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SHORT COMMUNICATION

Short communication articles are short scientific entities often dealing with methodological problems or with byproducts of larger research projects. The style is the same as in original articles

Age determination in fallow deer *Dama dama* neonates

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In this article, we evaluate the reliability of a set of easy-to-collect qualitative traits, such as appearance of the umbilical cord, position and behavioural reactions before and after handling, for ageing fallow deer *Dama dama* neonates. Using correspondence analysis, we found that the studied set of traits can distinguish the age of fawns during the hiding period, which, in this species, lasts only a few days. Ageing criteria are necessary to improve our understanding of mother-infant relationships, which may have important relevance when analysing the population dynamics of this ungulate, typical of Mediterranean habitats in Italy.

Key words: ageing, Dama dama, fallow deer, fawns, hiding

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Ungulates are characterised by a U-shaped mortality curve with low newborn survival (Caughley 1966). Variations in juvenile survival are much higher than variations in adult survival, so that "high yearly variability in juvenile survival may play a predominant role in population dynamics" (Gaillard et al. 1998). In several species of temperate ungulates, fawn mortality is concentrated in the perinatal period and is related to the fawn's body weight at birth (e.g. Coulson et al. 2003).

In order to get unbiased estimates of important phenotypic traits, such as birth date and birth weight, we need to know the age of captured newborns. This datum allows us to evaluate the birth date and to obtain an estimate of birth weight, whether a linear growth function may be estimated using multiple recaptures (e.g. Pelliccioni et al. 2004).

Fallow deer *Dama dama* adopt a hiding strategy and fawns lie hidden in vegetation, isolated from conspecifics, for about 4-5 days at which time they become able to follow their mothers. During the short hiding period, the mother visits the fawn several times a day (more often at twilight) to nurse and move it to a new bed site (Chapman & Chapman 1975).

Since 1988, the Istituto Nazionale per la Fauna Selvatica has developed a long-term monitoring programme for the fallow deer population in the Preserve of Castelporziano (Roma, Italy; Focardi et al. 2001). As part of this programme, we have captured and marked neonates since 2000 in order to investigate juvenile survival in this species.

For several species of ungulates it is possible to estimate the age of newborn fawns (white-tailed deer *Odocoileus virginianus*: Haugen & Speake 1958; roe deer *Capreolus capreolus*: Jullien et al. 1992; fallow deer: Pélabon 1995). Pélabon (1995) concluded that hoof abrasion, status of the umbilical cord and coat appearance were unlike to yield accurate age estimates for fallow deer fawns >2 days old. The aim of our study was to evaluate if there is a set of traits which can be used to reliably estimate the age of fawns during the hiding phase and which can be collected easily during captures in natural conditions.

The simplicity of the method is very important in order to reduce the handling time of fawns. By minimising stress at capture, we should get unbiased data on survival and growth. Because of this requirement, we excluded the use of blood samples and complex biometrical measurements. In the light of experience made on roe deer using Jullien's et al. (1992) ageing method (Pelliccioni et al. 2004), we adopted a similar approach to calibrate neonate age of fallow deer.

Study area, material and methods

Observations were made in the deer research facility 'Antonio Servadei' (University of Udine, Pagnacco, Italy) during two experimental periods in 2003 and 2007. In both years, 18 individually-marked adult females (different individuals in the two study years), were captured and weighed to determine their pregnancy status. The animals were divided into two groups. Each group was allocated to a 1.0-ha paddock and was allowed to graze a pasture dominated by tall fescue *Festuca arundinacea*, with the presence of foxtail bristlegrass *Setaria italica*, hairy crabgrass *Digitaria sanguinalis*, meadow buttercup *Ranunculus acer* and nettles *Urtica* spp. Observations were made from vantage points during 28 May-13 August, 2003, and during 7 June-24 July, 2007.

In some cases, parturition was observed or the doe showed signs of recent delivery. Females were always checked morning and evening from a distance, so that we could establish if parturition had occurred.

Most first captures were performed after observation of the fawn, but for several recaptures, fawns were detected upon systematic search.

Fawns were captured and inspected each day after birth when it was possible to capture them using a long-handled (150 cm) circular net (diameter 80 cm). To avoid desertion by the mother, we always waited some hours after delivery before capturing the newborn and latex gloves were always used when handling animals. During the first capture, fawns were marked with a numbered plastic ear-tag.

At capture the following parameters were recorded: status of the umbilical cord, behavioural reactions during capture and release, reaction to ear-tagging and/or handling and fawn's resting position at detection, before capture.

The umbilical cord was described by its status (protruding or in regression), appearance (wet, withered), solidity (soft, hard) and colour (pinkish, dark brown, black). Blood was defined as fresh, dry or absent. According to Jullien et al. (1992), we distinguished between three resting positions: 1) curled-up with head tucked against the flank, either with the belly down or lying on the flank (position lovée), 2) lying as an adult with the rear legs behind the belly, fore legs stretched along the ground and the head erect (position couchée) and 3) moving/standing up.

Like Pélabon (1995), we also used correspondence analysis (Andersen 1994) to evaluate whether or not recorded traits can provide a reliable estimate of fawn age. Using correspondence analysis (PROC CORRESP; SAS Institute, Inc. 2000) makes it possible to explore the structure of a multi-way contingency table (for such a table the usual χ^2-test is not appropriate). The derived structure is illustrated in a point diagram, which represents the categories of the studied variables on a plane. A profile is a vector obtained by dividing a cell frequency for the totals of its row or column. It is possible to compute both observed and expected profiles under the null hypothesis of no association between variables. This method allows us to reduce the number of variables, since only the two largest eigenvalues of the residual matrix (Dimension 1 and 2, respectively) usually

Table 1. Distribution of the different categories of the studied variables in the first five days of life of newborn fallow deer, reared in the research facility at the University of Udine, Italy.

Age	Umbilical cord						Behaviour	
(days)	Status	Solidity	Appearance	Colour	Blood	Position	During capture	At release
1	Present	Soft	Wet	Pinkish	Fresh	Lovée	Freeze	Freeze
2	Regression	Hard	Withered	Dark brown	Dried	Lovée	Calls	Unsteady flight movements
3	Regression	Hard	Withered	Dark brown	Dried (vestige)	Lovée/Couchée	Flight attempts, calls	Flight straight on
4	Regression	Hard	Withered	Dark brown/ black	Absent	Couchée	Flight, wriggle actively, calls	Trying to reach the mother
5	Regression	Hard	Withered	Black	Absent	Couchée/ movement	Calls and struggles to get free	Running towards the mother

explain a large part of sample variance. In the interpretation of the plot, three main rules have to be followed (*cf*. Focardi et al. 2000):

1) the categories near the origin have a frequency distribution close to the expected one; the larger the distance from the origin, the larger the residuals are;
2) to compare categories of the same variable it is possible to use the between-point distance; the larger the distance, the greater the difference;
3) the association between categories of different variables cannot be compared with distances, but the orientation of the line connecting the point to the origin can be used; categories with similar orientation are positively associated and categories with angular differences around π are negatively associated. Angles around π/2 between two categories denote that the observed frequencies are those expected under independence.

Results and discussion

The first births were recorded on the 18 June in 2003 and on 11 June in 2007, while the last births were recorded on 8 August and 18 July in 2003 and 2007, respectively. During the fawning period, 15 (six males and nine females) and 13 fawns (eight males and five females) were born in 2003 and 2007, respectively. In 2003, we excluded two fawns from our analysis because they died prematurely and three were excluded because they were born after the observation period ended. In 2007, we excluded five fawns; one because it died during the experiment and four because they were born before the observation period started.

Thus, a total of 18 newborn fawns (eight females and 10 males) were monitored in the two years and data were recorded systematically during the first three days of life; but on Days 4 and 5, we were able to capture 13 and nine fawns only.

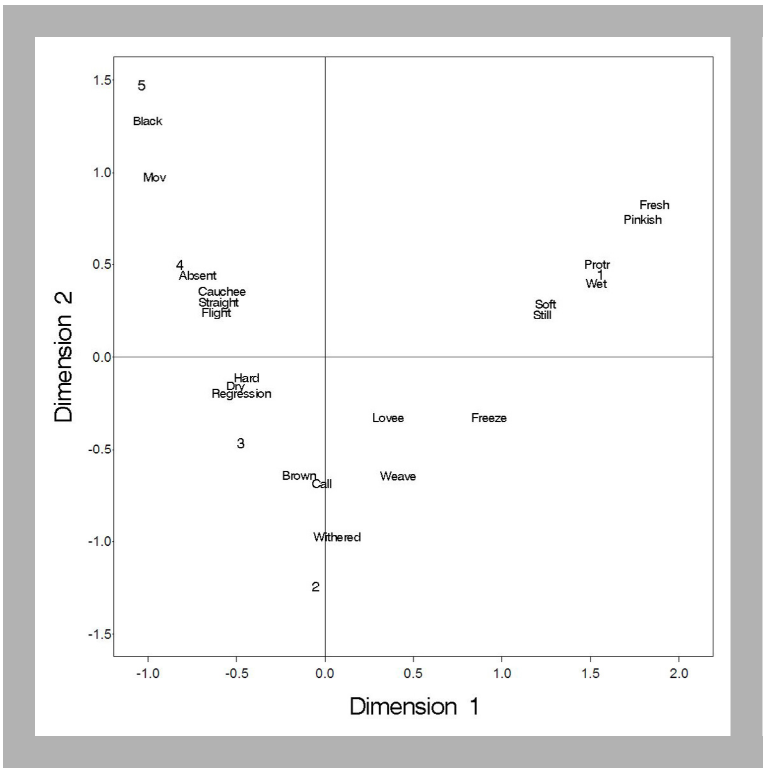

Figure 1. Categories of the studied variables plotted into the space defined by the two largest eigenvalues corrected by Greenacre adjustment. The numbers (1-5) represent the age (in days) of neonates. See text and Table 1 for definitions of the categories. To improve readability when two words overlapped, their position was shifted the minimal amount necessary to avoid overlapping text.

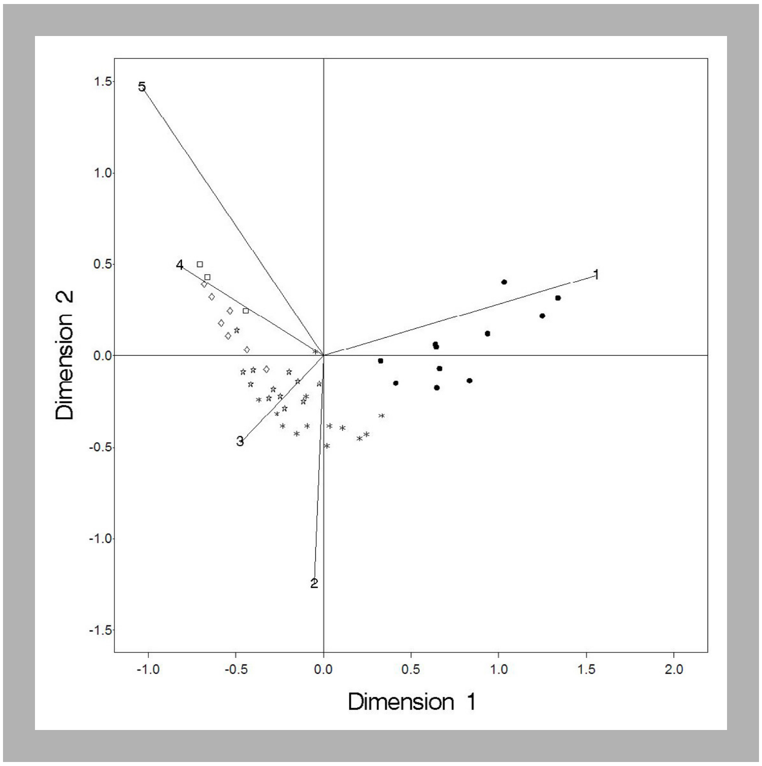

Figure 2. Coordinates of the individual animals and the age of neonates (in days; 1-5) plotted into the space defined by the two largest eigenvalues, to show the differences among individuals of the same age. Different symbols refer to different ages (i.e. ●= Day 1, *=Day 2; ☆=Day 3, ◇=Day 4 and □=Day 5). A line connecting categories of the variable Day to the origin allows an easier understanding of the discrepancies between each animals and the expected profile for each day. Note that some individual points overlap completely (because animals presented exactly the same features). True sample sizes for Days 1-5: 18, 18, 15, 13 and 9, respectively.

Only in one case (a very light newborn fawn weighing 3 kg) were we able to recapture a fawn after Day 5, but data for later days were omitted from the present analysis.

Inspection of the data showed systematic variations in the recorded traits as a function of age (Table 1). During the first and sometimes even the second day of life, we were able to capture the fawns by hand; on later days, it was necessary for us to use the net because flushing distance increased. Observations made during the study showed that during the first two days flushing distance was lacking or very short, and the fawn froze when somebody got close. At the age of 3-4 days the flushing distance was ≥ 2 m, but it was still possible to capture the fawns using the net. For older fawns capture probability decreased very rapidly.

Sometimes the fawns emitted distress calls when marked or handled inducing some specific reaction in the mothers, which usually were observing from some distance away.

Dimension 1 and Dimension 2 accounted for 42.0 and 17.0% of total sample variance, respectively. The association of the categories of the different variables showed a clear difference among the five one-day classes of age (Fig. 1). Day 1 was very different from the other days, which were arranged in a series showing continuous variations in the associated traits. Day 1 was also characterised by a non-random distribution of traits and it was associated with a pinkish, fresh, wet and protruding umbilical cord. Day 2 was associated with 'position lovée', withered blood and weaving movements. Day 3 was characterised by a dark brown cord and the presence of distress calls. Interpretation of traits associated with Days 4 and 5 followed straightforwardly.

Between-individual variability is shown in Figure 2, and so are the coordinates of the five age classes (note that some symbols overlap). Comparing the angle of each individual point with the angle of the appropriate category of variable Day, one can see that even at the individual level the different ages are clearly differentiated, particularly during the first three days of life. The overlapping between Days 3 and 4 is, however, small. More overlapping is present between Days 4 and 5. There are no significant between-sex differences (Wilcoxon test: Z=-0.40, P=0.69 for Dimension 1, and Z=0.64, P=0.52 for Dimension 2). The between-day differences of cosine of the angles connecting each individual point to the origin is highly significant (Kruskal-Wallis ANOVA: $\chi^2_4 = 63.8$, $P < 0.0001$).

Our simple study shows that it is possible to reliably age fallow deer neonates during their first five days of life using a set of easy-to-collect variables. Our sample size was too small to extend our analysis further (for instance to compute probabilities of correct classification), but we believe that the bias introduced by systematic ageing errors, in future analysis of fallow deer demography under field conditions, will be very limited. Interestingly, this classification is valid for both sexes. As it happens for many qualitative traits, there is some degree of subjectivity in their definition, and it is clearly necessary that the members of the capture team standardise procedures of data collection.

On Day 1 fawn age is clearly identifiable (Pélabon 1995). In field studies performed in enclosures or in very open landscape (e.g. Birgersson et al. 1998, Mc Elligott et al. 2002), it is often possible to detect and capture fawns during their first day of life, when they can be aged 'by eye'. The importance of having a suitable ageing methodology for fallow deer fawns was clearly evidenced by San José et al. (1999) who could not capture all fawns on their first day of life as they were working in a wild area. At Castelporziano, which is characterised by dense woods, only 9.2% (N = 76) of fawns were captured on their first day of life, so a reliable age calibration is necessary (A. Galli & S. Focardi, pers. obs.).

According to Lent's (1974) classification, fallow deer is a species (e.g. like red deer *Cervus elaphus*) with a quite reduced hiding period. From an evolutionary point of view (Fisher et al. 2002) this trait is well explained by the preference of this cervid for relatively open habitats and by the tendency to form large groups (Focardi & Pecchioli 2005). Because does exhibit intermediate body size (45-50 kg) and give birth to one fawn only, neonates are born relatively large and in few days they can attain a size which allows them to follow their mothers. When fawns join a group they may attain a certain degree of protection against predators.

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