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How to continue measuring horn growth after capture in Alpine ibex

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ABSTRACT

Accurate measurement of secondary sexual characters are fundamental for understanding life history strategies and mating systems. Ibex horns grow continuously through life and thus provide insights on individual life history. Horn growth can also reflect changes in environment and resources availability. Horn length is usually measured by hand on skulls of dead animals during live captures or with the use of two parallel lasers with a known distance pointed on the horns of the animal. Here we propose a simple method to measure horn growth of marked Alpine ibex (*Capra ibex*) in the years following capture. We took pictures of marked animals when the side of the horn was perpendicular to the lens of the camera and we analysed pictures using the 4^{th} annulus measured during captures as a reference unit to estimate the length of the other annuli. We compared the annuli length estimated with this method with the length of the same annuli measured by hand. Our results show that the error of the estimate is very small and that there is a high repeat of annulus length estimate using pictures.

Keywords: remote measurement, secondary sexual traits, *Capra ibex*, long-term studies

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INTRODUCTION

The measure of morphological traits is a fundamental tool for both theoretical and applied researches on wild populations. Evolution and differences in morphological traits are often used in evolutionary studies (Stearns, 1992; Moller and Pomiankowski, 1993; Dearborn and Ryan, 2002; Stephenson and Ramirez-Bautista, 2012) but they are also useful for understanding status and health of populations (Festa-Bianchet, 2003; Rughetti and Festa-Bianchet, 2011, Pélabon and van Breukelen, 1998). Particularly, secondary sexual traits (SST) are broadly used in wildlife evolutionary studies (Simmons and Scheepers, 1997; Yezerinac and Weatherhead, 1997; Mysterud *et al.*, 2005; Clements *et al.*, 2010). SST are costly to develop and to maintain (Zahavi, 1975; Reznick *et al.*, 2000) and they are under strong sexual selection. They thus present high intra- and inter-population variability and are used in evolutionary studies and to better understand mating systems (Reznick *et al.*, 2000; Ditchkoff *et al.*, 2001; Bergeron *et al.*, 2008; Vanpè *et al.*, 2007). For the same reasons SST can be affected by changes in resource availability and may offer important indications on the status of the population (Perez *et al.*, 2011). Most of these studies are based on repeated surveys that can detect changes in the populations over time (Ozgul *et al.*, 2009; Mysterud *et al.*, 2005; Coltman *et al.*, 2003). In ungulates, horns and antlers are often considered among the most important SST. Ibex horns grow continuously through life and thus provide a unique opportunity to understand individual life history; they are also considered an honest signal of individual quality (Bergeron *et al.*, 2008, 2010; von Hardenberg *et al.*, 2007). It is not easy, however, to monitor horn growth (Sarasa *et al.*, 2012): the main method used is to measure horns directly on dead animals or during live-captures (Toigo *et al.*, 1997; von Hardenberg *et al.*, 2004). This method, however, is not completely satisfactory: dead animals are often difficult to find and to identify. Live-captures on the other hand are often expensive, time consuming and in some case the methods used to capture the animal can involve some risk for its safety (Bassano *et al.*, 2004; Pelletier *et al.*, 2004). For this reason animals are usually captured only once for marking so that it is difficult to monitor life-long horn growth. In recent years methods to measure life history traits without handling animals have been proposed. Bergeron (2007) proposed a new technique to measure horn growth of live animals without handling: two parallel lasers with known distance are pointed to the horn of animals as reference unit and then horn length is calculated from digital pictures. This method, however, is difficult to apply in field conditions because it requires perfect parallelism of the lasers and also minor changes in instrument precision can lead to large errors.

Here we propose a new and fairly simple method to estimate yearly horn growth of marked Alpine ibex over the years following capture. We present results obtained testing the method both in controlled conditions and in natural conditions using hand measures obtained during live-captures and pictures of live animals to test the method and its effective applicability in the field.

STUDY AREA

The study was conducted during summer 2011 and spring 2012 in the area of Levionaz, Valsavaranche valley, Gran Paradiso National Park (North-western Italian Alps; 45° 25′ N, 07° 34′ W). The study area is above the tree line, between 2,300 and 3,500 m a.s.l. and is characterised by high-altitude alpine meadows (mainly *Festuca varia*), moraines, rock cliffs and glaciers. In the study site most ibex are captured and individually marked with coloured plastic ear tags (Allflex®: Allflex Europe (UK), 77 Greenchurch Street, London) in the framework of a long-term study on the life history of the species (Bassano *et al.* 2003; Grignolio *et al.* 2004; Bergeron *et al.* 2010). In 2011 in the Levionaz area there were 60 male ibex, 46 of which marked (76.7%), while in 2012 there were 65 males, 51 of which marked (78.5%).

METHODS

We took pictures of horns of marked animals for which we already had hand measures of the first few annuli which are used as reference units to estimate the size of other annuli (Figure 1). We chose the 4th annulus measured by hand as the reference unit; as a matter of fact, most animals are captured at this age or older, therefore we used this measure for all the marked animals. Moreover, this is the first well evident annulus which is not affected by horn curvature nor by the risk of wrong measurement. After estimating all annuli's length, we tested the method comparing the estimated and hand-measured lengths. We tested the method on pictures of skulls and of free-ranging animals.

Hand measurement

For both skulls and live-captured animals we measured annuli using a calliper to the nearest 0.5 mm along a central line on the side of the horn.

Pictures

We used different common digital cameras (Kodak Easy Share DX6490, Canon EOS3D; focal: Sigma 70–300 mm) to take pictures of skulls and marked male Alpine ibex.

Skulls

We took two series of pictures of both horns of 6 skulls. The first series of pictures was taken with the skulls in horizontal position (respecting the natural angle of the horn when the animal is in 'upright' position). The second series of pictures was taken with the horn perpendicular to the lens of the camera.

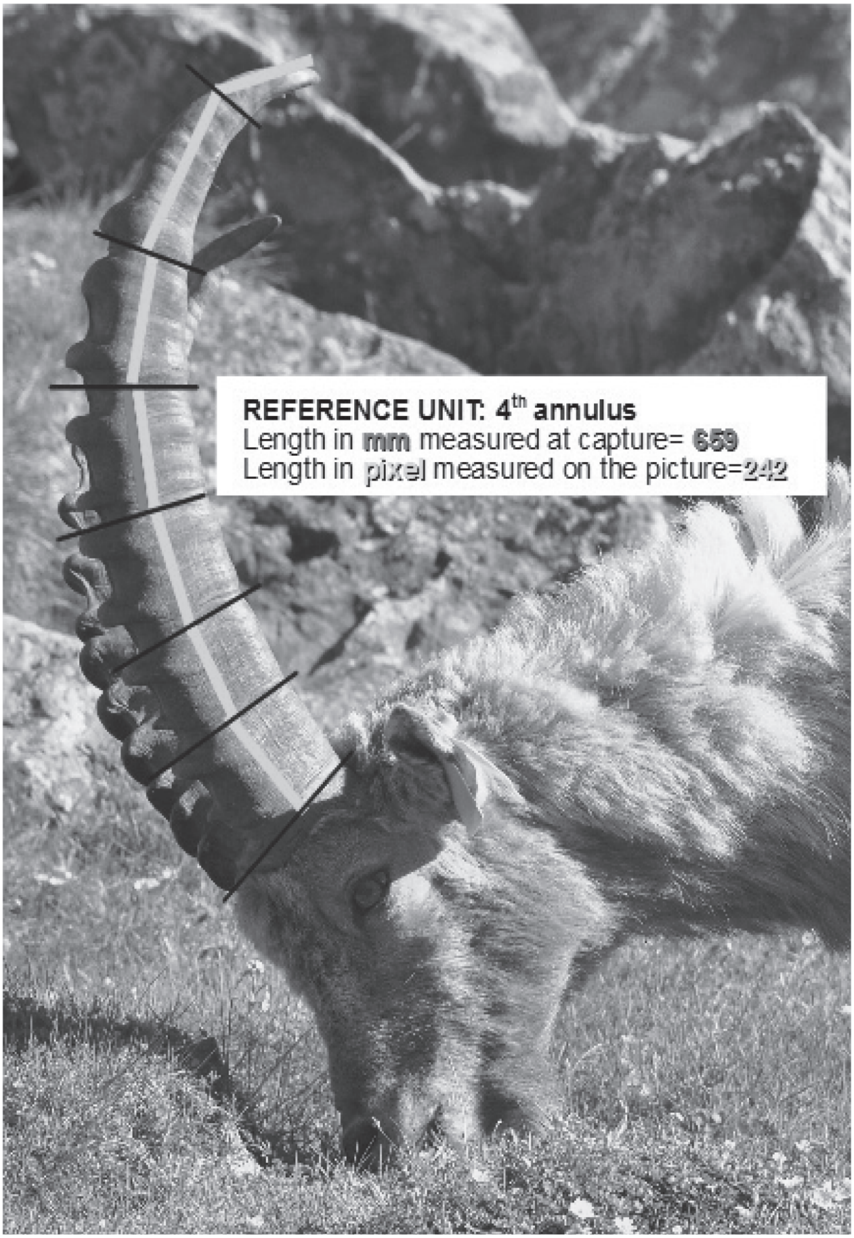

Figure 1 A marked Alpine ibex showing the 4th annulus measured by hand (in mm) and on the picture (in pixel) and used as reference unit to estimate the length of subsequent annuli.

Live animals

We approached ibex from the side and to a distance of up to 50 m trying not to disturb them. We took several pictures of both sides of the animals trying to have the horn perpendicular to the camera (we took pictures of 24 live animals).

Annuli estimates

We uploaded the pictures in Paint (Microsoft Windows® version 6.0) and measured the length, in pixels, of each annulus along a central line on the side of the horn (Figure 1). We then estimated the length in cm of each annulus using the known length of the 4^{th} annulus measured by hand during capture using the following formula:

$$\text{length } N^{th} \text{ annulus (mm)} = \frac{\text{length } N^{th} \text{ annulus (pixel)} \times \text{length } 4^{th} \text{ annulus mm}}{\text{length } 4^{th} \text{ annulus (pixel)}}$$

Obtained from this proportion:

$$\text{length } 4^{th} \text{ annulus (pixel)} : \text{length } 4^{th} \text{ annulus (mm)} = \text{length } N^{th} \text{ annulus (pixel)} : X$$

where X is the length in cm of the N^{th} annulus.

STATISTICAL ANALYSIS

We used R version 2.1.0 for the repeatability analysis (R Development Core Team, 2011). To test this method, i.e. to compare estimated annuli with hand measured annuli, we used linear regression forced through the origin. We first ran the analysis using pictures and hand measures taken on skulls. We tested separately the two series of pictures, perpendicular and slightly non-perpendicular, to see if there were differences in annuli estimates accuracy between them (as pictures taken in the field usually are usually non-perfectly perpendicular). We then ran the analysis using live-captures hand measures and pictures taken in natural conditions. For this analysis we did not consider the first two annuli because they were often broken or worn and thus difficult to identify from pictures. We also excluded from the analysis the 4th annuli because, as they were used as the reference unit, the estimate is equal to the measured length and thus they would fictitiously have improved the results. After testing the method we tested repeatability of estimates from different pictures of the same animals using ANOVA (Nakagawa and Schielzeth, 2010).

RESULTS

Skull estimates

The comparison between hand- and picture-measured annuli clearly showed a strong correlation suggesting that the estimates are unbiased compared to hand measures (Figure 2). We obtained very similar results both for Perpendicular ($N = 61$; $\beta = 0.993$; S.E. = 0.004; $p < 0.001$; $R^2 = 0.999$) (Figure 2a) and Upright pictures ($N = 61$; $\beta = 1.026$; S.E. = 0.013; $p < 0.001$; $R^2 = 0.990$) (Figure 2b).

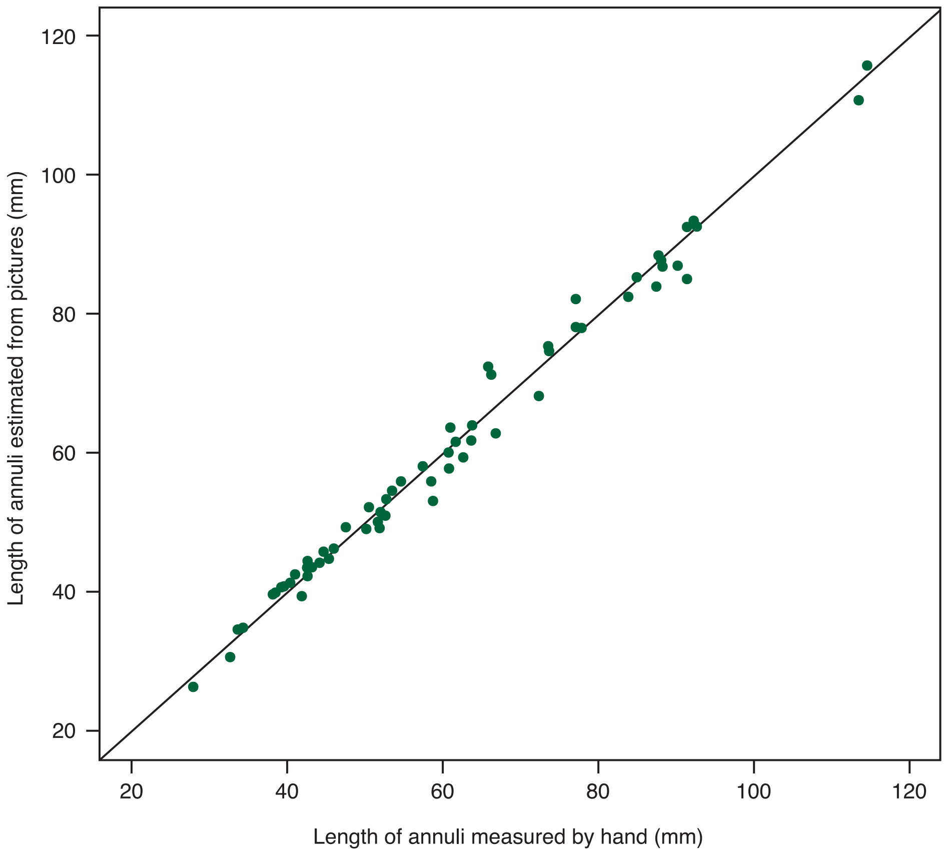

Figure 2a Perpendicular Pictures of Skulls: comparison between hand measured annuli and pictures measured annuli (in mm) by means of regression forced through the origin (picture length~real length)

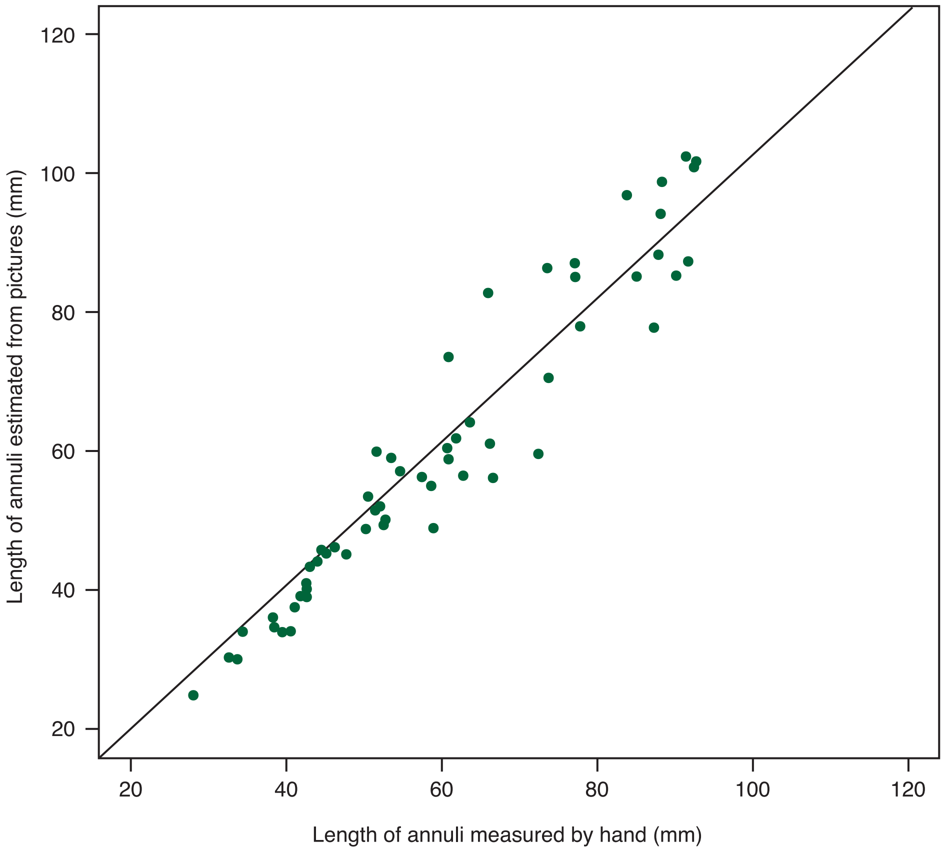

Figure 2b Upright Pictures of Skulls: comparison between hand measured annuli and pictures measured annuli (in mm) by means of regression forced through the origin (picture length~real length)

Live animals estimates

The comparison between hand-measured and pictures-measured annuli gave results similar to the skulls estimates even if the precision of the estimates was slightly lower.

($N = 654$; $\beta = 0.964$; S.E. = 0.006; $p < 0.001$; $R^2 = 0.973$) (Figure 3).

Repeatability between different pictures of the same animal

The repeatability of the annuli estimated from different pictures of the same animal was very high: (R = 0.924; S.E. = 0.006; C.I. = 0.913–0.935; $p < 0.001$).

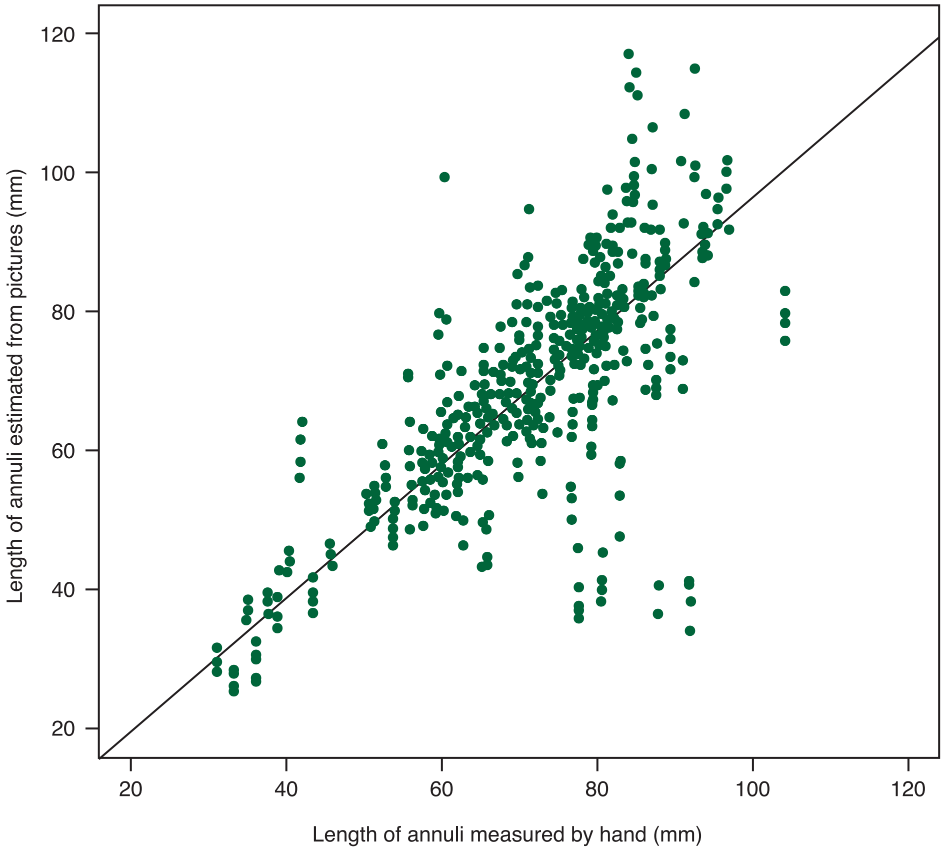

Figure 3 Live Animals pictures: comparison between hand measured annuli and pictures measured annuli (in mm) by regression forced through the origin (picture length~real length)

DISCUSSION AND CONCLUSIONS

Measuring secondary sexual traits is fundamental for understanding the mating system, life history strategy and ecology of populations of wild species (Bergeron *et al.*, 2008; Clements *et al.*, 2010; Stephenson and Ramirez-Bautista, 2012). In the current scenario of environmental changes, moreover, they can represent a useful signal to predict possible variations in resource availability (Ozgul *et al.*, 2009). Since SST are not always easy to measure, we propose a simple method to continue measuring horn growth of animals after captures. Our results showed that the method provides very accurate estimates of yearly horn growth. Perpendicular and non-perpendicular pictures in laboratory conditions gave similar results and provide evidence that slight variations from orthogonality in pictures can be tolerated without making the estimates worse. Pictures and estimates from pictures of free ranging animals also gave satisfactory results, especially if we consider that measurement during live captures are often taken in critical situations, by different operators, and that pictures estimates are done along time after capture, without the chance to have the horn close enough to see the exact partition of the different yearly horn growth. The method could be used also for measuring horns of other ungulates that have a shape similar to Alpine ibex horns (e.g. *Capra* genus: *Capra nubiana*, *Capra sibirica*, *Capra aegagrus*). It would be very useful as well to improve the method for species whose horns have a different shape (e.g. *Ovis musimon*, *Ovis canadensis*) inserting some geometrical adjustment for inclination and curvature of the horns. Another development of the technique could be to use horn length as a reference unit to estimate dimension of other parts of the body (i.e. leg length, total height, etc.).

This technique is suitable only for marked animals that have already been measured once but it has the advantage that it is extremely cheap and it does not require further effort since it is possible to also use pictures taken for other purposes. A way to improve the results can thus be to select only the pictures in which the animal looks perpendicular to the camera, for this study indeed we used all the pictures showing the side of the horn even if they were not perfectly perpendicular. The principal advantages of this method, compared to hand-measurement and to the parallel lasers method, are that it allows to continue collecting data that otherwise would be lost (such as horn growth in the years after capture), it is extremely cheap, easy to apply and it does not disturb the animals. This method, together with other remote measurement techniques such as scales baited with salt (Bassano *et al.*, 2003), behavioral observations (Grignolio *et al.*, 2004) and faecal samples collection (von Hardenberg, 2005), allows researchers to best exploit the big effort made for marking animals, collecting data during the whole life time of the organisms and for providing a very useful long-term series of data.

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