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A first survey on hair cortisol of an Alpine ibex (*Capra ibex ibex*) population

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Biometric measurements of horn, body and physiological variables of 35 Alpine ibex, *Capra ibex ibex*, (14 females and 21 males, age range: 2-15 years) were analysed in relation to gender and area of origin. Moreover, for the first time hair cortisol concentration was evaluated in this species. The ibexes were live-captured in spring in the Maritime Alps Natural Park. All the horn and body morphometric measurements were significantly different between genders and a statistical difference was found between horn and body data considering the area of origin of ibexes, with the exception of horn circumference and height as well of body and hock length. Among the physiological variables, female ibex showed higher heart rate and glycemia, but tended to have lower respiratory rate than male. The mean hair cortisol concentration was 22.40±1.44 pg/mg (±SE). A different distribution was described for the male hair cortisol concentrations compared to that in females. Obtained data indicate that the geographical area where Alpine ibex lives could affect the endurance of an ibex population but further investigations are needed to understand if intra- and inter-specific competition and different environment could be the basis of the HPA axis activation. Hair cortisol evaluation together with biometric values could be an interesting way to obtain information on the adaptation that the animal attempts to reach in relation to environmental factors and pursuing organism homeostasis. This feature could be important in attempting to preserve wildlife biodiversity that also depends from the state of animal welfare.

KEY WORDS: allostatic load / Alps / ibex / biometry / hair cortisol

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General information on the ecology of the Alpine ibex (*Capra ibex ibex*) can be found in the literature [Couturier 1962, Mustoni *et al.* 2002, Parrini *et al.* 2009]. A number of authors have described the morphometric details of this specie particularly in some Swiss and French populations [Couturier 1962, Giacometti 1988, Giacometti *et al.* 1997, Lüps *et al.* 1986, Michallet *et al.* 1994, Ratti 1981, Zumbach and Lüps 1987] or in the Gran Paradiso Natural Park population [Bassano *et al.* 2003; Grignolio *et al.* 2003; Von Hardenberg *et al.* 2007]. Although the Park of the Maritime Alps is one of the areas where the largest populations of Alpine ibex are concentrated, biometric data are not available. Being the biodiversity conservation of the local species dependent from the general well-being of the population, biometric helps to monitor the health status of a population [Mattioli and De Marinis 2009]. Biometric studies allow to describe quantitatively physical characteristics, representing the central tendency and variability of different populations. The increase or decrease of the species density, changes in food availability due to climatic variations or errors in managerial planning, can be evaluated by the measurement of the body weight and the length of the hock or jaw. In addition, biometrics is aimed at defining the quality of the individual or a population in order to provide useful tools to understand the mechanisms that govern the dynamics and schedule it for the proper conservation and management [Mattioli and De Marinis 2009].

Along with biometric information on the body and horn, data regarding hair cortisol concentrations (HCC) (key hormone of the hypothalamic–pituitary–adrenal (HPA) axis), provide an assessment of the allostatic load in a population. Animals at freedom survive by responding with adaptive and appropriate changes in their behaviours and physiological variables and these changes are also related to the endocrine status [Lowry and Moore 2006]. Adrenal corticoids, mainly cortisol, elicit physiological adjustments that enable animals to tolerate stressful conditions [Christison and Johnson 1972].

Cortisol concentrations are routinely determined from blood [Santiago-Moreno *et al.* 2007, Casas-Diaz *et al.* 2008, Probo *et al.* 2011], faeces [Huzzey *et al.* 2012], urine [Ferranti *et al.* 2013], milk [Fukasawa *et al.* 2010] and saliva [González *et al.* 2010]. These methods provide a measurement of the cortisol concentration at a single point in time or within 12 hours [Palme *et al.* 1996]. Instead, hair cortisol analysis reflects cortisol secretion over longer periods of time and it has a complementary meaning to monitor the HPA axis and, therefore, it can be associated with chronic stress [Kalra *et al.* 2007; Moya *et al.* 2013]. Unlike previous methods, the analysis of cortisol in hair is unaffected by circadian variations in the hormone or by factors inducing short-term variations. Shavings and resampling of hair in the same area allow to monitor the cortisol concentrations for weeks or months by providing a “window into the past.” This permits to retrospectively examine cortisol production [Kirschbaum *et al.* 2009, Russell *et al.* 2012].

The collection of hair is simple and non-invasive, and the sample does not decompose like other body fluids or tissues [Balíková 2005]. Hair is a relatively stable medium known to incorporate hormones through passive diffusion from blood capillaries on the basement membrane during its active growth phase [Pragst and Balíková 2006]. In humans [Ito *et al.* 2005, Slominski *et al.* 2005, Slominski *et al.* 2006] and in guinea pigs [Keckeis *et al.* 2012] cortisol synthesis in melanocytes and keratinocytes has been described, but to our knowledge, there are no data concerning a local production of cortisol in ruminants. Cortisol measurement in hair samples has been already reported as a validated method in ruminants species [Comin *et al.* 2013, Peric *et al.* 2013, Battini *et al.* 2015] and non-ruminants [Comin *et al.* 2012b, Montillo *et al.* 2014, Peric *et al.* 2016]. To date no data concerning the assessment of cortisol in hair of Alpine ibex are available.

The aim of this study was to evaluate the activation of the HPA axis of Alpine ibex living in different areas by measuring HCC along with the recording and comparison of their morphometric, biometric and physiological variables giving the basis to monitor the condition of an animal population.

Material and methods

Study areas and animals

The Italian Alpine range was subdivided into 17 ibex management units (MUs) including areas with similar habitat characteristics; the lowest average MU extension is that of Maritime Alps (AMNP, Cuneo, Italy − 44°10′45″N, 7°18′18″E) with 930 km^2 with an estimated population size of 1500 ibexes [Duprè and Pedrotti 2001]. The Maritime Alps are at the South-West extremity of the main watershed of the Alpine chain. This sector lies between two important international passes the Colle di Tenda (1871m − 44°8′57″N 7°33′43″E) and the Colle della Maddalena (1996m − 44°25′18″N 6°53′55″E). Twinned with the French Mercantour National Park since 1987, it preserves an alpine area of 100,000 hectares. All six alpine ungulate species are found in the Maritime Alps Nature Park including chamois (*Rupicapra rupicapra*), ibex, roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and mouflon (*Ovis orientalis*).

A total of 35 ibexes (*Capra ibex ibex*) were sampled in the AMNP, before the animals migration between valley and high pastures. Ibexes of both genders (14 females and 21 males, age range: 2-15 years) were captured in 5 areas in which AMNP has been divided. The areas were: Valle Gesso della Valletta (Matto-Valasco) (A) (2 females and 9 males); Valle della Rovina (B) (4 females and 4 males); Valle Gesso della Barra (C) (4 females and 4 males); Moncolombo (D) (4 females and 4 males); Sabbione (E) (7 males) (Fig. 1). Ibexes captured in the last area (E) were only males, therefore they were not considered in the statistical analyses.

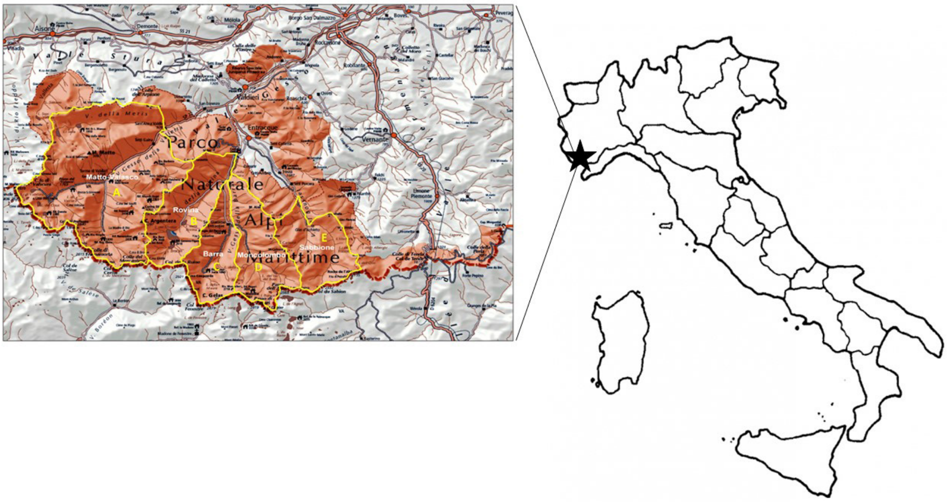

Fig. 1. Locations of the Alpine ibex (*Capra ibex ibex*) population inside the Maritime Alps Natural Park. Park was divided in 5 areas: A, Valle Gesso della Valletta (Matto-Valasco); B, Valle della Rovina; C, Valle Gesso della Barra; D, Moncolombo; E, Sabbione.

The main characteristics of the sampling areas are summarized in Table 1. Population densities were estimated on the basis of the results of the block count census (Management Committee of the Maritime Alps Natural Park). All the five areas are characterized by median population densities (Tab. 1) as defined by Mustoni *et al.* [2002] and by high mean altitudes, which are responsible for a mountain climate. In the Maritime Alps Natural Park, annual thermal excursion (difference between the average temperature in the warmer month and the average temperature in the colder one) is influenced primarily by geomorphology and varies from 21.2°C (low

Table 1. Characteristics of the five sampling areas (A, Valle Gesso della Valletta (Matto -Valasco); B, Valle della Rovina; C, Valle Gesso dellaBarra; D, Moncolombo; E, Sabbione) in terms of pre-reproductive ibexes density and altitude

Sampling area	Ibex density (ibex/km^2)[a]	Altitude (meters a.s.l.)		
		mean	min	max
A	4.62	2023.5	960.0	3087.0
B	8.87	2135.0	980.0	3290.0
C	6.60	2061.5	980.0	3143.0
D	3.93	2135.0	1200.0	3070.0
E	2.81	1997.0	1150.0	2844.0

[a] Data referred to pre-reproductive densities in 2014 (data provided by the Management Committee of the Maritime Alps Natural Park).

altitudes) to 20.9°C (alpine environment). Two rainfall regimes are described: the alpine (or continental) one, with one summer peak, and the sublitoral-alpine one, with two peaks (in spring and autumn). Snowiness and persistence of snow cover vary significantly with altitude, morphology, and exposure; furthermore, they vary from year to year. The permanence time of snow cover increases about 10 days/100 m of altitude. Substantially, in Cuneo Province we can identify three types of climate: (i) sub-alpine (cold season, lasting four months); (ii) alpine (above the tree line, with harsh winters, lasting six months); (iii) glacial (with temperatures under 0°C, almost exclusively snowy precipitations and almost absent vegetation). Climatic conditions and vegetation cover are consequently similar in the four areas, as a result of similar altitudinal ranges. The main tree and herbal species characterizing the different altitudinal levels are summarized in Table 2.

Table 2. Summary of main vegetal species at different altitudinal levels [Ferloni 2012]

Altitudinal level	Altitude range (m a.s.l.)	Tree species	Herbal species
Sub-mountain	500-1,000	*Fagus sylvatica* *Abies alba* *Larix decidua* *Picea abies* *Sorbus aucuparia* *Cytisus laburnum* *Calluna vulgaris*	*Trisetum flavescens* *Trifolium montanum* *Ranunculus montanus* *Campanula barbata* *Trolius europaeus*
Mountain	1,000-1,400	*Picea abies* *Larix decidua* *Vaccinium* spp. *Rhododendrum ferrugineum* *Rubus idaeus*	*Festuca ovina* *Melampyrum sylvaticum* *Campanula barbata* *Veronica officinalis* Mosses *(Hylocomium splendens, Rhytidiadelohus triquetus)*
Sub-alpine	1,400-1,800	*Larix decidua* *Pinus cembra* *Pinus montana var. mughus* *Picea abies* *Alnus viridis* *Juniperus communis var. nana*	*Festuca ovina capillata* *Nardus stricta* *Trifolium montanum* *Trifolium alpinum* *Carex* spp. *Juncus* spp.
Lower alpine	1,800-2,400	*Rhododendrum ferrugineum* *Vaccinium* spp. *Pinus montana var. mughus* *Alnus viridis*	*Nardus stricta* *Carex ferruginea* *Salix pentantra* *Salix purpurea*
Alpine	2,400-2,700	-	*Carex curvula* *Carex firma* *Carex elyna*
Nival	> 2,700	-	*Carex curvula* *Carex firma* *Carex elyna* *Saxsifraga panicolata* *Saxifraga aizoon* Mosses and Lichens

Moreover, in all the areas the anthropic disturbance is very mild due to tourism and no disturbance is given by domestic herds that reach the high pastures during the summer.

Supplementary food was never provided to the animals. Only wolfs as large predators on adult animals are present in the considered areas.

All animal handling was performed in accordance with the Italian legislation DL116/92 conforms to EU Directive 2010/63/EU for animal experiments.

Capture protocol

The ibexes were approached and anesthetized by Telinject® G.U.T. 50 (Telinject Inc. Remote Injection Equipment, CA, USA) with Rompun® (Xylazine) 10% from 1 to 1.6 ml according to gender and age of the animal. Once reached, the ibex was secured and positioned on the right decubitus to allow data collection and sampling. During manipulations, the eyes of the ibexes were covered to reduce stress and legs were restrained. At every capture, the geographical area in the Natural Park was identified by the GPS coordinates and the altitude was also recorded. To avoid the recapture of the same subject, the ibexes were marked with colored and number ear plates. At the end of samples collection, the ibex received, in relation to the body weight and the time elapsed from the capture, a dose (1 and 2 ml) of Antisedan® (Atipamezole), as the antagonist of Xylazine. Afterwards the ibex was freed and monitored until the awakening.

Horn measurements

To measure the horn, the anatomic reference points described by Mattioli and De Marinis [2009], were used. All measurements were carried out by the same person in order to avoid between-individual variations. From each ibex length and circumference measurements were seized by a tape rule, while the width measurements using a calibrated wooden caliper. The horn height was the distance between the skull and the tip on the vertical median; the tip-to-tip distance was the greatest distance between tips; the length was the distance from the lowest point in front (appoximately above the eye) to the tip; circumference was the measure at the base at right angles to the axis of the horn; spread was the distance between the lowest point of the base and the tip; curvature was the perpendicular distance between the horn spread and the widest point of the horn.

Body measurements

Morphometric values of the body were seized from each ibex. The measurements included total body length, as the distance from the most anterior point of the snout to the first coccygeal vertebra on the median body line; neck circumference, as the distance around the mid region of the neck, at the level of the third to fourth cervical vertebrae; mandible length, as the distance from the oral margin of the alveolus of the first incisor to the most aboral point of the angle of the mandible; left hock length, as distance from the tip of the calcaneus to the tip of the hoof, along the ventral surface of the hind limb [Mattioli and De Marinis 2009, Mustoni *et al.* 2002]. Also the animals' weight was recorded.

Physiological data

A set of physiological indicators were recorded from each ibex by stethoscope and glucose meter Lifescan OneTouch® (Johnson & Johnson Medical S.p.a., Pomezia, Italy). Heart rate, expressed as beats per minute; respiratory rate, expressed as number of breaths (inhalation − exhalation cycles) within one minute; glycemia, expressed in milligrams per deciliter (mg/dL).

Moreover, ibex health conditions and the presence of ectoparasites were evaluated. Since the seasonal operating time, pregnancy was confirmed in females by abdomen palpation.

Hair collection

Hair samples were carefully obtained from the left shoulder of ibexes using clippers. The hair samples were stored in dry tubes at room temperature until analysis.

Hair cortisol assay

Hair strands were washed in 5 ml isopropanol as proposed by Davenport *et al.* [2006] to minimize the risk of extracting cortisol from outside the hair and also to ensure the removal of any steroids on the surface of the hair due to sweat and sebum. Hair cortisol was extracted with 3 ml of methanol per 30 mg of hair for 18 h at 37°C. Samples were then centrifuged (15 min/1000 rpm) and the supernatant collected and transferred to a 12-mm glass test tube. The supernatant was dried at 37°C under a gentle stream of nitrogen gas and reconstituted with 0.3 ml of phosphate buffer. HCC were measured using a solid-phase microtiter RIA assay. In brief, a 96-well microtitre plate (OptiPlate, Perkin-Elmer Life Science, Boston, MA, USA) was coated with goat anti-rabbit γ-globulin serum (diluted 1:1000 in 0.15 mM sodium acetate buffer at pH 9) and incubated overnight at 4°C. The plate was then washed twice with RIA buffer (pH 7.5) and incubated overnight at 4°C with 200 µL of the anti-cortisol serum diluted 1:12,000. The rabbit anti-cortisol antibody was obtained from Biogenesis (Poole, UK). After washing the plate with RIA buffer, standards (5-300 pg/well), a quality control extract, the test extracts (10 mg), and tracer (Hydrocortisone (Cortisol, [1,2,6,7-3H (N)]-), Perkin-Elmer Life Sciences, Boston, MA, USA) were added, and the plate was incubated overnight at 4°C. Bound hormone was separated from free hormone by decanting and washing the wells in RIA buffer. After the addition of 200 µL/well scintillation cocktail (Microscint 20, Perkin- Elmer Life Sciences), the plate was counted using a beta-counter (Top-Count, Perkin-Elmer Life Sciences). Data were expressed in pg/mg.

Assay validation

To validate the method the sensitivity, specificity, precision, accuracy, and parallelisms were investigated. As the biological validation of the assay in a free-living wild species is particularly complicated, it was not possible to carry it out on ibex, and for this experiment, we referred to validation carried out in a domestic ungulate species [cow; Peric *et al.* 2013]. The assay sensitivity (defined as the hormone concentration producing a displacement of the labeled hormone at least 2 standard deviations from maximal binding) was 0.60 pg/well. The specificity of the method, estimated by calculating the percentage cross-reaction with different steroids, was: cortisol 100%, corticosterone 1.8%, and aldosterone <0.02%. The precision of the method estimated by repeatedly assaying samples in the same assay and in independent assays was expressed by intra-assay and inter-assay coefficients of variation (CV%) of hair sample. The intra- and inter-assay coefficients of variation were 5.7 and 9.8%, respectively.

To evaluate assay accuracy, possible interference of components within the extract with antibody binding was analysed through recovery of exogenous cortisol added to pooled ibex hair extracts. Each of four reconstituted hair extracts were divided in three independent aliquots and spiked with three different known cortisol concentrations, mixed, and assayed. The percentage of recovery was determined as follows: amount observed/amount expected × 100, where the amount observed is the value obtained from the spiked sample and the amount expected is the calculated amount of standard hormone added plus the amount of endogenous hormone in the unspiked sample. Recovery rate was 102.7±4.5% (mean ±SD). The measured hormone concentrations in the spiked samples correlated with the expected concentrations: r was 0.99 and the model was given by the equation $y = 1.030x - 0.222$. To determine the parallelism between cortisol standards and endogenous cortisol in ibex, hair samples containing high concentrations of endogenous cortisol were serially diluted in phosphate-buffered saline (PBS) 0.05 M, pH 7.5. The relationship between hair cortisol and standard cortisol curve determined through linear regression was linear: the correlation coefficient r was 0.99 and the model was given by the equation $y = 0.9347x + 2.58$.

Statistical analysis

The statistical analysis was performed using SPSS version 7.5.21 [1989-1997] and R software version 3.2.1, [R core team, 2015]. Morphologic variables were analysed by a model that includes age as covariate, gender and area of origin as fixed factors. The same model, without covariate, was used for the physiological variables. In these cases, the covariate was not considered because there was not a significant linear correlation between age and the variables [Pituch and Stevens 2016]. For variables which data violated the assumptions of analysis, robust analysis approaches were considered [lmRob function of the robustbase package; Finger 2010]. For multiple comparisons, the *P*-values were adjusted using the Ryan-Holm-Sidak procedure as suggested by Atkinson [2002] and Ludbrook [1998]. The *P*-values lower than 0.05 were considered significant, while *P*-values lower than 0.10 were considered as a tendency.

The probability density functions to assess the hormones distribution were obtained with the package fitdistrplus [Delignette-Muller and Dutang, 2015]. The density functions: normal, lognormal, logistic, exponential, and gamma were tested. The more appropriate density functions were selected on the bases of Akaike Information Criterion [AIC] and on Bayesian Information Criterion [BIC].

HCC classes were created on the basis of HCC values (5 classes: <15; 15-19.99; 20-24.99; 25-29.99; >29.99 pg/mg), in order to allow a better graphical representation of data.

Results and discussion

The estimated marginal means of horn biometric and body morphometric variables recorded in the ibexes in relation to the gender and the area of origin are reported in Table 3. As expected, all the horn biometric variables were greater in male than female (P<0.01). Considering the area of origin, ibexes from C area showed higher horn curvature and tip-to-tip distance than those from D area (P<0.05). Moreover, ibexes from C area tended to have greater horn spread and length than ibexes from D area (P<0.10). As expected, male ibexes had greater mandible length and neck circumference, hock length, weight and total body length than female (P<0.01). Considering the area of origin, animals from C area had greater neck circumference (P<0.01) and tended to have higher body weight than those from D area (P<0.10), which showed lower mandible length than animals from B area. Conversely body and hock length were not influenced by animals' area of origin (P>0.05).

Table 3. Estimated marginal means* of horn biometric and body morphometric variables in Alpine ibex (*Capra ibex ibex*)

Item	Gender		Area of origin				RRSE
	F	M	A	B	C	D	
Left horn spread (cm)	15.1^{A}	35.1^{B}	$25.4^{\alpha\beta}$	$24.1^{\alpha\beta}$	29.3^{β}	21.7^{α}	7.20
Left horn curvature (cm)	1.6^{A}	7.8^{B}	5.0^{AB}	4.0^{AB}	6.2^{B}	3.5^{A}	2.26
Left horn length (cm)	17.3^{A}	51.1^{B}	$35.2^{\alpha\beta}$	$32.7^{\alpha\beta}$	39.9^{β}	29.0^{α}	11.59
Left horn circumference (cm)	12.3^{A}	21.6^{B}	17.0	16.9	17.1	16.9	4.27
Horn height (cm)	14.1^{A}	33.7^{B}	24.3	23.3	26.4	21.7	6.85
Horn tip-to-tip distance (cm)	12.2^{A}	32.4^{B}	24.0^{ab}	20.0^{ab}	26.1^{b}	19.1^{a}	8.79
Mandible length (cm)	17.4^{a}	18.7^{b}	17.2^{ab}	18.7^{b}	18.3^{ab}	17.8^{a}	1.55
Neck circumference (cm)	32.1^{A}	44.1^{B}	39.1^{AB}	38.2^{AB}	39.7^{B}	35.6^{A}	5.95
Total body length (cm)	126.3^{A}	140.0^{B}	137.4	132.9	134.1	128.1	10.98
Left hock length (cm)	28.2^{A}	30.7^{B}	29.9	30.1	29.5	29.1	1.62
Weight (kg)	32.9^{A}	49.4^{B}	$42.0^{\alpha\beta}$	$43.5^{\alpha\beta}$	45.5^{β}	33.6^{α}	11.27

*Calculated considering age as covariate; F – female; M – male; RRSE – robust residual standard error.
Area of origin: A − Valle Gesso della Valletta (Matto-Valasco); B − Valle della Rovina; C − Valle Gesso della Barra; D – Moncolombo.
$^{AB}P<0.01$; $^{ab}P<0.05$; $^{\alpha\beta}P<0.10$.

The estimated marginal means of physiological variables recorded in the Alpine ibexes in relation to the gender and the area of origin are reported in Table 4.

Table 4. Estimated marginal means of physiological variables in Alpine ibex (*Capra ibex ibex*)

Item	Gender		Area of origin				RRSE
	F	M	A	B	C	D	
Heart rate (BPM)	75.1^{b}	60.7^{a}	81.7	64.5	65.3	60.3	16.44
Respiratory rate (number/min)	67.6$^{\alpha}$	88.0$^{\beta}$	66.6	79.8	84.0	80.8	29.48
Glycemia (mg/dL)	136.8^{b}	102.6^{a}	122.7	128.4	123.2	104.6	37.95
Hair cortisol, (pg/mg)	23.2	21.6	24.1	20.2	22.2	23.2	6.29

Area of origin: A – Valle Gesso della Valletta (Matto-Valasco); B –Valle della Rovina; C – Valle Gesso della Barra; D – Moncolombo.

Male ibexes had lower heart rate (P<0.05) and glycemia level (P<0.05), and tended to have higher respiratory rate (P<0.10) than female. An effect of the area of origin on these variables was not found (P>0.05).

The hair cortisol concentration was not affected by gender and animal's area of origin (P>0.05), while the average value recorded was 22.40 ±1.44 pg/mg (±SE).

Following both AIC and BIC criterium, the hair cortisol concentration frequency was described by a lognormal distribution in male (Fig. 2) and by a gamma distribution in female, however, in female, the normal, logistic and log-normal distribution are considered similar to gamma distribution since the ΔAIC and ΔBIC was lower than 2 [ΔAIC=0.44, Burnham and Anderson, 2010; ΔBIC=0.44, Kass and Raftery, 1995] (Fig. 3). Parameters regarding the two hair cortisol distributions are shown in Table 5.

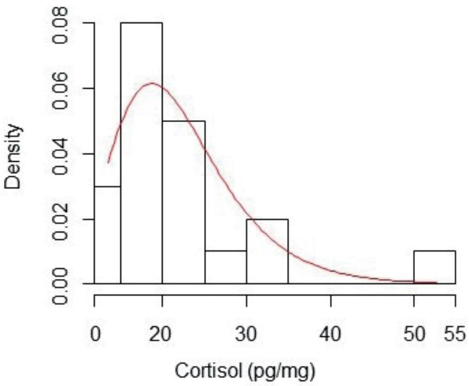

Fig. 2. Frequency distributions of hair cortisol concentrations in male ibexes with the line best-fitting the probability density curve (lognormal distribution).

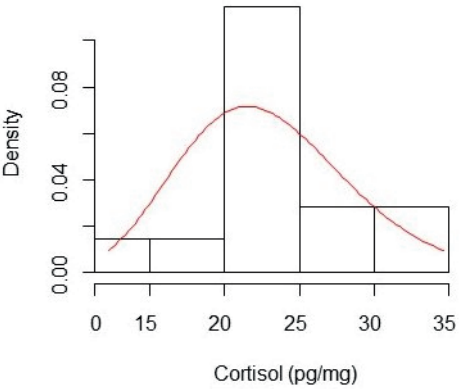

Fig. 3. Frequency distributions of hair cortisol concentrations in female ibexes with the line best-fitting the probability density curve (gamma distribution).

Table 5. Best-fitting probability density functions to hair cortisol distribution

Function	Parameter 1 (±se)	Parameter 2 (±se)	AIC	BIC
Male				
log-normal	mean: 3.04 (0.07)	sd: 0.33 (0.05)	137.66	139.66
gamma	shape: 8.36 (2.59)	rate: 0.38 (0.12)	140.49	142.48
logistic	location: 20.58 (1.58)	scale: 4.20 (0.82)	143.70	145.69
normal	mean: 22.12 (1.99)	sd: 8.92 (1.41)	148.27	150.27
exponential	rate: 0.045 (0.010)	-	165.85	166.84
Female				
gamma	shape: 16.12 (6.03)	rate: 0.70 (0.27)	91.99	93.27
normal	mean: 22.98 (1.50)	sd: 5.61 (1.06)	92.00	93.28
logistic	location: 22.76 (1.46)	scale: 3.15 (0.71)	92.12	93.40
log-normal	mean: 3.10 (0.07)	sd: 0.26 (0.05)	92.43	93.71
exponential	rate: 0.044 (0.012)	-	117.76	118.40

AIC – Akaike Information Criterion; BIC – Bayesian Information Criterion.

The percentages of ibexes in each HCC class are shown in Figure 4. All the HCC in ibexes from the D area belonged to HCC classes higher than 15.0 pg/mg.

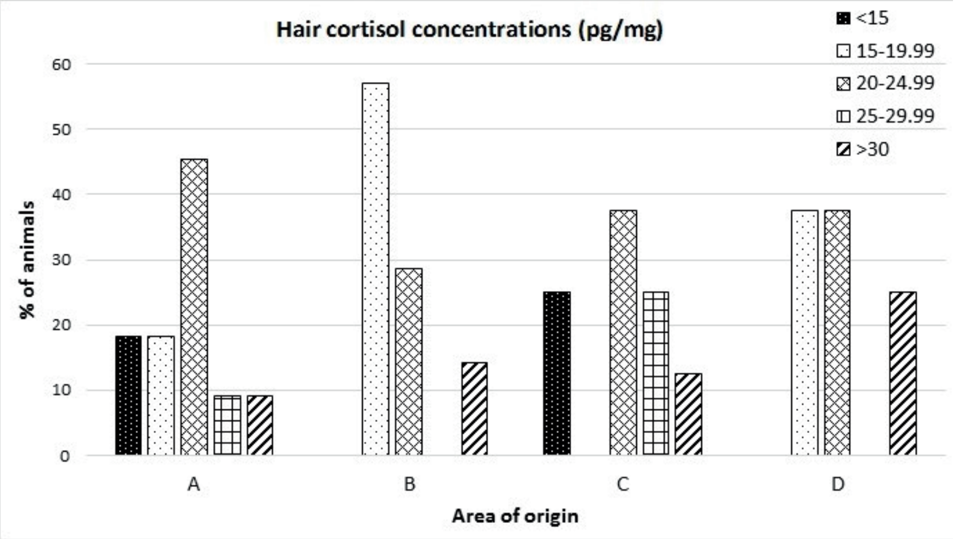

Fig. 4. Percentage of ibexes from each sampling area in each hair cortisol concentration class.

The present study provide biometric data from ibexes that are often caught to repopulate other areas in the Alps where the species was missing or was poorly represented. It is well known that the horn biometric evaluation is a valid index of the well-being of the individual, population and the environment as a whole [Mattioli and De Marinis 2009], moreover understanding the organization of animal in space is a fundamental issue in ecological studies [Scillitani *et al.* 2012]. Although the D and C areas are very similar in eco-climatic condition, the horn development could have been affected by the different behaviour that characterized the ibexes. Usually, flocks of male and female usually undertake long movements in search of high altitude pastures [Büntgen *et al.* 2014], however, as observed [L. Martinelli *personal comunication* 2015], some individuals of AMNP did not move and entire families showed a 'sedentary lifestyle'. This could entail that limited vegetative growth was available for ibexes, resulting in a food shortage that could have been reflected on the horn growth. Moreover, individuals that did not move to the South/South-West slopes during the winter could not start in feeding earlier on the newly growth vegetation. Thus, the renewal of horn in spring could be affected by the low available energy, which is saved for the reinstatement of metabolic reserves lost during the winter. Ibexes of the D area tended to have lower body weight than those of C area. As described by Mattioli and De Marinis [2009], the body size of Ungulate may be influenced by the population density. Even the competition with other ungulates, such as chamois, could cause an overlap of the diet and therefore a limitation of the trophic resources. It is usual to spot two species closely while sharing the pastures. Data concerning sexual dimorphism have been confirmed by body and horn measurements. It is well known that Alpine ibex is characterized by a remarkable sexual dimorphism, in contrast to other species that share the same mountain habitat as e.g. the Alpine chamois. Present data showed that body length of both genders were in agreement with the length data reported by Mustoni *et al.* [2002]: adult male about 130-160 cm, while the adult female 120-130 cm. Moreover, sexual dimorphism could be also related to neck circumference and the length of the left hock, as reported also in this study. Ibex males hold largest and stately horns, the data recorded in this study confirmed highly significant differences between genders for all the different horn variables. According to literature, males presented horn with greater spread, curvature, length, circumference, height and tip-to-tip distance than females [Mustoni *et al.* 2002].

The lowest heart rate was recorded, even if only numerically, in ibexes from the D area. Despite ibexes received the same treatment, anesthesia certainly affected the heart rate in term of absolute value. There are no studies describing the possible modifications based on gender in *Capra ibex ibex*; in human [Karason *et al.* 1999], cows [Hagen *et al.* 2005] and dogs [Lamb *et al.* 2010] lower body weight was linked with a slower heart rate. Moreover, the decrease in heart rate physiologically occurs when the physical activity decreases accordingly to basal metabolism [Sherwood *et al.* 2010]. From the present data, a statistically higher heart rate in females than in males was observed, which could be due to the pregnancy of females that led to an increase of this variable [Nagel *et al.* 2012]. Glycemia concentration was in accordance with the study in *Capra pyrenaica* [Casas-Diaz *et al.* 2008]. Females had higher glycemia values than males. The hormonal status in pregnant females may lead to an alteration of the glucose metabolic mechanisms.

To date, no data are available concerning the assessment of cortisol in hair of Alpine ibex. Casas-Diaz *et al.* [2008], Pérez *et al.* [2003], and Sartorelli *et al.*

[1997] determined blood cortisol concentration in *Capra pyrenaica* and *Capra ibex*. However, as described by Russel *et al.* [2012], this value cannot be compared with those obtained in hair because the blood cortisol concentration derives both from its circadian pulsatile secretion and from acute events as the capture and anaesthesia that cause anomalous values compared to the normal circadian rhythm. In hair, instead, cortisol concentrations are given by an accumulation over time of this molecule and can thereby provide a tool for assessing the HPA axis activity on the long term [Meyer and Novak 2012]. Being this the first study on HCC in Alpine ibex, the only possible comparison can be *interspecies* and, even if not *ceteris paribus*, this can be the only way to build up some considerations. HCC in *Capra ibex ibex* were in agreement with those found in calves by Comin *et al.* [2008], but they were markedly higher than values detected by Comin *et al.* [2011, 2012a, 2012c, 2013] in domestic animals. In cows and adult rabbits, the highest concentration were around 10 pg/mg, while in this study was, on average, around 22 pg/mg. Although the number of ibexes evaluated in this study did not allow to determine the physiological range of HCC characteristic for the species, a different activation of HPA axis could be claimed to explain the difference with domestic animals. Increased activation of the HPA axis may be induced by different factors such as predation pressure, human disturbance, males fighting in the breeding season and food scarcity in different seasons [Martin and Réale 2008, Romano *et al.* 2010]. HCC measured in ibex females were numerically higher than those recorded in males and had different frequency distribution. This is in agreement with data obtained in female polar bear hair (*Ursus maritimus*], which showed the highest cortisol concentrations in comparison to that of males [Bechshøft *et al.* 2011]. Moreover, all the ibex females were pregnant and this could have affect the concentration, it has been shown that HCC in pregnant women increased from the first quarter until the delivery and then decreased in the postpartum period [D'Anna-Hernandez *et al.* 2011]. A different allostatic load and thus an activation of the HPA axis influenced by the area of origin of animals has not been shown but still the HCC evaluation provided an interesting indication. The HPA axis activation, evaluable by the highest HCC, was higher in ibexes of the A and D area followed by C and B areas, even if only numerically. Moreover, examining Figure 4 it is possible to observe that all the ibexes (100%) from the D area belonged to HCC classes higher than 15.0 pg/mg. The 'sedentary lifestyle' of ibexes and the food shortage that could derive from this behaviour could influence both horn growth and animals weight as described above and could have induced a higher allostatic load in the area D. This could indicate that the highest HPA axis activation of ibexes in this area, to maintain of homeostasis in case of aversive stimuli, could have impaired animal growth, as described by literature [Smith and Vale 2006]. Life-history theory is rooted in the principle of energy allocation, which specifies that the amount of energy available for an individual is limited and has to be allocated either to growth, reproduction or survival. Since the amount of energy assigned to one of these functions cannot be used for another one [Cody 1966; Williams 1966, Charnov 1993, Roff 2002], trade-off should occur between fitness components [Stearns 1992]. This is remarked even more in wild populations because the energy intake in these animals are sometimes limited.

Morphometric, biometric, physiological data and the hair cortisol determination carried out in this study indicate that the geographical area where Alpine ibex lives could affect the endurance of the Alpine ibex population but further investigations are needed to understand in more detailed way if intra- and inter-specific competition and the different environment could be the basis of the activation of the HPA axis in the population of *Capra ibex ibex* in the Maritime Alps Natural Park.

Conclusions

The paper reports a complete biometric data set of Alpine ibex in different areas of Maritime Alps Natural Park. For the first time HCC was evaluated in this species and it could be an interesting way to obtain information on the adaptation that the organism attempts to reach in relation to environmental factors and pursuing organism homeostasis. The use of this method could help in attempting to preserve the wildlife biodiversity that also depends from the state of the animal welfare. The future application of these techniques could be further enhanced by the possibility to collect hair samples from free-ranging living ibexes using hair snares as described for the red deer by Belant *et al.* [2007].

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