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Evaluation of radiotagging techniques and their application to survival analysis of Red-legged Partridge *Alectoris rufa* chicks

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A better knowledge of chick survival rate is required to enable understanding of the population dynamics of gamebirds and to develop management measures to conserve their populations. The Red-legged Partridge *Alectoris rufa* is a highly valued game species in Spain, but its populations have been in continuous decline in recent decades. However, a lack of appropriate monitoring methods has been a limitation in gaining information on the mortality among Red-legged Partridge chicks. We developed effective methods for radiotagging chicks in captivity, and applied these methods in the field in northern Spain to estimate their survival during the first five months of life. The most effective method for radiotagging captive chicks between three and eight days old involved gluing small tags directly to the skin in the interscapular space using cyanoacrylate adhesive. Backpack harness tags attached with elastic bands were the most effective method for radiotagging four-week-old chicks. Predation was the main identified cause of chick mortality during the field experiments. Survival between hatching and five months of age was estimated to be 16–21%. The lowest survival rates occurred during the first seven days of life (62–70% cumulative survival), and this period seems to be a major determinant in the life history of the species.

Keywords: cyanoacrylate, chick survival, gamebird management, predation, radiotracking, retention time, Spain, tag attachment.

Estimates of the survival rate of neonatal birds are an important component of population models (Potts & Aebischer 1995, Topping *et al.* 2010), but have rarely been accurately assessed. The most direct way to estimate survival rates and to identify the causes of death of neonatal birds is to recover dead chicks. However, young growing birds are difficult to monitor effectively without adversely affecting growth and survival (Hubbard *et al.* 1998). The development of techniques for radiotagging neonatal chicks would assist in the acquisition of knowledge for this poorly-known phase of the avian life cycle.

Chick survival is a key factor determining the population dynamics of many Galliformes, amongst which high mortality occurs shortly after hatching (Potts 1986, Hannon & Martin 2006, Gregg *et al.* 2007). Therefore, estimating mortality rates during this period, and identifying the causes of mortality are central to the management and conservation of galliform species. Studies of chick survival have been based on direct observations of the reduction of brood size over time (Green 1984, Hudson 1985, Léonard & Reitz 1998). However, it is sometimes difficult to determine the number of chicks in a brood, especially during the first days of life, because parents look for dense vegetation to minimise risks to the brood (Green 1984, Hannon & Martin 2006). In addition, this method does not provide information about the causes of chick mortality. Survival of gamebird chicks under ten days old had also been studied through radiotagging using various methods of attachment, such as gluing tags to the back (Kenward *et al.* 1993, Göth & Jones 2001, Bowman *et al.* 2002, Spears *et al.* 2002), gluing and suturing tags to the skin (Larson *et al.* 2001, Burkepile *et al.* 2002), implanting tags subcutaneously (Hubbard *et al.* 1998, Larson *et al.* 2001, Bowman *et al.* 2002, Gregg *et al.* 2007), or attaching tags as harnesses (Hubbard *et al.* 1998). However, data on the effects of transmitters on chick growth and survival are scant, and accurate estimates of transmitter retention times are also limited (but see Bowman *et al.* 2002, Spears *et al.* 2002, Steen & Haugvold 2009).

The Red-legged Partridge *Alectoris rufa* is a galliform species native to south-western Europe (Cramp & Simmons 1980), whose natural range is restricted to Spain, Portugal and France. Although it is globally listed as "Least Concern" (IUCN 2011), it is a "Species of European Conservation Concern" (SPEC, Tucker & Heath 1994). During recent decades, Red-legged Partridge populations have declined worldwide (Potts 1980, Office National de la Chasse 1986, Aebischer & Potts 1994). Causes of decline are multiple, including habitat loss (Buenestado *et al.* 2008), pathogens and genetic introgression resulting from restocking with farm-reared partridges (Barilani *et al.* 2007, Blanco-Aguiar *et al.* 2008, Villanúa *et al.* 2008), predation (Buenestado *et al.* 2009, Moleón *et al.* 2008) and hunting pressure (Blanco-Aguiar *et al.* 2003). Hunting and diseases in highly managed estates, and predation in unmanaged populations are the most prominent causes of adult partridge mortality reported in Spain (Buenestado *et al.* 2009).

Radiotracking has been used to study the breeding and survival of adult (Buenestado *et al.* 2008, Casas *et al.* 2009) and juvenile (Pérez *et al.* 2004) Red-legged Partridges, but knowledge of age-specific mortality rates and causes of mortality among Red-legged Partridge chicks is still lacking.

The effects of radiotags on Red-legged Partridge chicks of a few days of age have not been investigated to date, but the retention times and the growth and survival effects of various transmitter attachment techniques can be estimated using captive birds (Hubbard *et al.* 1998, Bowman *et al.* 2002). The aim of this study was to develop effective techniques for radiotagging Red-legged Partridge chicks, with the objective of tracking chicks in the wild to estimate survival rates and assess causes of mortality. We tested various methods of radiotag attachment to chicks in captivity, assessing retention times and the effects on growth and survival. Selected trialled methods were then used to estimate wild-chick survival in a field study in northern Spain, and to discuss whether chick survival could contribute to the observed population declines. This work represents the first attempt to estimate Red-legged Partridge chick survival in the field by tracking young chicks.

METHODS

Study areas

In the first phase of the study, we tested several methods for attaching radiotags to captive Red-legged Partridge chicks in an experimental farm facility in Ciudad Real (central Spain), belonging to the University of Castilla-La Mancha. In the second phase, selected tagging methods were tested on wild partridge chicks at two county communal hunting areas of Navarra (Corella, 6400 ha and Artajona, 6700 ha) in northern Spain during spring and summer 2007. Both study areas are dominated by crops (68%-77%), including olive (*Olea europaea*) and almond (*Prunus amygdalus*) tree groves, vineyards

(*Vitis vinifera*) and cereals, with natural vegetation (shrub and thicket) representing between 8% (Corella) and 32% (Artajona). Potential partridge predators in these areas include Domestic Cat (*Felis silvestris catus*), Red Fox (*Vulpes vulpes*) and other medium-size carnivores, Magpie (*Pica pica*), and some medium-to-large raptor species, such as Red Kite *Milvus milvus,* Common Buzzard *Buteo buteo*, Short-toed Eagle *Circaetus gallicus,* Booted Eagle *Hieraaetus pennatus,* Eagle Owl *Bubo bubo,* and three species of harriers (*Circus sp.*). The partridge density prior to breeding is estimated through drive counts as about 25 individuals/100 ha in each study area (Gestión Ambiental Viveros y Repoblaciones de Navarra, unpubl. data).

Tests in captivity

There were two objectives to the tests in captivity: (i) to compare the retention time of different tagging methods for partridge chicks and (ii) to assess the effects of transmitters on chick development by comparing the body mass between tagged and untagged chicks. Both tests were independently analysed for three groups of captive chicks based on their size and moulting phases: (i) three to five day-old hatchlings; (ii) 18-day-old chicks; (iii) 27-day-old chicks (one-third adult size). The tests in captivity were carried out from July to September 2006. Dummy tags of the same weight and size as commercial radiotags, minus the electronic components, were used with the aim of reducing costs and testing the most effective tagging system before buying the real tags. Chicks of unknown sex were provided by the experimental partridge breeding farm where the tests were performed. Chicks were kept in 3 x 2 m indoor rearing rooms with concrete floor, communicated with 3 x 18 m outdoor pens with ground soil surrounded by a 2.5 m high wire fence and nylon net on the top. Chicks were confined within the indoor rooms during the first three weeks of age, and they were allowed to access the outdoor pens afterwards. The chicks of a given experiment were kept in an independent rearing room; hence stocking density was 6.3 chicks/m^2 at most. All captive-bred chicks were supplied with food and water *ad libitum* during the tests and younger chicks were supplied with infrared heat lamps (R125IR 250 watt, Philips, Netherlands), following standard rearing procedures (Hodgson 2009). A qualified member of the research team visited the facility every day and visually checked the physical state and behaviour of the birds. During handling, each chick was carefully inspected for any ill-effects, related or not to the experiment. Chicks showing any injury other than localized feather loss or superficial skin abrasion were excluded from the experiment, and had their tags removed.

Thirty-eight chicks between three and five days of age (18.2 ± 1.0 g, average body mass ± se) were randomly assigned to one of three treatment groups: (1) *control* (no transmitter; *n* = 8); (2) *back-tagged* (*n* = 15); (3) *wing-tagged* (*n* = 15). All tagged chicks were fitted with a dummy transmitter weighing 0.45 g (2.5% of the mean chick body mass) with a 12 cm antenna; this mimicked the PIP-21 transmitter (Biotrack, Dorset, UK; guaranteed factory battery life 14 d). The dummy transmitter was glued to the interscapular region between the wings (*back* group) or under the left wing (*wing* group), with the antenna pointing caudally in each case. Feathers over a 2 × 1 cm area in the interscapular region (*back* group) were trimmed before attaching the tag. We tested two adhesion methods in each of the *wing* and *back* groups: (i) cyanoacrylate (Loctite®, Henkel, Germany) applied directly to the skin (*n* = 7), and (ii) cyanoacrylate applied on a layer of latex-based false eyelash glue (a non-irritant adhesive, *n* = 8). The chicks were weighed with a digital balance (± 0.1 g) at 4-day intervals, and we assessed the retention of transmitters and any external injury to the chicks over a period of 29 days.

Each of twenty-nine 18-day-old chicks (average body mass: 82.6 ± 2.5 g) was randomly assigned to one of three groups: (1) *control* (no tag, *n* = 6); (2) *back-tagged* (*n* = 12); and (3) *wing-tagged* (*n* = 11). We used dummy transmitters weighing 1.3 g (1.6% of the mean chick body mass) that mimicked the PIP transmitter (Biotrack, Dorset, UK; guaranteed factory battery life five weeks). Each of the same two adhesives as for the younger chicks was used for half of the tagged chicks (*n* = 6, except for wing-tagged directly with cyanoacrylate: *n* = 5). We weighed the chicks with a digital balance (± 0.1 g) every four days until all birds lost their tags.

Thirty 27-day-old chicks (body mass: 120.4 ± 3.1 g) were randomly assigned to one of three groups: (1) *control* (no tag; *n* = 10); (2) *harness* (*n* = 10); (3) *necklace* (*n* = 10). The harness tags weighed 4.5 g (3.8% of the mean chick body mass); they mimicked the TW-41 transmitter (Biotrack, Dorset, UK; guaranteed factory battery life 6.3 months). The necklace tags weighed 2.3 g (1.9% of the mean chick body mass); they mimicked the PIP transmitter (Biotrack, Dorset, UK; guaranteed factory battery life 5 weeks). In both the harness and necklace tags the lace was elasticized to allow for chick growth. The elastic was covered with braided cotton cord to avoid chafing of the skin. Tagged birds were weighed with a Pesola spring balance (± 5 g) every 3–4 days during the first

45 days, and once each week thereafter until 69 days, when the experiment was terminated.

We tested, independently for each group, differences in body mass over time among treatments, using linear mixed-effect models with absolute body mass (log-transformed) as the response variable. Tag type (including untagged as a group) was considered a fixed between-subject factor, individual a random factor and age a within-subject covariate. Statistical analyses were performed using the nlme package from R software v. 2.12.2 (R Development Core Team 2011). Following recommendations for animal testing, samples sizes of our tests in captivity were minimised using Optimal Design software (Raudenbush *et al.* 2011), assuming a standardized effect size of 0.8 and allowing for a power of 0.6.

Field tests

Wild partridge chicks were radiotagged in the field tests during spring and summer 2007, using the most appropriate method based on the tests in captivity. To locate and capture chicks, we identified nest sites by capturing and tagging adult partridges during April–May 2007. One of two capture methods was used: (i) cage traps containing a live adult partridge as a decoy; (ii) lamping/dazzling at night –using a large hand-held net and a powerful head-torch (Buenestado *et al.* 2009). Each adult partridge was fitted with a 10-g necklace radiotag (TW-51 model, Biotrack, Dorset, UK) and released at the capture site. We tracked the radiotagged adults every 24-72 h to establish the locations of the nests, from where we later captured the chicks.

We captured and radiotagged 37 wild chicks (2–8 per brood) between two and eight days old (age estimated from known hatching dates). Nineteen chicks from four broods (2, 3, 7 and 7 chicks/brood) were tagged in the Corella area, and 18 chicks from three broods (4, 6 and 8 chicks/brood) were tagged in the Artajona area. Chicks were captured by hand, fitted with a radio-transmitter (PIP-21 model, 0.45 g, Biotrack, Dorset, UK) and immediately released at the capture site. Radiotagged chicks were located every 24–48 h. When a detached transmitter was found, the surroundings were carefully inspected for possible causes of detachment (chick remains, tracks, feathers, carnivore scats). The transmitter was also carefully examined for marks on the body or the antenna that could indicate the cause of death (e.g. the type of predator).

We also captured and radiotagged 17 4-10 week-old chicks at night using large hand-held nets and spotlights. We tagged 12 chicks from four broods (1, 3, 3 and 5 chicks/brood) in the Artajona area, and five from three broods (1, 2 and 2 chicks/brood) in the Corella area. The captured animals were fitted with the most appropriate radio-transmitter and method of tag attachment based on the tests in captivity. Tagged chicks were located every 24–48 h during the first 2 months, and one–three times each week after the second month. Radiotracking allowed us to assess the cause of death based on an assessment of either the transmitter itself or the surrounding area. We radiotracked the chicks between July and November 2007, for a maximum of 118 days.

Cumulative survival curves for each chick age group were obtained using the Kaplan-Meier procedure (Kaplan & Meier 1958). We used the nest-survival model with the sin-link function in Program MARK (White & Burnham 1999) to estimate survival rates. Since survival of chicks within broods may not have been independent, this may cause underestimation of the confidence intervals (Flint *et al.* 1995). Therefore we estimated chick survival rates within broods, and employed a boot-strap resampling method with 1000 replicates to estimate confidence intervals of the among-brood average survival rates.

Ethical Note

All experiments, both in captivity and in the field, complied with current EU and Spanish regulations on animal experimentation and animal welfare. The corresponding permit (number PP1104-04) was issued by the Committee on Animal Research and Ethics from Castilla-La Mancha University. Authors were deemed qualified by Spanish rules to design (category C) and develop (category B) animal experiments, and moreover, an animal welfare specialist (Dr. F. Castro, category D) was on hand to supervise all the procedures carried out both in captivity and in the field.

RESULTS

Tests in captivity

All three to five day-old chicks in the *wing* group lost their tags between tagging and four days after attachment, regardless of the adhesive used (median retention time 2 days, Fig. 1). Chicks from the *back* group retained the tags longer (median 12 days; Mantel-Cox test *back vs. wing*: $C = -2.746$; $P < 0.01$), but all tags had detached 29 days after attaching (Fig. 1). There were no differences in the retention time based on the gluing method used for the back-tagged chicks (Mantel-Cox test: $C = -0.175$; $P = 0.86$), but the latex system involved longer chick handling than the cyanoacrylate method.

We excluded the *wing* group from the analysis of the transmitter effect on chick growth because of the short time over which chicks of this group retained their tags. The selected model for variation in chick body mass included chick age as a covariate and individual as a random factor. The addition of either tag type (control vs. back-tagged) or the interaction between age and tag did not improve the fit of the model (L-ratio test: 0.0007, $P = 0.98$ and 0.0072, $P = 0.93$, respectively). As expected, there was a significant effect of age on body mass ($F_{1,84} = 1013.55$, $P < 0.0001$). However, there was not a significant effect of tag type ($F_{1,17} = 0.0007$, $P = 0.98$; standardized effect size: -0.017, Fig. 2a), nor the interaction age x tag type ($F_{1,84} = 0.0071$, $P = 0.93$).

No chick showed evidence of external injuries attributable to the tagging method. Based on above results, gluing the tag (PIP-21) directly onto the back of the chick with cyanoacrylate was the method selected for tagging young chicks in field tests (Fig. 3a). This method was chosen because of the longer retention time, the apparent lack of any effect on chick body mass gain, and the shorter time required for chick handling relative to the latex system.

Only two of the 18-day old chicks (40%) tagged on the back with the latex system retained their tags for four days or longer (both had lost the tag at eight days). All other chicks, from both the *back* and the *wing* groups, lost their tags within four days. Due to the short time chicks remained tagged, it was not possible to analyse the transmitter effect on chick growth. No chicks showed evidence of external injuries attributable to the tagging method.

Five of the 27-day-old chicks (50%) with harness tags and two birds (20%) with necklace tags remained tagged until the end of the experiment (69 days). Of these seven birds, none of them showed evidence of injuries related to the transmitters. Thirty-five days after attachment, two birds (20%) from the harness group had lost the transmitter and the tag had begun to tighten on each of seven birds (70%) from the necklace group. These tags were removed from the birds and consequently censored from the experiment.

The chicks showed no signs of injury in the first month of tagging, but three birds from the harness group and two from the necklace group showed minor abrasions on day 32. These abrasions disappeared spontaneously and were not evident during later inspections. A more serious injury, a broken wing, was observed in a chick from the harness group, likely related to a deficiency in the experimental farm facility (a hole in the room fence where the bird could have entangled its wing), which has repaired upon detection. We isolated this bird from the rest of the chicks until its wing healed and the bird was able to fly normally. One chick from the control group and two chicks from the harness group died during the experiment, from causes not related to the tag. However, a chick from the necklace group died 52 days after tagging, apparently because the necklace was too tight which prevented it from eating, although other unidentified causes could have contributed to its death. As a whole 70% of necklaces were removed and 20% of harnesses were lost before the end of the experiment, these birds being censored from the analysis at the day the tags were removed. Retention time could not be statistically compared between harness and necklace tags because most chicks with necklaces were censored and none of the remaining chicks lost their tags before the end of the experiment. Median retention time was greater than the duration of the experiment (69 days) for both harness and necklace groups.

The effect of tag type on chick growth was analysed considering only data until day 35, before seven chicks from the necklace group were censored from the experiment. The selected model for body mass included chick age as a covariate and individual as a random factor. The addition of either tag type or the interaction between age and tag type did not improve the fit of the model (L-ratio test: 1.628, $P = 0.44$ and 6.417, $P = 0.17$, respectively). As expected, there was a significant effect of age on body mass ($F_{1,260} = 1586.71$, $P < 0.0001$). However, there was not a significant effect of tag type ($F_{2,26} = 0.90$, $P = 0.37$; standardized effect sizes: -0.02 and -0.019, for harness and collar, respectively; Fig. 2b), nor the interaction age x tag type ($F_{2,258} = 2.55$, $P = 0.08$). Nevertheless, the lack of statistical significance must be interpreted with caution, since reduced sample size limits statistical power of the test (5.7% and 6.6% for harness and collar, respectively). Body mass of chicks censored from the experiment at day 35 (295.4 ± 4.7 g) did not differ from the mass of birds remaining in the experiment (293.9 ± 5.6 g, $F_{1,27} = 0.042$, $P = 0.84$). The last live body mass of the bird with a necklace tag that died (250 g) was lower than the average body mass of the remaining birds measured at that same day (336.6 ± 6.4 g).

The harness tags (TW-41) fixed with elastic bands around the wings were selected for tagging of four-week-old chicks in field tests (Fig. 3b) because, unlike the necklace, they did not prevent chicks from feeding.

Field tests

From the 37 tagged chicks that were monitored during their first month of life, eleven (30%) remained alive and tagged beyond the expected life of the transmitters (14 days), although lost signals from another five transmitters could also correspond to living chicks. The longest any chick remained tagged with a working transmitter in the field was 19 days. Ten transmitters (26.7%) were found in the surroundings of the capture site between two and four days after tagging, detached from the chicks for reasons apparently not involving predation. Predation was the only identified cause of mortality, affecting at least eleven (30%) of the tagged chicks, but potentially 16 (43%) if all transmitter losses were due to predation. The majority of predated chicks (63%) were taken by raptors, based on evidence of the bent antennas (Larson *et al.* 2001). Seven chicks were predated upon the day after tagging, including six chicks from the same brood, and all were apparently predated upon by a raptor. The predation of the six chicks from the same brood was deemed to be predation of the entire brood, as the mother was located alone afterwards. The only other case of multiple predation involved two chicks from a brood.

To calculate survival curves we assumed two extreme scenarios: (i) all signal losses were due to transmitter failure (“maximum survival”); (ii) all signal losses were a consequence of chick death (“minimum survival”). Chicks were subject to high mortality during the first five days of monitoring, but subsequent survival was high (Fig. 4). We analysed chick survival using the nest-survival model of Program MARK, but disregarded the first two days of radiotracking because of the likely effects of capture and handling (Fig. 4). We used a constant survival rate for young chicks in the model, as use of different survival rates for the first week and the following weeks did not improve the model (delta AIC = 0.0017). Daily survival rates were estimated to be between 0.964 (minimum survival assumption; 95% confidence interval: 0.905-0.986) and 0.972 (maximum survival assumption; 95% confidence interval: 0.917-0.991).

From the seventeen tagged chicks that were older than four-weeks, six (35.3%) survived the entire period (113-118 days) with the functional transmitter, three (17.6%) were predated, and one (5.9%) was found dead with the transmitter entangled in bushes. Five transmitters (29.4%) were found entangled in vegetation with no evidence of predation. The signal from the remaining two transmitters (11.8%) was lost for unknown reasons. The median retention time for transmitters in the field was 79 days, although most transmitters (71%) of those retained after the first week remained on the chicks until the end of the study (>113 days).

One chick was predated upon by a raptor and another by a carnivore, and in the case of a third predated chick we could not determine the predator involved. On the assumption that all losses of radio signal were because of predation, we estimated that up to 30% of chicks between four weeks and five months of age may have been predated. Chicks were followed until the transmitter batteries were exhausted. Unfortunately, without the aid of transmitter signal, we were unable to relocate or recapture chicks. However, two chicks were shot during the following hunting season (November-December) and their transmitters recovered in good physical condition.

Analysis using the nest-survival model of Program MARK indicated a daily survival rate of 0.994 (95% confidence interval: 0.980-0.999) for the period between one and five months of age, under both the minimum and maximum survival assumptions.

Assuming a constant daily survival rate during the first month after hatching as the value estimated from radiotracking chicks between 3-19 days, and a constant survival rate between 1 and 5 months of age, between 16.2% (minimum survival assumption) and 20.7% (maximum survival assumption) of partridge hatchlings would survive for up to 5 months in the study areas.

DISCUSSION

Methods for radiotagging Red-legged Partridge chicks

The most effective method for radiotagging young Red-legged Partridge chicks was gluing the transmitter in the interscapular space. This finding is in agreement with previous studies on methods for tagging young chicks of other species of Galliformes (Kenward *et al.* 1993, Göth & Jones 2001, Bowman *et al.* 2002, Spears *et al.* 2002). We excluded various attaching methods to young chicks: attachment to the legs (Tabonsky & Tabonsky 1995) was excluded because of their fragility and harnesses were not considered because of (i) the likely effects on physical development (Hubbard *et al.* 1998), and (ii) the high risk of tag entanglement (Keedwell 2001). Suturing transmitters to the skin (Korschgen *et al.* 1996, Burkepile *et al.* 2002) and subcutaneous implants (Larson *et al.* 2001, Gregg *et al.* 2007) were also discarded because of the risks associated with surgery in the field, including post-operative pain, infections and/or higher stress levels associated with the long handling time required.

Gluing tags to the back of small chicks implies varying rates of tag loss (Kenward *et al.* 1993, Göth & Jones 2001, Bowman *et al.* 2002, Spears *et al.* 2002). The median retention time for glued transmitters on small chicks in our study (12 days) is within the range for 20 species of small-bodied shorebirds and land birds (10-31 days, review by Mong & Sandercock 2007). However, the radiotags were retained in our study for a shorter time than those attached to two day-old Wild Turkey (*Meleagris gallopavo*) chicks, using a similar method (29 days; Bowman *et al.* 2002). This difference could be related to the fact that Bowman *et al.* (2002) roughened the underside of the transmitter to improve adhesion and inserted a small piece of cheese cloth between the transmitter and the skin, whereas we did not. The two adhesion methods we tested yielded similar transmitter retention times. This result contrasts with previous studies reporting that inclusion of a layer of latex glue between the skin and the cyanoacrylate glue improved the retention of transmitters on Australian Brush-turkey (*Alectura lathami*) (Göth & Jones 2001) and Common Pheasant (*Phasianus colchicus*) chicks (Kenward *et al.* 1993).

We did not observe adverse effects of the glue on chick skin, contrary to earlier studies that suggested cyanoacrylate may be histotoxic (Woodward *et al.* 1965) and harmful to the skin (Göth & Jones 2001). In other studies cyanoacrylate has been widely used for radiotagging chicks, without detriment (Mauser & Jarvis 1991, Wheeler 1991, Bowman *et al.* 2002).

Transmitters glued to the backs of young partridge chicks did not affect their body mass gain, as has been reported in other studies using similar attachment systems (Göth & Jones 2001, Bowman *et al.* 2002), although we must treat this result with caution due to our low test power. According to Fig. 1 the effect of tags on growth in the 3-5 days old chicks is very unlikely. We could not test for an effect of transmitter attachment on chick survival in the wild as we could not estimate survival of untagged chicks, but the survival of Willow Ptarmigan (*Lagopus l. lagopus*) chicks was not affected by this type of transmitter (Steen & Haugvold 2009).

Transmitter detachment from young chicks in the field during the first four days following attachment (27%) was similar to that observed in tests on captive chicks (29%) with most detachment in the field (24%) occurring within the first two days. This may be due to (i) the greater activity of chicks in the field, (ii) the greater density of vegetation where younger chicks are found (compared to older chicks) resulting in potential entanglement, or (iii) attempts by the mother to remove the transmitters from the chicks (Green 1984).

We were unable to identify an efficient tagging system (in terms of retention time) for 18-day-old chicks. The transmitters tested had the same shape as those used successfully with younger chicks, but were larger, in relation to chick size. The extremely short retention time (< 4 days) may be because of the loss of down feathers, or to the greater pressure exerted against the transmitters by the growth of first moult feathers.

Although the differences in body mass among the 3 treatments (necklace, harness and untagged chicks) of chicks older than 27-days of age were not significant, this must be treated with caution due to low test power. In contrast with smaller chicks, tagged 27-day-old chicks showed a lower body mass at the end of the measurement period than the control group (see Fig. 1). The effect of the interaction age x tag type on body mass was nearly significant, which points to a likely detrimental effect on chick growth. Therefore, alternative methods for tagging chicks over 27-day-old should be evaluated. Necklaces, even with elastic bands, are particularly not recommendable for chicks of this age, since one month after being tagged with necklaces, the transmitter started to tighten on 70% of chicks which subsequently could prevent them from eating normally and might have done in one of the remaining birds where the necklace was not removed. Therefore, this method was not included in the field tests and is not recommended for radiotagging of growing partridge chicks.

Some captive partridge chicks tagged with dummy harnesses had slight abrasions on their wings during the first month following tag attachment, but no more severe injuries. In contrast, Hubbard *et al.* (1998) found that harnesses caused wing oedema and affected wing growth in captive Wild Turkey poults, which prevented the birds from flying. In our study, the harnesses did not prevent chicks from eating (unlike necklaces), rather they allowed a larger transmitter, with longer lifespan and range, to be used (Kenward 2001).

The retention time for transmitters in the field for chicks older than 27-days of age was probably reduced by the density of bushes, and exposure to environmental factors that probably increased wear on the elastic band. Göth and Jones (2001) considered the risk of chicks becoming entangled in dense undergrowth to be the main limitation in tagging Galliformes using a harness system. The death of one chick (5.9%) from this cause in our study confirms this risk, which should be carefully considered in future studies. We found another five transmitters (29.4%) entangled in the vegetation, but the lack of bird remains in the immediate surroundings leads us to think that the chicks managed to release themselves from the tag. This could raise another welfare issue on the method since we do not know how long each bird was entangled in the vegetation, something that would have raised stress levels as it was prevented from unrestricted movement, from foraging and evading predators. These risks, which could be reduced by tightening the harness elastic bands when attached to the bird, should be seriously considered when tagging galliform chicks with harnesses.

Survival rate estimates and causes of mortality

Several problems affect the use of radiotracking to study young chick mortality. Failure of transmitter electronic components is especially likely with small transmitters (Kenward *et al.* 1993) and the short range of small transmitters makes signal loss quite likely, especially if transported out of range by a predator (Spears *et al.* 2005, Steen & Haugvold 2009). For chicks less than one month of age, we had high tag detachment and signal loss rates and in order to account for these limitations, we considered two extreme scenarios, which provide the range within which true survival rates must be included (Whittier & Leslie 2009).

High mortality among chicks during the days following tagging might be related to the stress of capture and manipulation (Keedwell 2001), or to the time required to habituate to the transmitter (Mong & Sandercock 2007). Predation risk may thus increase immediately after transmitter attachment (Mong & Sandercock 2007). Indeed a large number of transmitters were lost during our initial period of radiotracking, so we excluded the two days immediately following capture of young chicks, thereby enabling more realistic survival rates to be obtained (Kenward 2001, Mong & Sandercock 2007).

The highest mortality among partridge chicks in our study occurred during the first seven days following hatching (Fig. 4), which is probably related to an inability to fly and the high dependence of chicks on their mothers (Spears *et al.* 2005, Steen & Haugvold 2009). Fledging in the Red-legged Partridge occurs at two–three weeks of age (Cramp & Simmons 1980), which corresponds to a decrease in mortality as a result of the increased ability of chicks to escape from predators. Similarly, the greatest mortality in chicks of other Galliform species occurs during the first two–four weeks after hatching (Jenkins 1961, Spears *et al.* 2005, Gregg *et al.* 2007, Steen & Haugvold 2009). Survival of Red-legged Partridge hatchlings up to four weeks of age estimated in our study area (33-43%, according to daily survival rate estimates) was within the values reported by Léonard and Reitz (1998) in Central France (4-47% up to 4 weeks) based on direct observations of broods over time. Duarte and Vargas (2004) estimated in southern Spain the survival of Red-legged Partridge chicks during the first ten days after hatching as 9%, although this estimation was based on radiotracking of only 11 chicks from the same brood.

Predation was identified to be the primary cause of mortality among Red-legged Partridge chicks, as has been reported for individually marked chicks of other Galliformes (Riley *et al.* 1998, Hubbard *et al.*1999, Larson *et al.* 2001, Gregg & Crawford 2009). Predation by raptors was the predominant cause of death in young chicks, even if predation by carnivores had been underestimated because of signal loss. In Spain, raptors have been identified as the primary predator of released two to three month old partridges (Pérez *et al.* 2004). Similarly avian predators have also been reported to be the main cause of death of Red Grouse (*Lagopus l. scoticus*) (Redpath 1991) and Ruffed Grouse (*Bonasa umbellus*) chicks (Larson *et al.* 2001). In contrast, carnivores are the main predators of Sage Grouse (*Centrocercus urophasianus*) chicks (Gregg *et al.* 2007). We found that among older partridge chicks, both raptors and carnivores seem to contribute equally to chick predation. However, even for a single species the main predator groups can differ among areas, depending on their relative abundance (Buenestado *et al.* 2009).

Factors other than predation can also determine chick survival. For instance, the survival of Red-legged Partridge chicks, which feed mainly on insects and seeds, has been correlated with the abundance of Coleoptera and grass seeds in the United Kingdom (Green 1984). The survival of Sage Grouse chicks is largely explained by factors related to their main food (Lepidopteran larvae) and preferred habitats (Gregg & Crawford 2009). Habitat characteristics also affect the survival of Wild Turkey chicks, through refuge and food availability (Hubbard *et al.* 1999), as does the physical condition of the hen (Spears *et al.* 2005). Such factors could also be relevant to Red-legged Partridge chicks. For this reason it is important to distinguish between periods with different chick survival, because combining productivity data with data on habitat use data from different periods that may differ in survival rates will lead to erroneous conclusions on the relationships between chick survival and habitat characteristics (Spears *et al.* 2005).

An accurate estimate of chick survival is essential for determining the rate of recruitment into the autumn and spring population (Gregg *et al.* 2007). Our estimates of chick survival from hatching to five months old (16.2-20.7%) can contribute to explain the declining trend of Red-legged Partridge populations. Considering the average clutch size (10.65) and nest success (47%) estimated in the study area (authors unpubl. data), hatching success in central Spain (86%, Casas *et al.* 2009), and assuming an even sex ratio at hatching and no sex-biased mortality, approximately 0.35-0.45 female offspring per clutch would recruit into the autumn population. Considering winter survival rates of first-winter partridges estimated in central and southern Spain (39-66% Buenestado *et al.* 2009), between 0.14 and 0.29 females would be recruited into the spring breeding population for each clutch laid the previous spring, a value likely insufficient to replace annual adult losses. Consequently, the observed decline of Red-legged Partridge in the region of study could be related, at least partially, to low offspring survival, although the small sample size prevents us from drawing firm conclusions. Population models confirm that chick survival plays a predominant role in the population dynamics of Red-legged Partridges in France (Ponce-Boutin *et al.* 2001). Juvenile survival is a key factor determining population size of bird populations, being poor survival of young chicks reported as the driver of population declines for threatened species (Aldridge & Brigham, 2001). Annual fluctuations of grey partridge populations were largely attributable to annual variations in chick survival (Potts & Aebischer 1995). In a review of 13 studies that examined the relative effect of varying grouse (subfamily Tetraoninae) vital rates on population growth, four indicated that early chick survival (before independence) was a major factor and seven that juvenile survival from autumn to spring was an important predictor of population growth (Hannon & Martin 2006). A wider study by radiotracking a larger number of chicks would allow a more accurate estimation of chick survival rates and an evaluation of the role of juvenile survival in the population dynamics of this species. Moreover, the development of effective methods for radiotracking Red-legged Partridge chicks will enable subsequent research to focus on the relative role of “ultimate factors” determining their survival, especially during the first two weeks of life, when most mortality occurs.

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FIGURE LEGENDS

Figure 1: Retention time of dummy transmitters on three to five day-old Red-legged Partridge chicks in captivity. Tests were carried out in the experimental partridge farm facility in Ciudad Real (central Spain) between July-September 2006. Retention times based on the tagging method: (i) glued to the back with cyanoacrylate over a latex layer (open triangles and continuous line, *n* = 8) or directly onto the skin (open squares and dashed-dotted line, *n* = 7); (ii) glued under the wing (open circles and dashed line, *n* = 15) with cyanoacrylate over a latex layer or directly on the skin.

Figure 2: Effect of transmitters on the body mass (± standard deviation) of (a) three to five day-old and (b) 27-day-old Red-legged Partridge chicks in captivity. Tests were carried out in the experimental partridge farm facility in Ciudad Real (central Spain) between July-September 2006.Body masses are for control (white circles, *n* = 8 in (a), and *n* = 10 in (b)), *back-tagged* (black circles, *n* = 15), harness (black triangles, *n* = 10), and necklace (black squares, *n* = 10) tags. The points slightly offset along the x-axis for the sake of clarity.

Figure 3: Three-day-old Red-legged Partridge chick (a) with glued-on transmitter (model PIP-21, Biotrack, Dorset, UK) and eight-week-old Red-legged Partridge chick (b) with harness transmitter (model TW-41, Biotrack, Dorset, UK). Fieldwork was carried out in Navarra (N. Spain) in spring-summer 2007. Photographs courtesy of Ainhoa Mateo-Moriones – Instituto de Investigación en Recursos Cinegéticos.

Figure 4: Cumulative survival curves (Kaplan-Meier) of partridge chicks in the field (Navarra, N. Spain) during the first weeks of life under the maximum (continuous line) and minimum (dotted line) survival assumptions. Triangles and circles represent deaths for the two survival assumptions, respectively, and X represent censored events (e.g detached radiotags or signal loss).

Figure 1

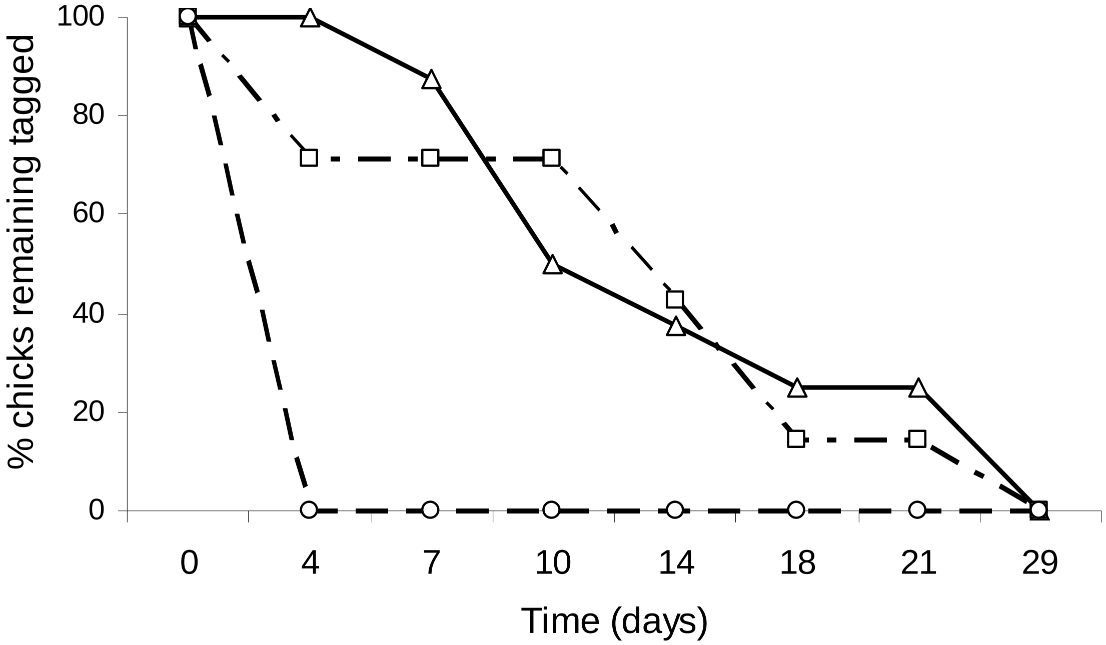
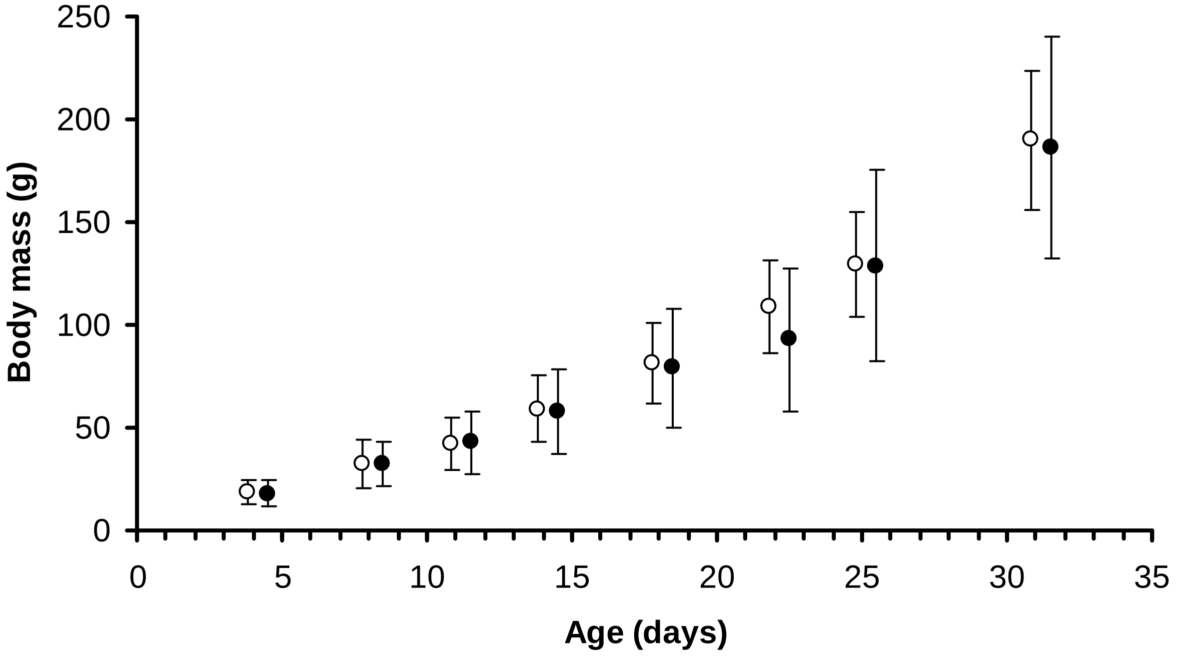

% chicks remaining tagged
100
80
60
40
20
0
0
4
7
10
14
18
21
29
Time (days)

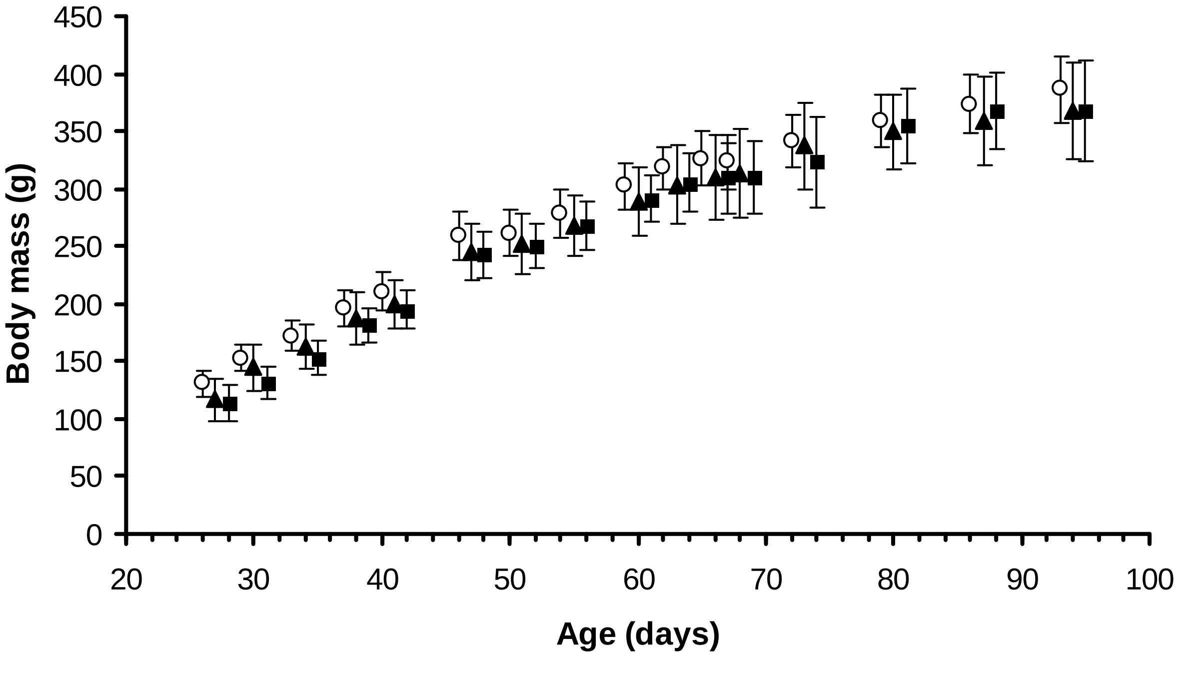
Figure 2

a)



Body mass (g)
0
50
100
150
200
250
0
5
10
15
20
25
30
35
Age (days)

b)



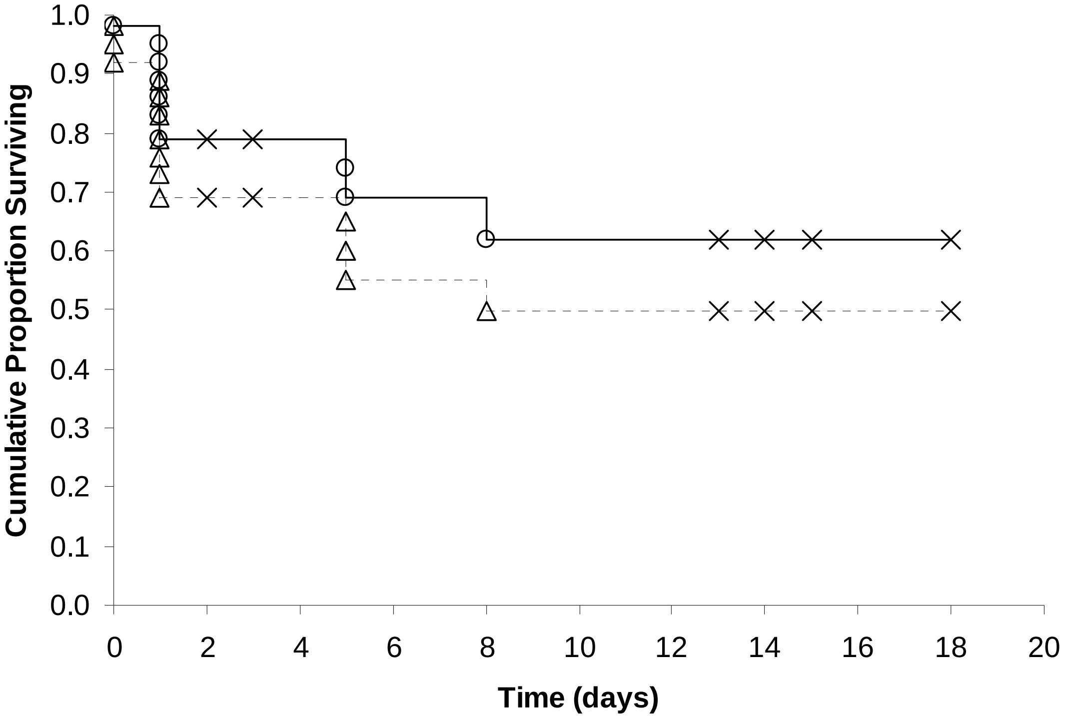
Body mass (g)
0
50
100
150
200
250
300
350
400
450
20
30
40
50
60
70
80
90
100
Age (days)

Figure 3

a)

b)

Figure 4


Cumulative Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (days)