## Understanding invaders: how easy indices of fecundity and body condition may aid management

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Most of invasive alien species (IAS) need to be controlled to avoid or reduce damage on native species and ecosystems, agriculture and livestock, human health and infrastructures. Since IAS population growth rate may vary across sites depending on intrinsic and habitat characteristics, investigating population dynamics parameters specific to the area of intervention is important to precisely quantify impacts of IAS and plan effective control strategies. In particular, knowledge of the reproductive output is essential for the definition of adequate harvesting quotas, while measures of body condition may help to pinpoint populations that are thriving and identify factors promoting invasiveness, thus allowing for the definition of priority areas or periods. Unfortunately, most methods commonly used to investigate reproductive output (e.g. capture-mark-recapture) and body condition (e.g. body mass-length ratios) in mammals are often time consuming and/or imprecise. Here we propose two less known methods that may be easily used on carcasses obtained from control campaigns to gain information on IAS fecundity and body condition: uterine scars counts and bone marrow fat proportion. To evaluate the feasibility and reliability of these techniques, we made use of carcasses of two invasive tree squirrel species introduced to Italy, Eastern grey squirrels (Sciurus carolinensis) and Pallas's squirrels (Callosciurus erythraeus), that were collected between 2013 and 2014 within a European Community LIFE project (LIFE09 NAT/IT/00095 EC-SQUARE).

To determine fecundity (n. of youngs weaned/year) of female squirrels we adapted a staining technique that allows to distinguish the number of scars left at birth by the detachment of each embryo's placenta from the endometrium. We collected uteri from 44 adult grey squirrels and 31 adult Pallas's squirrels trapped at the end of the last breeding season (late autumnearly winter) and stored them at -20 °C in clean tap water until later examination. Each uterus was then opened over its entire length and stained by the Turnbull reaction, which reveals dark pigments of macrophages involved in tissue repair processes. Scars were examined under a microscope within one hour from staining and classified based on their coloration, with lightstained scars indicating earlier litters and darker scars more recent parturitions. Analysis of uterine scars revealed that grey squirrels produced a maximum of two litters/year, with total fecundity between 0 and 8 youngs/year and an average of 3.4 scars/female. Number of uterine scars differed between breeding seasons, with females that reproduced in both seasons producing on average a larger summer than spring litter. Pallas's squirrels produced up to 3 litters/year, with total fecundity between 0 and 9 youngs/year and an average value of 3.1 scars/female. In this species, fecundity was positively affected by female body mass, foot length and age. Overall, our data show that fecundity of both squirrel species in the introduction range is comparable or even higher than values reported for their native ranges.

Body condition was evaluated on 58 grey squirrels of both sexes by determining the proportion of fat contained in femoral bone marrow, a parameter that had been previously applied on ungulates where it is considered a good index of body condition. Bone marrow was extracted directly from frozen femurs, weighed to nearest mg and then dried at 60 °C for 24 h. After drying, each sample was weighed again and the percentage of fat contained in bone marrow was obtained based on the ratio between dry and wet sample weight. Proportion of fat in femoral bone marrow of grey squirrels ranged between 21% and 87%, it significantly increased with body weight and was affected by season, reaching maximum values in spring and a minimum peak during autumn. Statistical analysis showed that, compared to body weight, bone marrow proportion has both a higher coefficient of variation and a stronger relationship with biological and biometric variables that may influence individual nutritional condition, indicating that this measure represents a reliable parameter to define squirrel's body condition.

Overall, our results indicate that uterine scars counts and bone marrow fat content offer good estimates of fecundity and body condition in squirrels. As a consequence, they represent two practical and cost-effective methods that may help to improve impact assessment and management strategies of IAS by providing key data for estimating population growth rates essential to define establishment likelihood and control measures.