adaptability to contingent non-natural stimuli, and these changes could be interpreted as a loss of functionality in the wild (disadvantages).

Animals

For our experiment, because the critical conservation status of this species prevents the capture of large number of wild birds, and with the aim to exclude the genetic effect on the exterior morphology, we used two experimental groups of related chicks hatched from the eggs laid by the

female of the same wild breeding pair.

Wounded wild birds captured in Si-rente-Velino Mountains were assigned to an authorized fosterage and released into a dedicated rehabilitation aviary (50 m² wide, 3 m high), where scattered rocks and composite vegetative cover were available. Seeds and feed specifically intended for breeders (21 % CP, 2750 Kcal/kg ME) were provided *ad libitum* with a feeder hopper protected by a hut; water was also provided *ad libitum*.

The birds were maintained, for two successive reproductive seasons (April–June), in standard conditions. These wild birds mated freely in the aviary, with the female laying 39 eggs overall. Eggs were collected daily, weighted, stored as described in González-Redondo (2010) and artificially incubated in the third decade of June. Overall, thirty-three (33) eggs hatched after 23 days of artificial incubation.

Chicks were assigned to two groups, G1 and G2, consisting respectively of sixteen and seventeen individuals. Three chicks in G2 died during the first week post hatching. At sexual maturity, a 1:1 sex ratio (8 males and 8 females) was verified in the G1 whereas in the G2 there were more males than females (8 males and 6 females).

Experimental design

The birds in G1, that was the treatment group, were reared under intensive conditions. After hatching, the chicks were placed in a heated room in a cage at density of 16 chicks/m² for up to 14 days post-hatching (DPH). Heating was conducted as described in Günlü et al. (2007). From 15 to 35 DPH, the available indoor area was enlarged to allow density of 2 chicks/m². From hatching to 35 DPH,

they were kept indoors on a concrete floor covered with straw. Starting at 36 DPH, the indoor-outdoor phase began in an adjacent aviary of 25 m². Henceforth, 2 m² per bird were available until the end of the trial. The group was fed *ad libitum* with a starter diet (28 % CP and 2850 Kcal/kg ME) during the first three weeks, and subsequently with a second diet (22 % CP and 2850 Kcal/kg ME). Water was available *ad libitum* throughout the trial, and during the first day after hatching, it was fortified with sugar (5 % w/v) and minerals (0.05 % w/v).

The birds in G2, that was the control group, were reared in a semi-natural complex environment under the guidance of their natural parents. After hatching, the chicks were immediately placed under the hen that continued to incubate surrogate eggs. The parental couple accepted the chicks without incident, and the family group remained in a 50 m² aviary in a complex semi-natural environment (alternation of composite vegetative cover, draining ground, stones, rocks and ramps) until the end of the trial. The presence of ants, which were attracted by some up-turned plant pots positioned as suggested by Buner and Aebischer (2008), was verified in the aviary. Edible seeds (*Triticum aestivum* L., *Hordeum vulgare* L., *Sorghum vulgare* Pers., *Pisum sativum* L. and *Vicia faba* var. *minor* Beck) and green foods (*Medicago sativa* L. and *Trifolium incarnatum* L.) were available *ad libitum*, but during the first three weeks, chicks ate almost exclusively the arthropods (pupae and eggs) naturally present in the aviary and the moths that were artificially provided as recorded by direct observations. No mixed feed was given to the animals. Water was available *ad libitum* throughout the trial.

Data recording

Chicks were individually identified with non-toxic colour markers on the *patagium* of the right wing. Starting at 14 DPH and continuing up to 98 DPH, the birds were weighed and measured fortnightly by the same researcher for eight external body traits (BTs). BTs recorded were body mass (BM), tarsus length (TL), tarsus depth (TD), tarsus width (TW), head width (HW), head length (HL), wing length (WL) and beak length (BL). TL was the distance between the proximal and the distal ends of the tarsus-metatarsus (Pis 2012). TD and TW were, respectively, the depth and the width of the tarsus-metatarsus at the distal end of the spur scale. HW was the distance between the two orbital faces of the frontal bone, and HL, between the midpoint of the occipital and the nasal face of the frontal bone (Çağlayan et al. 2011). WL was the distances between the wrist joint (radio-carpal) and the longest primary (Bernard-Laurent et al. 2003), and BL, the length of the straight line along the upper margin of the beak (Pis 2012). Partridges were weighed with an electronic balance (± 0.01 g accuracy). All linear measures were taken to the nearest 0.01 mm with a digital calliper except for WL, which was measured by a ruler (± 1 mm accuracy). All bilateral measurements were taken on the left side of the bird.

Statistical analyses

Data analysis was performed with R (R Development Core Team 2011). The normality of the data distribution was tested in each group using Q-Q plot and the Shapiro-Wilk test.

With the aim to assess the morphological changes and the occurrence of SSD at

different stage of growth, an unbalanced 2×2 (group and sex) full factorial ANOVA was performed. The GLM procedure (F-test) with type III sums of squares was used because it is independent of sample sizes. The effects of the group and of the sex were investigated by age in all the BTs separately as well as in their interaction (group \times sex). Once verified the occurrence of not significant interactions between the main factors, the analysis was

repeated with the type II sums of squares that, for non-significant interaction, gives a more powerful test. When the effects of the group were significant, the Tukey HSD post hoc test, Tukey-Kramer for unbalanced cases, were used in pairwise comparisons.

The Sexual Size Dimorphism Index (SSDI) (Calabuig et al. 2011) was used to assess the magnitude of the sexual dimorphism in size:

$$\text{SSDI} = \frac{\text{mean size of the males} - \text{mean size of the females}}{\text{mean size of the females}} \times 100.$$

For all the statistics, 1 % significance level ($p = 0.01$) was accepted.

WL and BL were then excluded from the protocol because of inaccurate measures not reliable for group comparisons.

Results

At 14 DPH, in G1, difficulties were found in measuring WL and BL in a standardized and replicable way, because the tip of the longest primary and of the beak were often irregularly worn with indented edges.

Morphological comparisons between groups

The group and sex interactions were never significant. Instead, the separate effects of the sex and of the group were significant in some cases (Table 1).

Table 1. Effects of the group and sex (GLM, F-test at $p = 0.01$) on BTs at 14, 28, 42, 56, 70, 84 and 98 DPH.

Body traits:		BM		TL		TD		TW		HW		HL	
DPH	Effects	F	p	F	p	F	p	F	p	F	p	F	p
14	Group	11.71	0.002	4.39	0.061	4.13	0.069	0.04	0.922	23.78	0.000	2.80	0.098
	Sex	4.66	0.040	5.60	0.025	2.77	0.111	0.95	0.337	1.13	0.298	0.99	0.328
28	Group	1.72	0.200	2.45	0.129	0.15	0.129	1.56	0.222	27.60	0.000	2.350	0.137
	Sex	32.24	0.000	49.42	0.000	10.38	0.003	10.72	0.002	5.10	0.032	6.82	0.014
42	Group	4.24	0.054	0.30	0.514	3.44	0.055	0.03	0.693	97.39	0.000	0.01	0.903
	Sex	11.72	0.002	91.12	0.000	13.17	0.001	19.25	0.000	9.46	0.050	33.33	0.000
56	Group	9.06	0.015	2.06	0.333	1.680	0.109	0.50	0.337	61.14	0.000	0.04	0.845
	Sex	40.49	0.000	56.19	0.000	24.35	0.000	8.78	0.006	6.09	0.020	31.30	0.000
70	Group	6.06	0.047	1.08	0.564	2.73	0.051	5.79	0.011	10.48	0.004	0.03	0.970
	Sex	27.51	0.000	59.33	0.000	31.39	0.000	14.26	0.000	5.13	0.032	14.36	0.000
84	Group	1.10	0.530	0.04	0.724	9.80	0.000	0.80	0.161	5.22	0.046	10.50	0.000
	Sex	15.00	0.000	34.09	0.000	39.16	0.000	23.25	0.000	5.23	0.030	18.89	0.000
98	Group	0.86	0.617	0.00	0.540	6.29	0.004	8.71	0.001	3.29	0.143	121.39	0.000
	Sex	16.88	0.000	37.21	0.000	35.54	0.000	28.19	0.000	10.33	0.003	28.22	0.000

Note: significant differences are bolded.

The group's effect was graphically represented (Fig. 1) aiming to provide a complete figure of this effect.

Birds in G1 were constantly heavier than birds in G2 although not always at the chosen statistical significance level.

BM was significantly different between groups at 14 DPH.

TL was not sensitive to the intensive rearing; in fact, the average values of both groups overlapped somewhat consistently during the entire observation

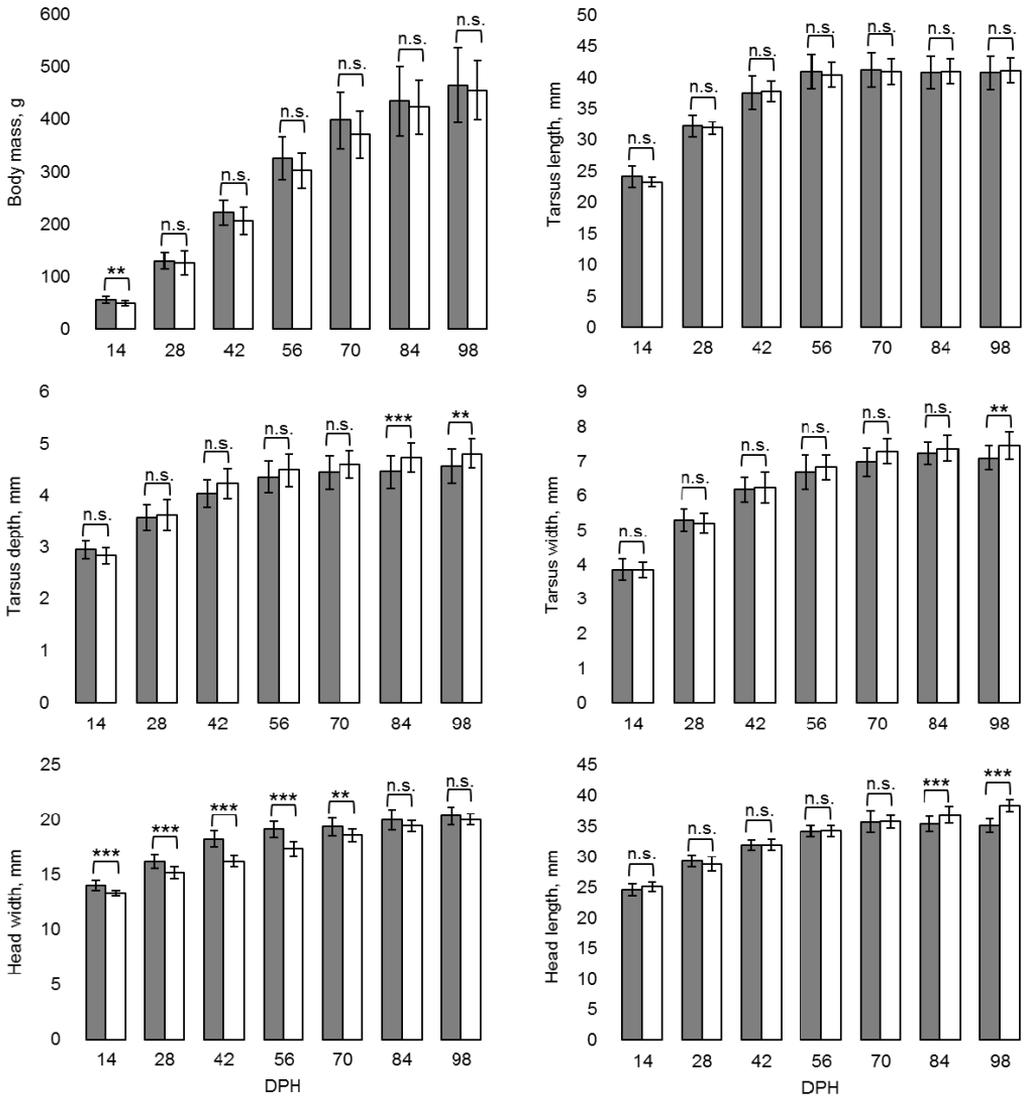


Fig. 1. Comparisons (GLM F-test at $p = 0.01$) between G1 (grey bars) and G2 (white bars). Note: vertical bars denote standard deviations (SD); ** $p < 0.01$, *** $p < 0.001$.

period. TD and TW showed the first significant difference between groups at 84 DPH and 98 DPH respectively. These measures were higher in G1 at an early stage of growth but at 28 and 42 DPH, respectively, the average values of G2 exceeded those of G1. Birds in G1 had wider heads (HW) than birds in G2, but this difference, which was greatest at 42 DPH, narrowed progressively and maintained significance until 70 DPH but not after. HL was not sensitive to the intensive rearing until 84 DPH; in fact, in the period between 14 DPH and 70 DPH, the average values of the groups constantly

overlapped. After 70 DPH, HL reached a plateau in G1 and continued to grow in G2. Differences in HL between groups became significant at 84 DPH.

Sexual dimorphism in size

A significant effect of sex was detected starting from 28 DPH (Table 1) and a descriptive statistic of each BT, is also provided by sex and group in tables 2 and 3. For sex comparisons $p = 0.05$ significance level would allow to highlight differences between sexes 14 days earlier than 0.01 level.

Table 2. Descriptive statistics (mean \pm SD) of males ($n = 8$) and females ($n = 8$) of G1.

Body traits	Sex	Days post hatching					
		28	42	56	70	84	98
BM	♂	141.91 \pm 8.4	237.57 \pm 21.2	355.12 \pm 20.3	430.30 \pm 37.5	469.11 \pm 64.4	507.11 \pm 64.4
	♀	118.32 \pm 11.2	206.83 \pm 12.4	295.65 \pm 29.8	365.15 \pm 46.8	403.61 \pm 54.1	427.42 \pm 57.1
TL	♂	33.70 \pm 0.7	39.72 \pm 1.0	43.14 \pm 1.6	43.52 \pm 1.2	42.98 \pm 1.9	43.03 \pm 1.7
	♀	30.83 \pm 1.3	35.12 \pm 1.4	38.83 \pm 1.6	39.06 \pm 1.7	38.94 \pm 1.5	38.80 \pm 1.6
TD	♂	3.75 \pm 0.1	4.20 \pm 0.2	4.56 \pm 0.3	4.66 \pm 0.3	4.71 \pm 0.3	4.81 \pm 0.3
	♀	3.39 \pm 0.2	3.87 \pm 0.1	4.14 \pm 0.1	4.22 \pm 0.1	4.23 \pm 0.1	4.35 \pm 0.1
TW	♂	5.49 \pm 0.2	6.40 \pm 0.3	6.85 \pm 0.5	7.18 \pm 0.4	7.45 \pm 0.3	7.36 \pm 0.2
	♀	5.13 \pm 0.3	5.94 \pm 0.3	6.51 \pm 0.4	6.76 \pm 0.4	7.04 \pm 0.2	6.88 \pm 0.3
HW	♂	16.57 \pm 0.6	18.62 \pm 0.4	19.42 \pm 0.6	19.65 \pm 0.8	20.40 \pm 1.0	20.81 \pm 0.9
	♀	15.82 \pm 0.4	17.90 \pm 0.8	18.82 \pm 0.7	19.08 \pm 0.8	19.60 \pm 0.7	19.96 \pm 0.5
HL	♂	29.87 \pm 0.6	32.56 \pm 0.5	34.86 \pm 0.5	36.74 \pm 1.2	36.14 \pm 0.5	36.08 \pm 0.7
	♀	28.69 \pm 0.9	31.36 \pm 0.5	33.58 \pm 0.7	34.78 \pm 1.6	34.82 \pm 1.4	34.33 \pm 0.7

Table 3. Descriptive statistics (mean ± SD) of males (n = 8) and females (n = 6) of G2.

Body traits	Sex	Days post hatching					
		28	42	56	70	84	98
BM	♂	139.43±15.1	215.58±17.1	324.41±16.4	401.68±17.7	455.41±32.2	489.02±45.1
	♀	107.43±16.5	194.62±31.6	273.82±26.1	329.25±33.5	378.86±37.9	409.31±31.1
TL	♂	32.63±0.7	39.01±0.6	41.88±1.2	42.39±1.4	42.19±1.6	42.32±1.7
	♀	30.96±0.4	36.26±0.9	38.61±0.7	39.14±0.9	39.50±1.1	39.54±1.1
TD	♂	3.70±0.3	4.36±0.2	4.67±0.2	4.77±0.2	4.92±0.2	5.00±0.2
	♀	3.52±0.2	4.05±0.3	4.25±0.3	4.37±0.1	4.48±0.1	4.55±0.1
TW	♂	5.32±0.2	6.48±0.2	7.06±0.2	7.51±0.3	7.61±0.3	7.70±0.2
	♀	5.06±0.3	5.90±0.4	6.51±0.3	7.01±0.2	7.05±0.2	7.12±0.3
HW	♂	15.24±0.6	16.46±0.5	17.57±0.3	18.83±0.6	19.60±0.5	20.27±0.4
	♀	15.17±0.5	15.96±0.3	17.02±0.8	18.27±0.2	19.21±0.4	19.68±0.5
HL	♂	29.07±1.2	32.51±0.5	34.83±0.6	36.34±0.7	37.69±0.7	38.86±0.7
	♀	28.44±1.10	31.31±0.79	33.51±0.80	35.03±0.9	35.71±1.2	37.68±0.1

In both G1 and G2, SSDI showed larger features in males than in females (figs 2 and 3).

In both G1 and G2, the SSDIs in BM showed a peak at 28 DPH (SS-

DIG1 = 19.94, SSDIG2 = 19.80) and then decreased before reaching the maximum values at 56 DPH in G1 (SSDI = 20.12) and at 70 DPH in G2 (SSDI = 22.00). The SSDI in TL reached its maximum in G1

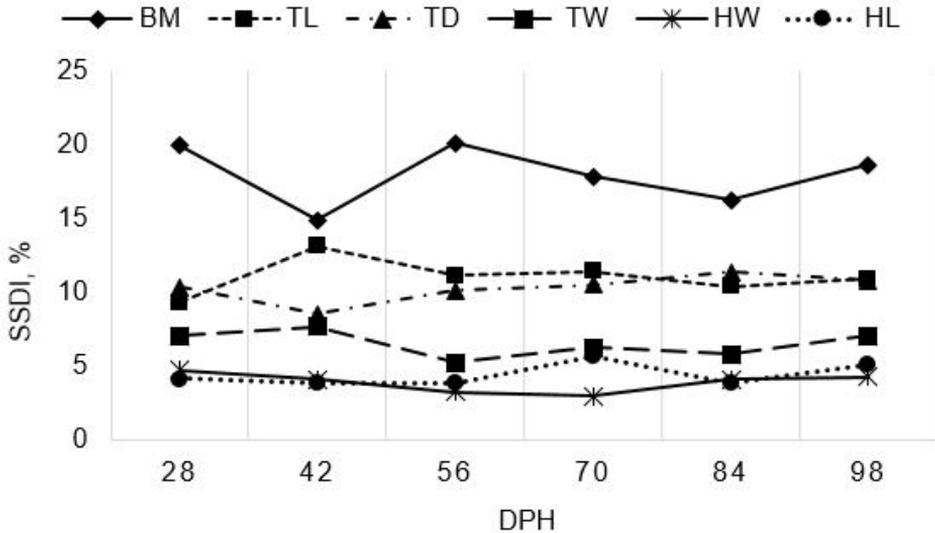


Fig. 2. Trends of Sexual Size Dimorphism Index in G1.

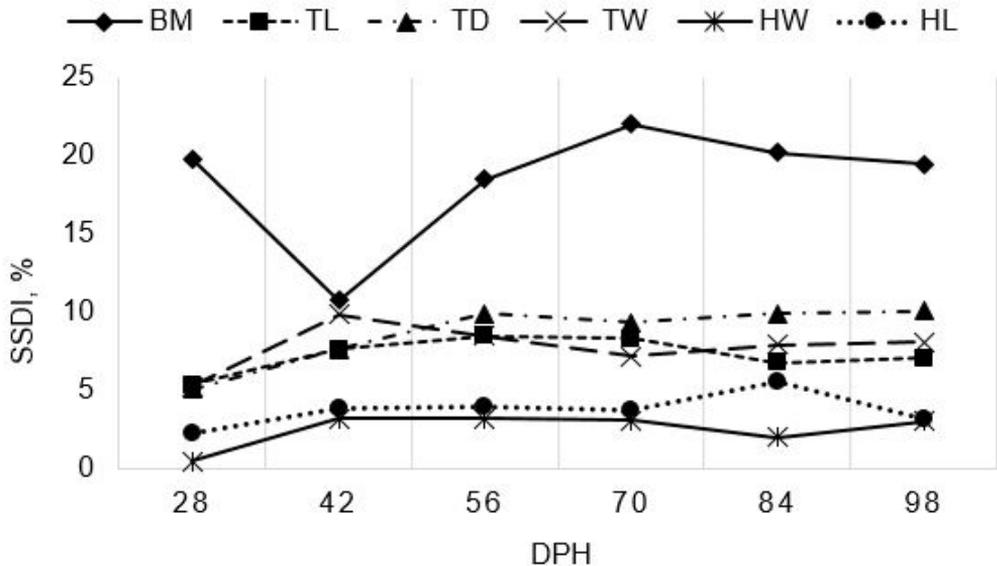


Fig. 3. Trends of the Sexual Size Dimorphism Index in G2.

and G2, respectively, at 42 DPH (SSDIG1 = 13.10) and 56 DPH (SSDIG2 = 8.46). For TD, the maximum SSDI occurred in G1 and G2, respectively, at 84 DPH (SSDIG1 = 11.38) and 98 DPH (SSDIG2 = 10.05). The third measure of TW reached its maximum dimorphism in size at 42 DPH in both groups (SSDIG1 = 7.68, SSDIG2 = 9.83). The maximum difference between sexes in HL occurred in G1 and G2, respectively, at 70 DPH (SSDIG1 = 5.62) and 84 DPH (SSDIG2 = 5.54). Overall, the maximum SSD did not exhibit a very different magnitude (SSDI) in the groups but was always observed at least two weeks earlier in the treatment group than in the control one.

Discussion

The slight wounds and cuts on the beaks' tips and primary feathers recorded in G1 during the indoor phase are probably con-

sequences of aggressiveness and agonistic interactions between captive chicks housed at high densities without shelters or shields. In these conditions, aggressive behaviour is well known (Duncan and Hawkins 2009). These injuries impair the use of BL and WL as reliable morphological indicators. Unfortunately, our data do not allow the assessment of the resilience times of these injuries that, in the case of permanence until the time of release, could represent a limiting factor for the birds' survival in the wild.

At least up to 28 DPH, the birds in G1 showed larger size than those in G2, although not always significantly. However, according to the BTs considered, the birds in G2 compensated, and in some cases surpassed the measurements of the birds in G1 (Fig. 1). This evidence suggests that the intensive rearing system minimizes energy costs and provides more resources for growth at an early stage, but also suggests that life in an enriched semi-nat-

ural habitat allows for progressive and greater development of the muscles (Whiteside et al. 2016, Santilli and Bagliacca 2017) which mitigate the overall differences in BM.

In contrast with Whiteside et al. (2016) and Santilli and Bagliacca (2017) who reported, respectively, that 7- and 5-week-old pheasants grown in an environment enriched with perches were heavier than those subjected to standard commercial rearing conditions, we found differences in BM between groups only at 14 DPH with birds in G1 heavier than birds in G2. These authors explained their results highlighting that when natural or artificial perches are available, young pheasants are incentivized to fly to perches for roosting or to escape chases and that a frequent take-off allows for the marked development of pectoral muscles; differently, in Rock partridges, the tendency to use perches is unknown in both nature and captivity. In G2 we found that the use of wings was limited to occasional short flights and Wing Assisted Incline Running (Dial 2003). Our result is in accordance with that of Akşit et al. (2017) who recorded significant differences between broiler chickens provided with perches and conspecifics without perches only at 14 DPH. These authors hypothesized that the lower body weight of chickens with perches could be associated with their higher activity and that this is a transitory condition because activity tended to decrease as birds aged according to Bailie et al. (2013).

In our study, birds in G2 moved inside the semi-natural aviary principally on foot, walking or running on the rough and uneven ground, enriched with rocks and ramps.

The exercise in this complex environment allowed for a greater development of the muscles of the limbs according to

Whiteside et al. (2016) and Santilli and Bagliacca (2017). In fact, birds in G2, showed wider and deeper tarsi than those observed in G1 at 84 and 98 DPH respectively.

In our study, TL consistently overlapped between the groups. Likewise, Bernard-Laurent et al. (2003) did not observe significant differences in TL between hand-reared and wild *A. g. saxatilis* (Bechstein, 1805) males. Most likely, this result is due to the heritability of this trait (Kondra and Shoffner 1955, Lerner 1989).

The higher values of HL observed in G1 at 70 DPH are quite difficult to explain because we have not specifically focused on brain mass and cranial volume. However, other authors found these parameters positively correlated with the cognitive abilities of the examined animal and that the complexity of the environmental stimuli is a crucial factor in this regard (Sol et al. 2005, 2007; Møller 2010; Whiteside et al. 2016).

Concerning the wider head (HW) recorded in G1 until 70 DPH, we suppose, according to Mani (2008), it may depend on a slight irritation of the respiratory mucosa, due to ammonia in the air during the indoor phase, with consequent swelling of the eyelids or eyebrows.

As expected, our results reveal sexual dimorphism in size (SSD) in both G1 and G2 with males larger than females and without notable differences between groups. However, SSD occurred earlier in G1 than in G2 as well as the maximum distance between males and females that occurred first in G1 and at least 2 weeks later in G2. Anderson et al. (1993) proved that in sexually dimorphic bird species, the larger sex grows faster than the smaller one and requires more energy during growth. Evidently, intensive rearing, artificially heating, low exercise and *ad libitum*

availability of industrial feed amplify the differences between sexes in growth rate at least at an early stage of growth.

Conclusions

In this paper, we present evidences that intensive rearing determines significant morphological changes in Rock partridges, particularly at the tarsus level, that are recordable, with high statistical significance ($p \leq 0.01$), starting from 84 DPH. Furthermore, we cannot exclude that feathers and beaks usury, recorded on intensive reared birds, could persist and limit their survival probability when released in the wild.

Therefore, the semi-natural rearing approach should be encouraged because it can mitigate the negative effects of the intensive rearing, allowing for a better survival rate after release.

However, breeders rarely exploit this type of complex and expensive rearing strategy, then, where intensive reared birds are the only possible choice, we recommend their translocation from strict captivity to wild-like pre-release acclimatization site, where they can exercise before the final release into the wild, within 70 DPH. Within this time limit, birds seem not significantly corrupted by the intensive rearing, at least morphologically, and the chance of fostering by barren wild pairs could be preserved where Rock partridges are still present, as suggested by Buner and Aebischer (2008) in the guidelines for re-establishing Grey partridge (*Perdix perdix* Linnaeus, 1758) through releasing.

In addition, although molecular sex determination methods are already available (Vucicevic et al. 2013), the magnitude of SSD suggests the possibility of a cheap, expeditious and early accurate sex de-

termination facilitating the translocation of sex-balanced groups. In this view, further researches should be oriented on the development of early sex determination methods based on SSD.

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References

- AKŞIT M., KAÇAMAKLI YARDIM Z., SERVET Y. 2017. Environmental enrichment influences on broiler performance and meat quality: effect of light source and providing perches. *European Poultry Science* 81: DOI: 10.1399/eps.2017.182.
- ALONSO M.E., PÉREZ J.A., GAUDIOSO V.R., DIEZ C., PRIETO R. 2005. Study of survival, dispersal and home range of autumn-released red-legged partridges (*Alectoris rufa*). *British Poultry Science* 46: 401–406.
- AMICI A., PELOROSSO R., SERRANI F., BOCCIA L. 2009. A nesting site suitability model for rock partridge (*Alectoris graeca*) in the Apennine Mountains using logistic regression. *Italian Journal of Animal Science* 8(2): 751–753.
- AMICI A., SERRANI F., PRIMI R., ADRIANI S., VIOLA P., BONANNI M. 2013. Status of rock partridge *Alectoris graeca* in Lazio Region, Central Italian Apennine: six years of monitoring. *Avocetta* 37: 119–124.
- ANDERSON D.J., REEVE J., MARTINEZ GOMEZ J.E., WEATHERS W.W., HUTSON S., CUNNINGHAM

- H.V., BIRD D.M. 1993. Sexual size dimorphism and food requirements of nestling birds. *Canadian Journal of Zoology* 71: 2541–2545.
- ARMSTRONG D.P., SEDDON P.J. 2008. Directions in reintroduction biology. *Trends in Ecology & Evolution* 23: 20–25.
- BAILIE C.L., BALL M.E., O'CONNELL N.E. 2013. Influence of the provision of natural light and straw bales on activity levels and leg health in commercial broiler chickens. *Animal Welfare* 7: 618–626.
- BERGERO D., DEBERNARDI M., MUSSA P.P., PAGANIN M. 1995. Effetto di diete a diverso contenuto in fibra ed in energia sulle prestazioni riproduttive delle coturnici (*Alectoris graeca*). *Rivista di Avicoltura* 9: 37–39.
- BERNARD-LAURENT A., CORDA E.M., SOYEZ D. 2003. Sex differences in body measurements of Rock partridges (*Alectoris graeca saxatilis*) inhabiting the southern French Alps. *Avocetta* 27: 181–186.
- BIRDLIFE INTERNATIONAL 2018. Species factsheet: *Alectoris graeca*. Available at: <http://www.birdlife.org> on 21/02/2019.
- BUNER F., AEBISCHER N.J. 2008. Guidelines for re-establishing grey partridges through releasing. *Game and Wildlife Conservation Trust, Fordingbridge*, ISBN 978-1-901369-17-5. 18 p.
- ÇAĞLAYAN T., KIRIKÇI K., GÜNLÜ A., ALAŞAHAN S. 2011. Some body measurements and their correlations with live weight in the rock partridge (*Alectoris graeca*). *African Journal of Agricultural Research* 6: 1857–1861.
- CALABUIG C.P., GREEN A.J., FERRER M., MURIEL R., MOREIRA H. 2011. Sexual size dimorphism and sex determination by morphometric measurements in the coscoroba swan. *Studies on Neotropical Fauna and Environment* 46: 177–184.
- CATTADORI I.M., RANCI-ORTIGOSA G., GATTO M., HUDSON P. 2003. Is the rock partridge *Alectoris graeca saxatilis* threatened in the Dolomitic Alps? *Animal Conservation* 6: 71–81.
- COCCHI R., RIGA F., TOSO S. 1998. Pheasant biology and management. Technical documents, 22, National Institute for Wildlife (INFS), Ozzano dell'Emilia, ISSN 1121–4120. 146 p.
- DIAL K.P. 2003. Wing-assisted incline running and the evolution of flight. *Science* 299: 402–404.
- DUNCAN I.J., HAWKINS P. 2009. The welfare of domestic fowl and other captive birds. *Animal Welfare* 9. DOI 10.1007/978-90-481-3650-6_12.
- ELLIS D.H., DOBROTT S.J., GOODWIN J.G. 1978. Reintroduction techniques for Masked Bobwhites. In: Temple S.A. (Eds). *Endangered birds: management techniques for preserving Endangered birds*: University of Wisconsin Press, Madison: 345–354.
- FRANKHAM R., HEMMER H., RYDER O.A., COTHRAN E.G., SOULÉ M.E., MURRAY N.D., SNYDER M. 1986. Selection in captive populations. *Zoo Biology* 5: 127–138.
- GAUDIOSO V.R., PÉREZ J.A., SÁNCHEZ-GARCÍA C., ARMENTEROS J.A., LOMILLOS J.M., ALONSO M.E. 2011a. Isolation from predators: a key factor in the failed release of farmed red-legged partridges (*Alectoris rufa*) to the wild? *British Poultry Science* 52: 155–162.
- GAUDIOSO V.R., SÁNCHEZ-GARCÍA C., PÉREZ J.A., RODRÍGUEZ P.L., ARMENTEROS J.A., ALONSO M.E. 2011b. Does early antipredator training increase the suitability of captive red-legged partridges (*Alectoris rufa*) for releasing? *Poultry Science* 90: 1900–1908.
- GONZÁLEZ-REDONDO P. 2010. Effect of long-term storage on the hatchability of red-legged partridge (*Alectoris rufa*) eggs. *Poultry Science* 89: 379–383.
- GÜNLÜ A., KIRIKÇI K., ÇETİN O., GARIP M. 2007. The effect of stocking density on growth performance and average cost in partridge rearing (*Alectoris graeca*). *Poultry Science* 86: 1800–1804.
- HESS M.F., SILVY N.J., GRIFFIN C.P., LOPEZ R.R., DAVIS D.S. 2005. Differences in flight characteristics of pen-reared and wild prairie-chickens. *The Journal of Wildlife Management* 69: 650–654.
- KONDRA P.A., SHOFFNER R.N. 1955. Heritability of some body measurements and reproductive characters in turkeys. *Poultry Science* 34: 1262–1267.
- LERNER I.M. 1989. Allometric studies of poultry. *Proceedings of the XIX World's Poultry*

- Congress 7: 86–88.
- LIUKKONEN-ANTTILA T., PUTAALA A., HISSA R. 1999. Does shifting from a commercial to a natural diet affect the nutritional status of hand-reared grey partridges *Perdix perdix*? *Wildlife Biology* 5(1): 147–156.
- LIUKKONEN-ANTTILA T., SAARTOALA R., HISSA R. 2000. Impact of hand-rearing on morphology and physiology of the capercaillie (*Tetrao urogallus*). *Comparative Biochemistry and Physiology. Part A. Molecular & Integrative Physiology* 125: 211–221.
- MANI P. 2008. Hygiene and prophylaxis in partridge rearing. In: Bagliacca M., Fronte B., Galardi L., Mani P., Santilli F. Guidelines for grey and red-legged partridges rearing. ARSIA, Firenze, ISBN 978-88-8295-103-0: 55–66.
- MIHAJLOV C., GRUYCHEV G., STOYANOV S., ANGELOV E. 2018. Rock partridge (*Alectoris graeca*) recovery program: first evidences on survival and dispersion rate of semi-natural reared birds in 'Vrachanski Balkan' Nature Park. *Forestry Ideas* 24(2): 208–216.
- MILLÁN J., GORTÁZAR C., BUENESTADO F.J., RODRÍGUEZ P., TORTOSA F.S., VILLAFUERTE R. 2003. Effects of a fiber-rich diet on physiology and survival of farm-reared red-legged partridges (*Alectoris rufa*). *Comparative Biochemistry and Physiology. Part A. Molecular & Integrative Physiology* 134: 85–91.
- MØLLER A.P. 2010. Brain size, head size and behaviour of a passerine bird. *Journal of Evolutionary Biology* 23: 625–635.
- O'REGAN H.J., KITCHENER A.C. 2005. The effects of captivity on the morphology of captive, domesticated and feral mammals. *Mammal Review* 35: 215–230.
- PAGANIN M., MENEGUZZI P. 1992. Gut length of wild and reared rock partridges (*Alectoris Graeca*): its role in release success. *Gibier Faune Sauvage* 9: 709–715.
- PÉREZ J.A., SÁNCHEZ-GARCÍA C., DíEZ C., BARTOLOMÉ D.J., ALONSO M.E., GAUDIOSO V.R. 2015. Are parent-reared red-legged partridges (*Alectoris rufa*) better candidates for re-establishment purposes? *Poultry Science* 94: 2330–2338.
- PERONACE V., CECERE J.G., GUSTIN M., RONDININI C. 2012. Nesting birds Italian red list. *Avocetta* 36: 11–58.
- PIS T. 2012. Growth and development of chicks of two species of partridge: the grey partridge (*Perdix perdix*) and the chukar (*Alectoris chukar*). *British Poultry Science* 53: 141–144.
- PRICE E.O. 2002. Animal domestication and behaviour. CABI, Wallingford, ISBN 978-08-5199-597-7. 297 p.
- PUTAALA A., OKSA J., RINTAMÄKI H., HISSA R. 1997. Effects of hand-rearing and radiotransmitters on flight of gray partridge. *The Journal of Wildlife Management* 61: 1345–1351.
- R DEVELOPMENT CORE TEAM 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- RONDININI C., BATTISTONI A., PERONACE V., TEOFILI C. 2013. Vertebrates Italian red list. IUCN Italian Committee and Ministry of Environment and Protection of Land and Sea, Roma. 54 p.
- SÁNCHEZ-GARCÍA C., ALONSO M.E., TIZADO E.J., PÉREZ J.A., ARMENTEROS J.A., GAUDIOSO V.R. 2016. Anti-predator behaviour of adult red-legged partridge (*Alectoris rufa*) tutors improves the defensive responses of farm-reared broods. *British Poultry Science* 21: 1–11.
- SANTILLI F., BAGLIACCA M. 2017. Effect of perches on morphology, welfare and behaviour of captive reared pheasants. *Italian Journal of Animal Science* 16: 317–320.
- SOL D., DUNCAN R.P., BLACKBURN T.M., CASSEY P., LEFEBVRE L. 2005. Big brains, enhanced cognition, and response of birds to novel environments. *Proceedings of the National Academy of Sciences* 102: 5460–5465.
- SOL D., SZÉKELY T., LIKER A., LEFEBVRE L. 2007. Big-brained birds survive better in nature. *Archive of Proceedings of the Royal Society B: Biological Sciences* 274: 763–769.
- STANEVA A., BURFIELD I. 2017. European birds of conservation concern. Populations, Trends and national responsibilities. *BirdLife International*. 172 p.
- TOBALSKE B.W., DIAL K.P. 2000. Effects of body size on take-off flight performance in the *Phasianidae* (Aves). *Journal of Experimental Biology* 203: 3319–3332.

- TROCCHI V., RIGA F., SORACE A. 2016. National Action Plan for the rock partridge (*Alectoris graeca*). Quaderni di Conservazione della Natura, 40 MATTM – ISPRA – Institute for Environmental Protection and Research, Roma, ISSN: 1592–2901. 84 p.
- VUCICEVIC M., STEVANOV-PAVLOVIĆ M., STEVANOVIĆ J., BOSNJAK J., GAJIC B., ALEKSIC N., STANIMIROVIC Z. 2013. Sex determination in 58 bird species and evaluation of CHD gene as a universal molecular marker in birds sexing. Zoo Biology 32: 269-275.
- WHITESIDE M.A., SAGE R., MADDEN J.R. 2015. Diet complexity in early life affects survival in released pheasants by altering foraging efficiency, food choice, handling skills and gut morphology. Journal of Animal Ecology 84: 1480–1489.
- WHITESIDE M.A., SAGE R., MADDEN J.R. 2016. Multiple behavioural, morphological and cognitive developmental changes arise from a single alteration to early life spatial environment, resulting in fitness consequences for released pheasants. Royal Society Open Science 3(3): 160008 Available at: <https://doi.org/10.1098/rsos.160008>.