Allegato 2a_2

Effects of capture by telenarcosis on male Alpine ibex

Francesca Brivio[1]

[1] University of Sassari, Department of Science for Nature and Environmental Resources, via Muroni 25, I-07100, Sassari, Italy.

Corresponding author: Francesca Brivio
E-mail address: fbrivio@uniss.it

Abstract

The importance of capturing and marking wild animals for research or conservation projects is widely shared. However, only recently researchers paid attention to assess the risks and the negative effects of capture methodologies. During capture and manipulation animals undergo the risk of death or injury. There is a lack of knowledge about the direct and indirect effect of chemical immobilization, particularly on ungulates. We investigated the influence of captures by telenarcosis on ibex males in the Gran Paradiso National Park. For ten days after the capture we collected data on spatial behaviour (1 fix per 2 hours), activity rhythms (mean activity over the 2 hour) and hormone levels (twice per day). Our results showed that this capture methodology did not affect spatial behaviour, testosterone and cortisol levels of ibex males. Instead, up to two days after the capture, males showed reduced activity levels. The other environmental variables analysed influenced behavioural patterns accordingly with the previous knowledge. Our findings highlighted a reduced impact of the capture with telenarcosis on ibex biology. Nonetheless, we suggest to carefully check freed animals for some hours after the capture in areas characterised by high predator density. Moreover, researchers should avoid considering data of the first days after the manipulation in order to avoid biased information.

Introduction

A wide array of research, conservation and management programmes on wildlife require the capture and handling of animals. Certainly, the new technologies allow to obtain information using no- or less-invasive techniques (e.g. collecting faecal or hair samples for DNA analysis or hormones levels). Nonetheless, some information can be collected only by capturing animals (e.g. morphometric measurements, age determination, or serum biochemistry for population sanitary controls; Powell and Proulx 2003, Garshelis 2006). Captures are also important for marking animals: ecological and behavioural studies require data from individually recognizable animals. New technological advances such as global positioning system (GPS) collars (Cagnacci et al. 2010), heat sensitive vaginal implant transmitters indicating the birth of neonates (Bishop et al. 2007), and advanced physiological monitoring equipment (Laske et al. 2011) allow detailed and novel researches on wildlife but necessitate the capture and handling of the animals. Translocation is an important tool in conservation biology. Live capture are required for reintroductions, restocking of population or it may be an alternative to lethal control in management situations, where human safety or property may be at risk (Linnell et al. 1997).

While there are clear reasons to perform capture of wild animals, only in recent years researchers have begun to assess the risks and negative effects of this activity. The captures of wild animals have potential to cause mortality and reduction in survival probability (Jacques et al. 2009) or injury in focal individuals (Cattet et al. 2008). Mortality rate is absolutely important and rather easy to measure. Anyway, it cannot stand alone as the measure of capture success and immobilization safety. The full impact of capture and handling procedures cannot be determined without evaluating physical, behavioural, and physiological effects on animals at the time of capture and in the days that follow. Haematological and biochemical blood constituents show that capture and immobilisation of wild ungulates is likely to be one of the most stressful events in their lives (Wesson et al 1979a,b, Spraker 1993). Several studies have assessed capture effects on behavioural metrics in free ranging wildlife. The potential impacts include displacement from areas around capture sites (Chi et al. 1998, Moa et al. 2001), altered space and habitat use (Morellet et al. 2009), depressed movements (Cattet et al. 2008, Quinn et al. 2012, Nurthrup et al. 2014) and reduction in activity patterns (Morellet et al. 2009).

The duration and the magnitude of a stress play a key role affecting glucocorticoid physiology (Romero 2004). Moreover, corticoids regulated the behavioural responses and, consequently animal welfare (DelGiudice et al. 1990, Diverio et al. 1996, Ingram et al. 1999, Montané et al. 2003). Therefore, the effect of immobilization can differ considerably according to capture techniques (DeNicola & Swihart 1997, Langkilde & Shine 2006). Survival, behaviour and reproduction of mammals need not be affected by field techniques (Laurenson & Caro 1994). Researchers should reduce the effects of non-trivial handling choosing the best technique and partially modifying their capture protocol. Several authors analysed short and long term effects on survival, vital rates and behaviour of mammals captured by helicopter net-gun (Jacques et al. 2009, Nurthrup et al. 2014), box trap, rockets nets, dart guns (Haulton et al. 2001) and vertical drop nets (Morellet et al. 2009). However, to our knowledge, to date no study has evaluated the effects of chemical immobilization by telenarcosis on the ungulates behaviour. Researchers and managers consider less stressful the animal manipulation when them are chemical immobilized. Indeed, this methodology is very common used in species living in open area particularly. However there are not available analyses assessing the short and long term effects of telenarcosis on large mammals. Thus, in our study we investigated the influence of captures by means of telenarcosis in the ibex (*Capra ibex*) population of the Gran Paradiso National Park. We expected that ibex reduced their movements and total activity after the capture. As a substudy, we examined the effects of capture and handling procedures on the testosterone and glucocorticoid production during the days after the immobilization, using faecal steroids analysis.

Methods

Study area

The study took place in the Valsavarenche Valley, within the Gran Paradiso National Park (GPNP; 45°35'N, 7°12'E; north-western Italian Alps). The study area is a mountainous region with steep glacial valleys ranging from 1500 to 3300 m a.s.l.. Rock cliffs, moraines and alpine meadows are the dominant habitats. The vegetation of this area includes conifer woods (*Picea abies*, *Larix decidua* and *Pinus cembra*), scrubs (*Rhododendron* and *Vaccinium* ssp.) and grassland, where the most common grass genera are *Festuca, Carex*, *Poa*, *Achillea*, and *Trifolium*. The local climate is temperate, with snowfall mostly occurring from November to April, the warmest period generally occurs from June to September. An automatic station recorded temperature, radiation, precipitation and wind speed data (24 records⁄d, Property of Meteorological Service of Aosta Valley Region).

Data collection

Adult male ibex (8-13 years old) were captured between May and July 2013 as part of on going research on the ethology, ecology, and sanitary conditions of the only autochthonous population of this species. The captures were performed by a team with a long experience in the use of telenarcosis on mountain ungulates (more than 40 years of experience). The team is composed by the rangers (at least 3 people) and the veterinarian of the Park. To reduce considerable distress to the

animals during the first part of capture just one operator with a Dan-Inject dart-gun approached on foot the ibex. The shooter measured the distance from the animal to properly regulate the pressure of the shot of the dart. Animals were darted at a distance of 26.6 ± 6.3 m (mean ± SD) far from cliffs to prevent potentially dangerous situations. In order to immobilize the ibex we used a mixture of xylazine HCl (Rompun, 20-40 mg) and ketamine (Inoketam, 50-100 mg). Xylazine is an alpha-2-adrenergic agonist acting as a nonnarcotic sedative analgesic, and ketamine is a dissociative anesthetic. The combination of the two drugs enables their dosages to be reduced, enhances muscle relaxation and duration of effect, and has been associated with faster and smoother induction (Lin, 1996). With the employed dose there is not evidence about specific adverse effects of this drugs (Peracino & Bassano 1993). Furthermore, this combination has been reported as effective for several wild species of Bovidae (Festa-Bianchet & Jorgenson 1985, Wiesner & von Hegel 1985, Fico 1988, Gauthier 1993, Dematteis et al. 2006).
The sedated ibex was observed by means of binoculars and about 10 minutes after the injection the ibex lay down. After further 5 minutes one operator approached it and if the animal did not show any signs of alert, three operators approached it simultaneously, hobbling and blindfolding it. Upon the capture the animal was placed in right lateral recumbency and its tongue was adjusted to ensure open air-ways. We collected biometric data, took biological samples and weighted the animals with a digital scale. Ibex was aged by counting the clearly separated annuli on its horn (von Hardenberg et al. 2004) and marked. During the manipulation, the ibex were constantly monitored to assess signs of stress related to capture. Heart rate, respiratory rate, and rectal temperature were recorded. Finally, in order to accelerate recovering of the animal and reduce the risks of hypothermia we injected a specific alpha-2-adrenergic antagonist, the atipamezole (1.5 cc), with the property of reversing the effects of xylazine; currently there are no effective antagonists for ketamine (Kreeger et al. 2002). We freed the ibex after about 45 min in the same place where it was captured.

We fit each male ibex with a GPS radio collar (GPS PRO Light collar, Vectronic Aerospace GmbH) set to attempt a relocation once every 2 hours. Moreover, these collars were equipped by an activity sensor that measure activity in two axes based on the true acceleration experienced by the collar. Axis X measures acceleration in forward/backward motions, axis Y measures sideways as well as rotary motion. Activity is measured four times per second simultaneously on each axis as the difference in acceleration between two consecutive measurements, and is given within a relative range between 0 and 255, characterizing the mean activity/acceleration (Krop-Benesch et al. 2011). Measurements are averaged over a sampling interval of 4 minutes and stored with the associated date and time. Data were downloaded by SMS and handheld terminal (VHF connection).

Faecal samples were collected from each animal captured. We collected 83 fecal samples from 9 males. For each marked ibex we collected one faecal sample just before or during the capture. After the capture, when the animal was detectable we collected samples twice per days for five consecutive days. We recorded the date and time of collection and stored in plastic bag at -20°C until the immune assay analysis.

Data analysis

We performed statistical analyses under 3 broad themes: effect of capture on mobility; effect of capture on activity pattern; and effect of capture on hormonal levels. To account for the nested nature of the data, we used hierarchical (i.e., random effects) models in all analyses performed.

Movement behaviour - Analysis focused on movements derived from relocations collected 7 days following the capture. We removed any locations with a dilution of precision (DOP) greater than 10. We used the resulting data to examine the effect of capture on movement behaviour. We calculated movement rates (m/h) for male ibex as straight-line distance (m) between consecutive locations divided by time interval (h), and we used it as depend variable in a Generalized Additive Mixed Model (GAMM). To explore the potential for temporal effects of capture and handling on movement rates, we used in the models the number of hours post-capture (HPC) as a fixed factor. To account for the daily and seasonal changes we also included the sampling time (hour) and the day (Julian day). In constructing models, we considered effects of other factors on movement rate: temperature (C°), radiation (W/m^2), precipitation (mm), wind speed (m/sec) and altitudinal differences between the arrival and the starting location (m). Meteorological data were calculated as mean, minimum and maximum values during the respective time interval of each displacement. We used AIC model selection to find which kind of values for each meteorological data fitted better the data.

Activity patterns – We calculated the mean activity values in the same time interval used for movement rates (2 hours). The mean activity was used as dependent variable in GAMMs. The effect of capture was explored using the same variables considered in the movement analyses, with the exception of the altitudinal differences between two consecutive localisations.

Hormonal levels – We extract steroid metabolites shaking, for 1-2 min in a handvortex, 0.5g of wet faeces suspended in 5 ml of 80% methanol (Palme et al. 2005). We centrifuged the samples for 15 min at 2,500 g and subsequently, 300 µl of the supernatant was dilute with assay buffer (1:10) and stored at -20°C until analysis with EIA (enzyme immunoassay). In all fecal samples collected, glucocorticoid metabolites (FGM, an indicator of stress levels) concentrations were measured using a validated EIA for 11-oxoaeticholanolone (first described: Palme & Möstl 1997), which have

previously been shown to provide reliable information on adrenal function in ibex (Posautz 2010). In addiction, in all fecal extracts, testosterone metabolites (FTM) concentrations were measured. In order to apply group-specific immunoassays for their measurement (Bamber et al. 2001, Sapolsky et al. 2000), a GNRH stimulation test was performed with two males of 4 years old at the Wildlife Park Langenberg, Switzerland. District veterinary office of the Canton of Zurich approved the treatment of the animals (N° 48/2013). The animals were narcotized and subsequently brought in an enclosure. Once narcotized, 0.01 μg of Buserelin (Receptal®, Intervet/Schering-Plough Animal Health) was injected intramuscular: as a GnRH-analogue, this drug stimulates, on top of the other, testosterone production. We monitored the treated males for 24h, during which all the fecal samples were collected. Samples were analysed by two different EIAs (to compared their performance in determining FTM levels in male ibex): 17β-OH-androgens and 17-oxo-androgens (first described: Palme & Möstl 1994). The assay that was more sensible to the variation of quantities of immunoreactive metabolites after the GnRH injection was 17-oxo-androgens, so we select this EIA to measure the level of FTM.
We performed statistical analyses on the level of FGM and FTM in two different sets of GAMMs. The number of hours post-capture (HPC) and the Julian date were included in both sets of models to account respectively for the effect of capture and seasonal changes on ibex hormonal levels.

The goodness of fit of each model (homoscedasticity, normality of errors and independence) was checked by visual inspection of residuals. When necessary, the dependent variables were transformed (ln [movement rates]; ln [mean activity]; ln[fecal cortisol metabolite], where ln=natural logarithm) to improve normality of residuals and reduce skew. Statistical analyses were implemented using R 2.14.1 (R Core Team 2013).

Results

We captured 10 male ibex, aging from 8 to 13 years, between the 7th of May and the 28th of June. For each marked male, during the 7 days following their capture we obtained 93.30 ± 13.23 valid relocations, from which we calculated the movement rates and the mean activity of ibex in the corresponding time interval. We collected 9.11 ± 3.14 faecal samples for males, from which we measured both testosterone and cortisol faecal metabolites levels.

Movement behaviour – The best model explaining ibex movement rate included six predictor variables: HPC, the hour of the day, the Julian day, the altitudinal difference, the maximum temperature and the mean precipitation during each time interval. According to the prediction of the

GAMM (Table 1) ibex movement rate increased linearly from the 7th of May to the 8th of July (β=0.01±0.002, P<0.001), but no influence of HPC was detected.

Table 1: Generalized Additive Mixed Model (GAMM) of the effects of the day of the year (Julian day), precipitation, number of hour following the capture (HPC), hour of the day, altitudinal differences (between the arrival point and the starting point) and maximum temperatures on movements rates in male Alpine ibex in the Gran Paradiso National Park, Italy.

Variables inserted with linear effect:				
	Coefficient	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.90	0.333	5.704	<0.001 ***
HPC	-0.0003	0.0005	-0.614	0.539
Julian day	0.009	0.002	4.305	<0.001 ***
precipitations	0.041	0.053	0.770	0.442

Approximate significance of smooth terms:				
	edf	**Ref.df**	**F**	**p-value**
s(hour of the day)	7.031	8.000	35.91	<0.001 ***
s(altitudinal differences)	8.556	8.556	64.04	<0.001 ***
s(temperatures)	2.596	2.596	3.39	0.024 *

R-sq.(adj) = 0.576 Scale est. = 0.92651 n = 933

The pattern of variation predicted by the model for movement rate as a function of the hour of the day was described by a sinusoidal curve with two positive picks at 9:30 and at 20:00 and a negative pick at 4:00 a.m. (Fig. 1). Temperatures significantly affected movements rates, with a pattern of constant movement rates till 15 °C and a pattern of decrease for higher temperatures (Fig. 2). Finally, results of the model showed that movement rates were minimum when ibex reached areas with low differences in altitudes and increased with increasing altitudinal differences till ±100 m of difference, beyond which was found a different pattern for positive and negative values, but with very high variance which makes it difficult to describe variation in ibex movement rates (Fig. 3).

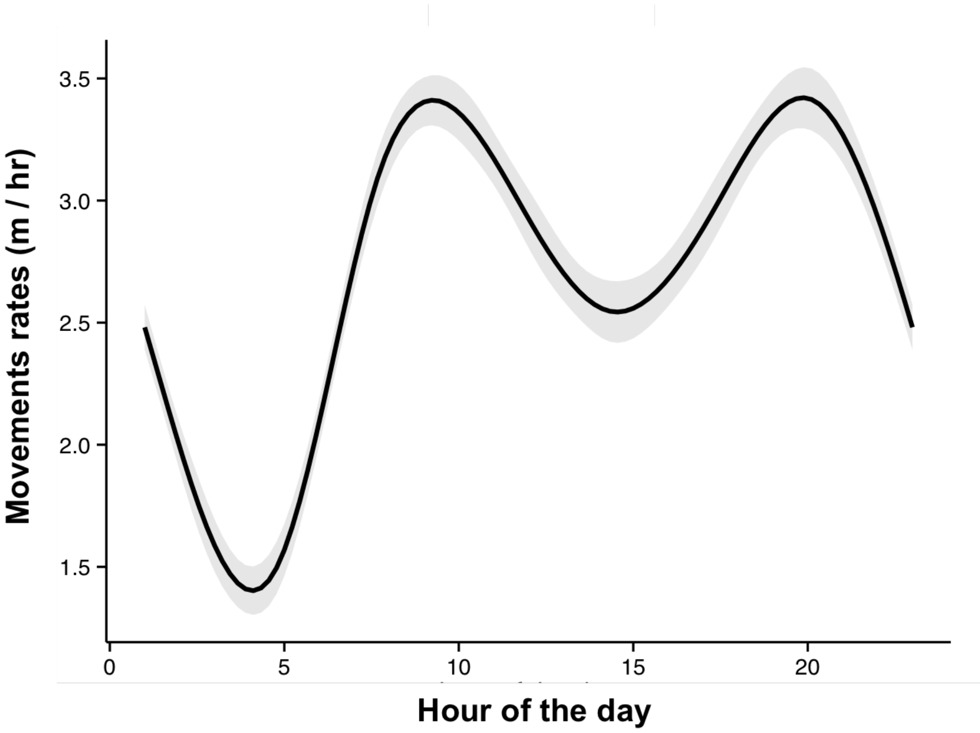

Figure 1: Individual movements rate predicted by the most-supported generalised additive mixed model for male ibex (including the individual as a random effect): as a function of the hour of the day, including a smoothing effect with a spline.

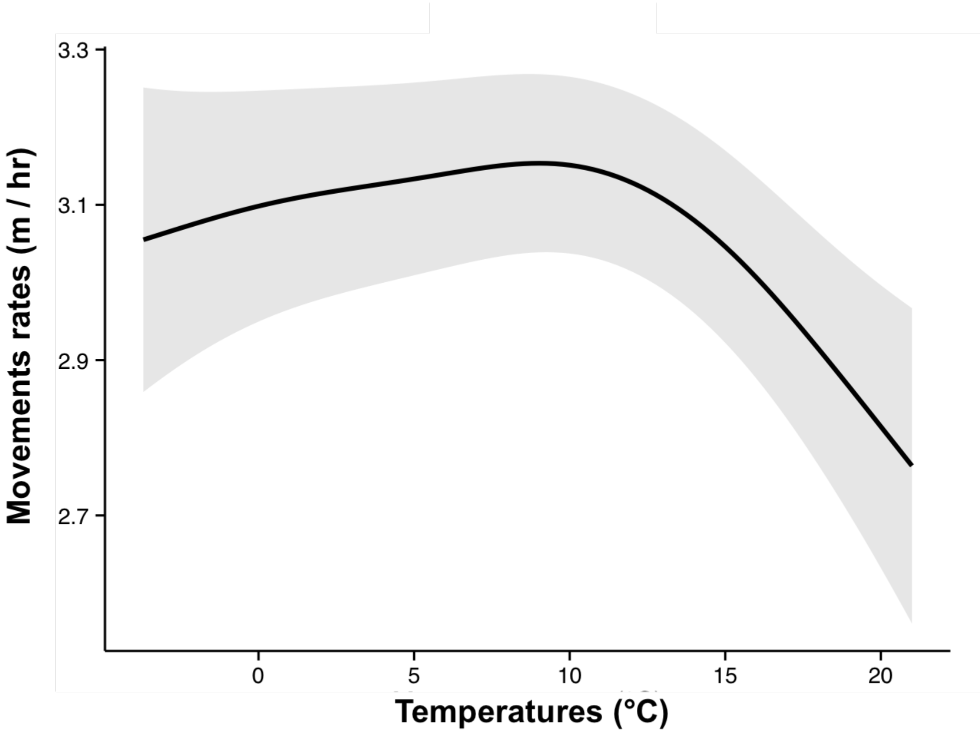

Figure 2: Individual movements rate predicted by the most-supported generalised additive mixed model for male ibex (including the individual as a random effect): as a function of the maximum temperatures, including a smoothing effect with a spline.

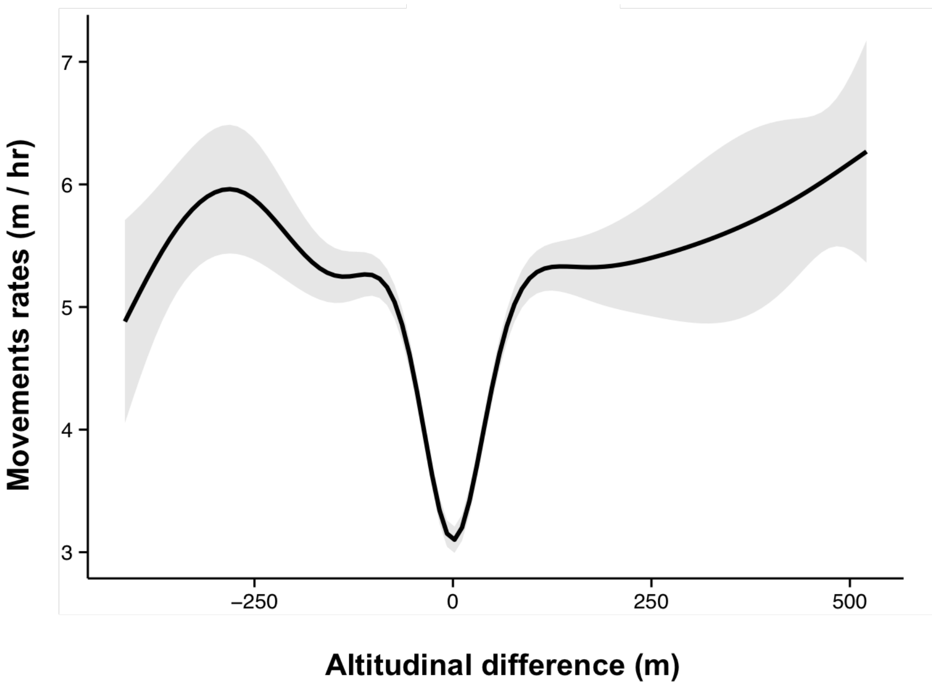

Figure 3: Individual movements rate predicted by the most-supported generalised additive mixed model for male ibex (including the individual as a random effect): as a function of the altitudinal differences, including a smoothing effect with a spline.

Activity patterns **-** The best model explaining activity patterns included five predictor variables: HPC, the hour of the day, the Julian day, the maximum temperature and the mean precipitation during each time interval (Table 2).

Table 2: Generalized Additive Mixed Model (GAMM) of the effects of the day of the year (Julian day), precipitation, number of hour following the capture (HPC), hour of the day and maximum temperatures on mean activity values in male Alpine ibex in the Gran Paradiso National Park, Italy.

Variables inserted with linear effect:				
	Coefficient	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	1.420	0.866	1.640	0.101
Julian day	0.011	0.006	1.895	0.058
precipitations	-0.171	0.060	-2.859	<0.001 ***
Approximate significance of smooth terms:				
	edf	**Ref.df**	**F**	**p-value**
s(HPC)	4.303	4.303	3.610	<0.001 ***
s(hour of the day)	7.375	8.000	70.664	<0.001 ***
s(temperatures)	4.119	4.119	4.361	<0.001 ***

R-sq.(adj) = 0.456 Scale est. = 1.1177 n = 933

According to the prediction of the model ibex activity was lower immediately after the capture and showed an increase over time up to about 48 hours, after which the activity appears to stabilize showing little variations (Fig. 4).

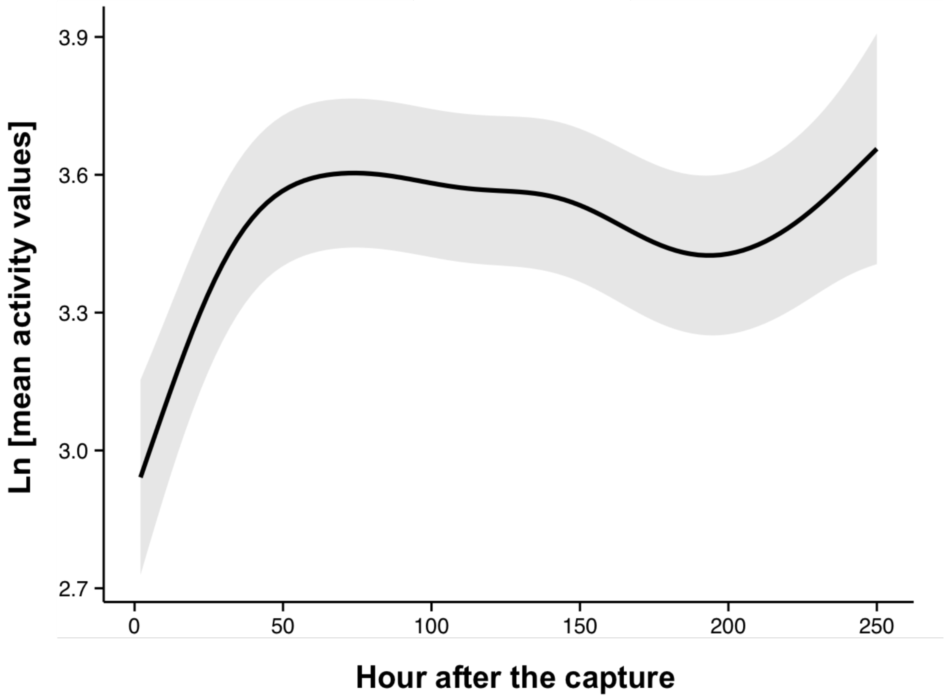

Figure 4: Individual daily activity (ln-trasformed) predicted by the most-supported generalised additive mixed model for male ibex (including the individual as a random effect): as a function of the hours after the capture, including a smoothing effect with a spline.

Daily pattern of activity showed by the model predictions is similar to that of movement rates: sinusoidal curve with two positive picks at 9:30 and at 20:00 and a negative pick at 4:00, for activity the pick of evening was higher than that of the morning (Fig. 5). Male activity increased with increasing maximum temperatures up to about 14°C, for higher temperatures ibex activity steeply decreased (Fig. 6). Activity linearly decreased with increasing precipitation levels (β= -0.171±0.060, $P<0.001$).

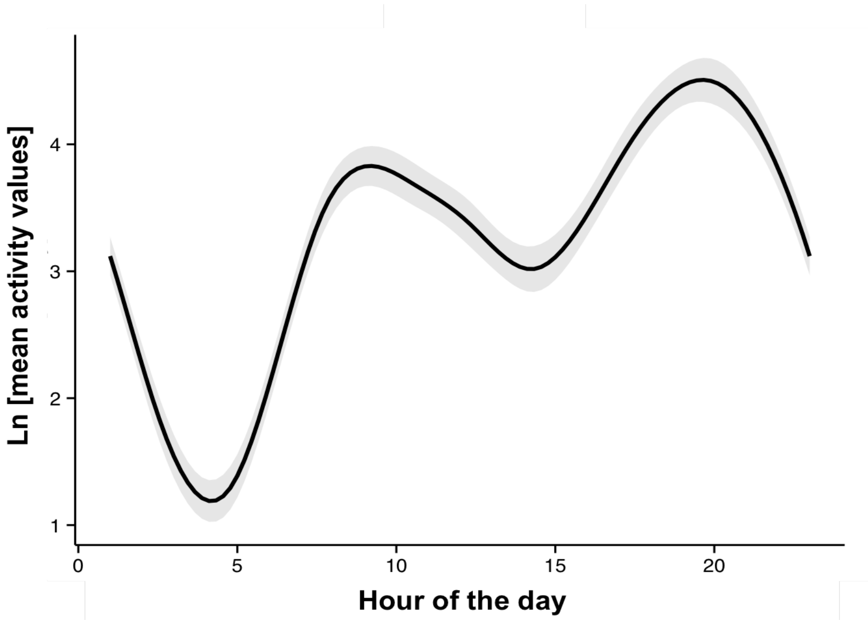

Figure 5: Individual daily activity (ln-trasformed) predicted by the most-supported generalised additive mixed model for male ibex (including the individual as a random effect): as a function of the hour of the day, including a smoothing effect with a spline.

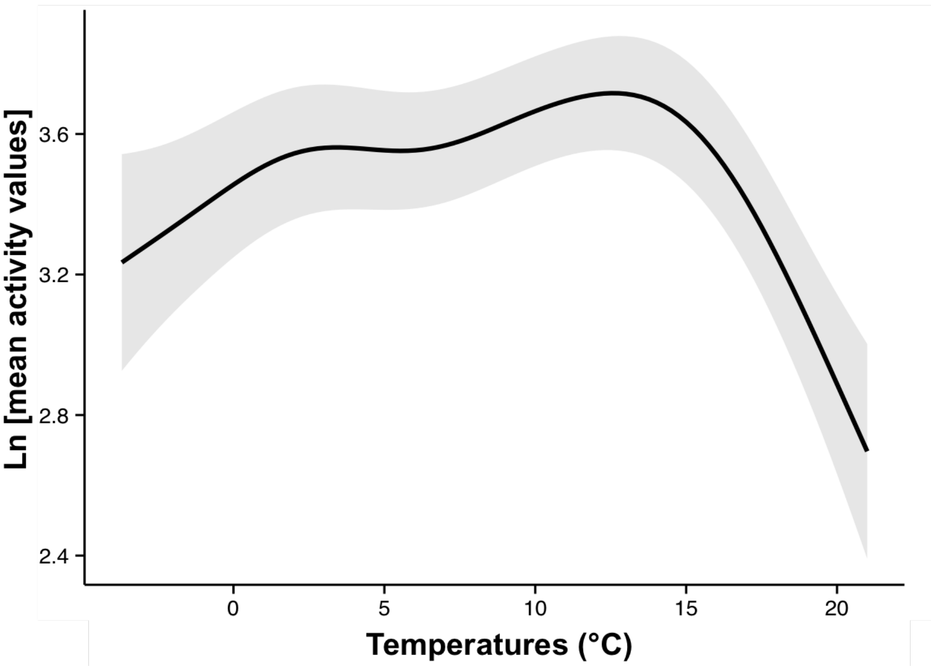

Figure 6: Individual daily activity (ln-trasformed) predicted by the most-supported generalised additive mixed model for male ibex (including the individual as a random effect): as a function of the daily maximum temperature, including a smoothing effect with a spline.

Hormonal levels – Results on hormone levels showed that both cortisol and testosterone production in ibex males was not affected by the capture as both FGM and FTM did not significantly change during the hours following the capture (Fig. 7). Moreover, no pattern was found investigating the influence of the day of the year on FGM and FTM.

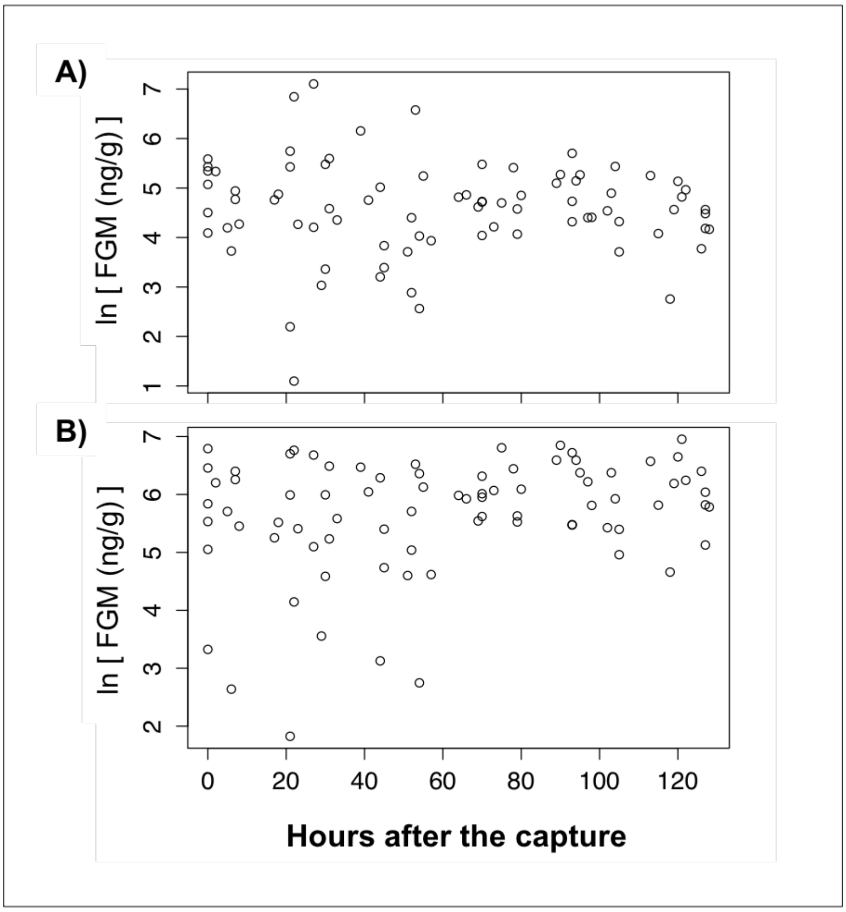

Figure 7: Variation of (A) Faecal Glucorticoid Metabolites (FGM, ln-trasformed) and (B) Faecal Testosterone Metabolites (FTM, ln-trasformed) in samples collected from the collared males as a function of time (in hours) after the capture.

Discussion

We found pronounced differences only in terms of overall activity level during the first days after capture in the monitored ibex. We did not find differences in terms of spatial behaviour (movement rates) and physiological responses (faecal metabolites of cortisol and testosterone). In our models we took into account some variables that are known to affect spatial behaviour of ibex: movements and activity were highest during morning and evening hours and negatively affected by high temperatures (more than 15 °C) and precipitations. The results confirmed the influence of these environmental variables, also in a brief period of analysis.

Our data highlighted that, after capture male Alpine ibex had movement rate comparable to the following days. In other words, the mobility of marked ibex was not affected by the mixture of xylazine and ketamine. This finding is partially surprising because we supposed that the stress of the capture and the undesirable effects of the drugs forced the animals to adopt a more cautious behavioural pattern. In fact, in case of risky situations, ibex moves to rocky slopes where they are safe and stay there for a long time (Grignolio et al. 2007).

The model on activity patterns provides results according to our expectations: both the environmental variables and the capture affected males' activity. The best model assessing the overall activity showed that the marked males were significantly less active and they needed two days to regain normal activity rhythms after capture. This result is not in contrast with our findings on spatial behaviour. The new marked males lived and moved together with groups of other males. But, whereas the other males fed, the marked males were lain down and slept, in spite of the injection of a specific alpha-2-adrenergic antagonist of xylazine. Marked males preferred make an effort to move with other males, rather than look for a refuge area and wait to feel better. Our conjecture is supported also by several occasional observations made by rangers and people of capture team. Immediately after the capture, the personnel of the Gran Paradiso National Park is usual to carry out short and repeated sessions of control by binoculars on captured animals. During these observations, it is easy to identify the marked males inside a foraging group, because often they are lying on the ground. A detailed analysis on habitat selection is necessary to support our hypothesis. Anyway, our findings highlight the key role of sociality as antipredator behavioural strategy in response to a stress event such as the captures, handlings and fitting of a collar.

This strategy to move in-group with other males, but reducing total activity, could be dangerous in area with high density predator population. A lying ibex inside a foraging group in a flat area undergoes higher predation risk in case of an attack of predators, for example a wolf pack. Consequently, the managers should take into account this potential behavioural effect of the capture by means of telenarcosis. In area characterised by high density of predator could be dangerous to free an animal without providing adequate precautions. For example, could be necessary to foresee a continuous direct control of the released individuals during the first hours after the capture in order to deter a potential attack by a predator. The behavioural changes induced by the capture did not affect significantly the energy balance. The analyses reported a reduction of total activity for about two days, but it is important to consider that ibex recover gradually a normal activity up to about two days (Fig 4). Thereby, it is clear that even if males reduced the foraging time for 48 hours, this cost rarely may be risky for the life history or the survival of the individual.

The analysis about hormone levels did not show significant systemic modifications. Certainly the capture and the manipulation induced a stress and an increase of cortisol level, but this alteration had a short duration. In fact, we are not able to measure the increase or decrease of hormone levels using faecal metabolites. Consequently, we can affirm that chemical immobilization did not cause hormone modifications that lasted for a long period and that could potentially be dangerous for the individual wellness.

Finally, our results point out that chemical immobilization, using a mixture of xylazine and ketamine, generated brief and slightly hazardous behavioural and physiological modifications. An expert and well-informed team can reduce the direct negative effects of this methodology (injury or death from dart shot to animal) and the indirect effect in the days after the capture event. The researchers should not use data collected in the first 2-3 days to avoid analyses with biased data.

References

Bamber E, Palme R, Meingassner JG (2001) Excretion of corticosteroid metabolites in urine and faeces of rats. Laboratory Animals 35:307–314.

Bishop CJ, Freddy DJ, White GC, Watkins BE, Stephenson TR, Wolfe LL (2007) Using vaginal implant transmitters to aid in capture of mule deer neonates. Journal of Wildlife Management 71:945– 954.

Cagnacci F, Boitani L, Powell RA, Boyce MS (2010) Animal ecology meets GPS-based radiotelemetry: a perfect storm of opportunities and challenges. Philosophical Transactions of the Royal Society B- Biological Sciences 365:2157–2162.

Cattet M, Boulanger J., Stenhouse G., Powell RA, Reynolds-Hogland ML (2008) An evaluation of long-term capture effects in ursids: implications for wildlife welfare and research. Journal of Mammalogy 89:973–990.

Chi DK, Chester D, Gilbert BK (1998) Effects of capture procedures on black bear activity at an Alaskan salmon stream. Ursus 10:563–569.

DelGiudice GD, Kunkel KE, Mech LD, Seal US (1990) Minimizing capture-related stress on white-tailed deer with a capture collar. Journal of Wildlife Management 54: 299-303.

Dematteis A, Menzano A, Canavese G, Meneguz PG, Rossi L (2009) Anaesthesia of free-ranging Northern chamois (*Rupicapra rupicapra*) with xylazine/ketamine and reversal with atipamezole. European Journal of Wildlife Research 55:567-573.

DeNicola AJ & Swihart RK (1997) Capture-induced stress in white-tailed deer. Wildlife Society Bulletin 25:500-503.

Diverio S, Goddard PJ, Gordon IJ (1996) Use of long-acting neuroleptics to reduce the stress response to management practices in red deer. Applied Animal Behaviour Science 49:83-88.

Festa-Bianchet M & Jorgenson JT (1985) Use of xylazine and ketamine to immobilize bighorn sheep in Alberta. Journal of Wildlife Management 49:162–165.

Fico R (1988) Variabilità di risposta alla sedazione in camosci appenninici *Rupicapra pyrenaica ornata* catturati in libertà ed in recinto. In Atti I Convegno Nazionale dei Biologi della Selvaggina, Bologna, Italy, 28–30 January 1988, pp. 569–575.

Garshelis DL (2006) On the allure of noninvasive genetic sampling—putting a face to the name. Ursus 17:109–123.

Gauthier D (1993) Pratiques francaises en matiere d'immobilisation par voie chimique: synthese des questionnaires et experience du Parc National de la Vanoise. In Proceedings: Techniques de capture et de marquage des ongules sauvages, Meze, Herault, France, 20–22 March 1990; Dubray D (ed.), FDC, l'Herault, Montpellier, France, pp. 7–17.

Grignolio S, Rossi I, Bassano B, Apollonio M (2007) Predation risk as a factor affecting sexual segregation in Alpine ibex. Journal of Mammalogy 88:1488-1497.

von Hardenberg A, Bassano B, Zumel Arranz MP, Bogliani G (2004) Horn growth but not asymmetry heralds the onset of senescence in male Alpine ibex (*Capra ibex*). Journal of Zoology 263:425-432.

Haulton SM, Porter WF, Rudolph BA (2001) Evaluating 4 methods to capture white-tailed deer. Wildlife Society Bulletin 29: 255-264.

Ingram JR, Crockford JN, Matthews LR (1999) Ultradian, circadian and seasonal rhythms in cortisol secretion and adrenal responsiveness to ACTH and yarding in unrestrained red deer (*Cervus elaphus*) stags. Journal of Endocrinology 162:289-300.

Jacques CN, Jenks JA, Deperno CS, Sievers JD, Grovenburg TW, Brinkman TJ, Swanson CC, Stillings BA (2009) Evaluating ungulate mortality associated with helicopter net-gun captures in the Northern Great Plains. Journal of Wildlife Management 73:1282–1291.

Kreeger TJ, Arnemo JM, Raath JP (2002) Handbook of wildlife chemical immobilization. International Edition, Fort Collins, Colorado, 412 pp.

Krop-Benesch A, Berger A, Streich J, Scheibe K (2011) Activity Pattern User's Manual. Version: 1.3.1.

Langkilde T & Shine R (2006) How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. Journal of Experimental Biology 209:1035-1043.

Laske T, Garshelis D, Iaizzo P (2011) Monitoring the wild black bear's reaction to human and environmental stressors. BMC Physiology 11:13.

Laurenson MK & Caro TM (1994) Monitoring the Effects of Nontrivial Handling in Free-Living Cheetahs. Animal Behaviour 47: 547-557.

Lin HC (1996) Dissociative anesthetics. In Lumb and Jones' veterinary anesthesia. Williams and Wilkins, Baltimore, Maryland, pp. 241–296.

Linnell J, Aanes R, Swenson J, Odden J, Smith M (1997) Translocation of carnivores as a method for managing problem animals: a review. Biodiversity and Conservation 6:1245–1257.

Moa P, Negard A, Overskaug K, Kvam T (2001) Possible effects of the capture event on subsequent space use of Eurasian lynx. Wildlife Society Bulletin 29:86–90.

Montané J, Marco I, Lopez-Olvera J, Perpinan D, Manteca X, Lavin S (2003) Effects of acepromazine on capture stress in roe deer (*Capreolus capreolus*). Journal of Wildlife Diseases 39: 375-386.

Morellet N, Verheyden H, Angibault JM, Cargnelutti B, Lourtet B, Hewison MAJ (2009) The effect of capture on ranging behaviour and activity of the European roe deer *Capreolus capreolus*. Wildlife Biology 15:278–287.

Northrup JM, Anderson JrCR, Wittemyer G (2014) Effects of helicopter capture and handling on movement behavior of mule deer. The Journal of Wildlife Management 78(4):731–738.

Palme R & Möstl E (1994) Biotin-streptavidin enzyme-immunoassay for the determination of oestrogens and androgens in boar feces. In: Advances of Steroid Analysis '93 (ed. Görög S), Akademia Kiado, Budapest 111-117.

Palme R & Möstl E (1997) Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. In: Zeitschrift fur Saugetierkunde, 62 (SUPPL. 2), 192-197.

Palme R, Rettenbacher S, Touma C, El-Bahr SM, Möstl E (2005) Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Annals of the New York Academy of Sciences, 1040:162–71.

Peracino V & Bassano B (1993) Bilan de 30 annees d'experience de capture des ongules sauvages- Bouquetin des Alpes (*Capra ibex ibex*) et chamois (*Rupicapra rupicapra rupicapra*) - dans le Parc National du Grand Paradis (Italie). In Proceedings: Techniques de capture et de marquage des ongules sauvages. Meze, Herault, France, 1990; Dubray D (ed.), FDC de l'Herault, Montpellier, France, pp. 37–44.

Powell RA & Proulx G (2003) Trapping and marking terrestrial mammals for research: integrating ethics, performance criteria, techniques, and common sense. Institute for Laboratory Animal Research (ILAR) Journal 44:259–276.

Posautz C (2010) Measurement of glucocorticoid metabolites in feces of Capricorns (*Alpine ibex*). Diploma thesis, University of Veterinary Medicine, Research Institute of Wildlife ecology, Vienna.

Quinn ACD, Williams DM, Porter WF. 2012. Postcapture movement rates can inform data-censoring protocols for GPS-collared animals. Journal of Mammalogy 93:456–463.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89.

Spraker TR (1993) Stress and capture myopathy in artiodactyls. In Zoo and Wild Animal Medicine: Current Therapy, 3rd ed. M. E. Fowler. Philadelphia, W.B. Saunders Co. pp 481–488.

Wesson JA, Scalon PF, Kirpatrick RL, Mosby HS (1979a) Influence of chemical immobilization and physical restraint on packed cell volume, total protein, glucose, and blood urea nitrogen in blood of white-tailed deer. Canadian Journal of Zoology 57, 756–767.

Wesson JA, Scalon PF, Kirpatrick RL, Mosby HS, Butcher RL (1979b) Influence of chemical immobilization and physical restraint on steroid hormone levels in blood of white-tailed deer. Canadian Journal of Zoology 57, 768–776.

Wiesner H & von Hegel G (1985) Praktishe hinweise zur immobilisation von wild und zootieren. Tierarztliche Praxis 13: 113–127.