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Short communication

Fat reserve assessment in Pyrenean chamois using body measurements

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ABSTRACT

Body condition is a measure of the energetic reserves stored in tissues and organs of animals in anticipation of periods of special energetic demands or to attenuate food shortages. For more than half a century, the kidney fat index (KFI) has been the most common invasive method for assessing body condition in ungulates. Since KFI requires animal necropsy, other non-invasive indicators based on body measurements have been suggested to assess body condition of mammals. These include the residuals from an ordinary least squares regression of body mass and linear measure of size (OLSr), the scores from a Principal Components Analysis on body measurements (PCAsc), and the scaled mass index (SMI). These indices, however, are often difficult to interpret and little effort has been made to confirm whether they are related to direct measures of fat reserves. We used the Bland-Altman method and linear models applied to biometric data to explore whether these three biometric indices can be used to assess body condition in 94 adult male and female Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*). Animals were hunter-harvested in the Eastern Pyrenees (Spain) during two contrasting periods of food availability. We found that OLS residuals from the regression between body weight and hind foot length were the best proxy for fat reserves in both periods of the year. This simple, low cost and non-invasive biometric indicator can be used for monitoring body condition of chamois populations and probably in other ungulate species with similar life strategies.

Mammals require energy to support the costs of basal metabolism and other biological activities including growth, reproduction and health maintenance. This energy can be stored in tissues and organs, increasing the weight of the animal. The aspect or weight of these reserves is generally considered by wildlife biologists to describe "body condition" in a broad number of animal species, including mammals (Stevenson and Woods, 2006).

Because of their wide distribution and their economic and recreational value, body condition of ungulates is commonly assessed in population management plans (Putman et al., 2011). Ungulates typically store energy in anticipation of periods of special energetic demands (e.g., mating season, migration, convalescence) or in anticipation of food shortage (Parker et al., 2009). This fact has a special relevance for population management plans since body condition integrates the response of herbivores to both temporal and spatial environmental heterogeneity. In practice, body condition can be considered a good indicator of ecological change and an indirect measure of habitat quality (Taillon et al., 2011). Numerous works have addressed the relationship between body condition and life history traits of ungulates, namely reproductive success (Parker et al., 2009), infection susceptibility (Beldomenico and Begon, 2010), and lifespan (Gaillard et al., 2003). On the other hand, population (e.g., density and interspecific interactions) and environmental factors (e.g., habitat quality and climate conditions) have a significant influence on the ability of individuals to store body reserves (Mautz, 1978; Parker et al., 2009). As a result, more than a hundred works about body condition assessment in 39 ungulate species have been published in the last eight decades (Serrano et al., 2008).

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In ungulates, invasive methods to evaluate body condition are the most common. During fasting, fat reserves are generally used sequentially starting with the burning up of subcutaneous deposits, followed by the mesenteric and kidney fat and finally bone marrow fat (Russel et al., 1968). This observation motivated the use of the weight of perirenal fat divided by the weight of the kidney (i.e., Kidney Fat Index, KFI, *sensu* Riney, 1955 and revised in Serrano et al., 2008) as an indicator of total fat reserves (Finger et al., 1981). This index is still being used in many ungulate species (e.g., López-Olvera et al., 2015; Santos et al., 2013; Simard et al., 2014).

Due to the latent limitations of KFI for monitoring body conditions in living animals, researchers have developed other non-invasive biochemical, physiological, and morphological indicators to evaluate the body condition of wildlife (e.g., Brown, 1996; Dobson, 1992; Stevenson and Woods, 2006). Among these non-destructive indices, the residuals from an ordinary least squares regression (OLS) of body mass and a linear measure of size is one of the more commonly used (Green, 2001; Schulte-Hostedde et al., 2001). In practice, an individual with positive residuals is considered to be in better physical condition (more absolute fat stores) than an individual with a negative residual (Schulte-Hostedde et al., 2005).

However, important assumptions must be considered before the broad implementation of OLS in wildlife to limit type I (e.g., rejection of the a true relationship between body condition and the selected life-history trait) and type II (acceptance of a spurious relationship between body condition and the selected life-history trait) errors (Green, 2001). These assumptions have been summarized in six points. The first is that the relationship between body mass and body measurement (Body Size Indicator, BSI) should be linear. Actually the decision to use linear regressions relies on the supposition that mass increases linearly with BSI length (following any transformations). On the other hand, (2nd assumption) that energy stores are independent of BSI length, that is to say small and big individuals have the same chance of being in good condition. These assumptions are not mentioned, tested or simply violated in most studies according to Green (2001). The third assumption is that BSI length must be an accurate measure of overall structural size, in other words high correlation with the BSI and other body measures summarizing structural size of the individual. Low correlations between BSI length and true size will result in low association between the residuals and true condition of the animal. Moreover, there should be no correlation between the size of BSI corrected by other structural components and the life history trait liked to true condition (fourth assumption). The fifth assumption indicates that BSI length should be strictly independent of body mass; and 6) BSI length is not subject to measurement errors. Violations of these assumptions are common, resulting in a poor relationship between OLS residuals and more direct measures of condition, such as the KFI. Hence, there is a serious danger of misinterpretation of ecological patterns when using OLS without care (Peig and Green, 2009).

One solution is the application of principal components analysis (PCA) aimed at summarizing the systematic patterns of variations in the data, in our case between body mass and chamois biometry (structural size *sensu* Green, 2001). Hence, a PC1 from a PCA of many linear measures (including body weight) can be considered a good indicator of the structural size of the animal. The PC1 scores can be a valid option to assess body condition as long as these variables are not subject to size correction themselves (Berner, 2011). The Scaled mass index (SMI) is another method recently proposed by Peig and Green (2009) that overcomes much of the limitations previously mentioned. This index is based on the central principle of scaling and is easily compared between different populations and is useful in studies with low sample sizes (Peig and Green, 2009).

In this work, we evaluated the uses of these three well-known and non-invasive methods of body condition assessment: the OLS residuals of body mass and tarsus length (OLS residuals, Toïgo et al., 2006; Zannèse et al., 2006), the scores of a Principal Components Analysis (PCAsc, Taillon et al., 2011), and the Scaled mass index (SMI, Peig and Green, 2010, 2009), to assess the kidney fat reserves (KFR, Serrano et al., 2011a, 2008) in the Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*). Kidney fat indices have positively been related to real fat content in other ungulates (Cook et al., 2005) and hence haven considered as proxy for body condition along this work.

Of the three methods, only the SMI has not been used as a proxy for body condition in ungulates but this index has been applied in other mammals, such as the eastern grey kangaroo (*Macropus giganteus*), the American beaver (*Castor canadensis*) and the deer mouse (*Peromyscus maniculatus*) (Gélin et al., 2016; Rodríguez-Estival et al., 2016; Severud et al., 2013).

The Pyrenean chamois is a medium-sized mountain ungulate inhabiting high seasonal environments. As in other mountain herbivore species, chamois are forced to spend most of their time grazing (hyperphagia) during the warm season in anticipation of food shortages in winter (Bruno and Lovari, 1989). This behaviour results in gains and losses in diet quality (Gálvez-Cerón et al., 2013), body weight (Garel et al., 2009) and hence, fat reserves (Pérez-Barberia et al., 1998), making this mammal an excellent model to explore the suitability of non-invasive indices of body condition.

During a 4-year sampling period (2012–2015), 94 adult Pyrenean chamois (58 females and 36 males), were shot-harvested in the Freser-Setcases National Game Reserve (FSNGR), Eastern Pyrenees, Spain (4° 21′ N, 2° 09′ E). Harvesting took place during two periods of contrasting food availability: March to April (spring) and October to December (autumn). These periods coincide with the vegetation onset (green-up period) and senescence (senescence greenness, according to Villamuelas et al., 2016) in this Alpine ecosystem. The FSNGR is a typical sub-humid alpine region covering an area of 20,200 Ha ranging from 1800 to 2,910 m.a.s.l. Summers are mild and dry, and winters cold, with an average annual temperature of 6.05 °C (range from −16.8 °C to 39.2 °C) and mean yearly accumulated rainfall of 963.38 mm (range from 520.6 mm to 1,324.8 mm; data from the Nuria station at 1,971 m.a.s.l. in the core FSNGR, Servei Meteorològic de Catalunya; www.meteocat.com). The population of chamois in the FSNGR has been estimated by the Reserve personnel to be approximately 3200 individuals.

After culling, sex was assigned by visual inspection and age assessed by counting horn segments (Corlatti et al., 2015). Before necropsy, basic biometry including the total length (Tl, Fig. 1A), wither height (Wh), and heart girth (Hg) were measured to the nearest 0.5 cm with a nylon tape measure. The hind foot length (Hfl) was also measured using a specific calliper (Garel et al., 2010). Subsequently, chamois were weighed with a spring scale (50 kg Weston scale 300-01) to the nearest 0.1 kg. Finally, the two kidneys with the surrounding fat were removed and stored in labelled plastic bags and kept refrigerated in a cooler box (4 °C). On arrival at the laboratory, kidneys were blotted dry with a paper towel and the fat surrounding both kidneys was removed by peeling away the peritoneal membrane. Finally, fat-free kidney mass and the associated fat were then weighed with a digital balance scale (5000 g Mechsonic 0.1 US1) to the nearest 0.1 g.

Different approaches were used to evaluate the suitability of OLS residuals, SMI and PCAsc to assess fat reserves of chamois. In a first step, we averaged the weight of kidneys without fat (KM) and the surrounding fat (KF). Later we estimated the kidney fat reserves (KFR), as the residuals from a linear regression between log-KF (response variable), according to Serrano et al. (2008). Sex was initially included as a covariate in the regression model to avoid