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Stress evaluation in hares (*Lepus europaeus* Pallas) captured for traslocation

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ABSTRACT

With the aim to evaluate the capturing techniques some haematic and physiological parameters were studied to discriminate stressed hares from non stressed hares.
A total of 66 wild hares (experimental group) were sampled in 14 different non-hunting areas, where hares are usually captured for later release in low-density areas. In the same season a total of 30 hares (about 1 year old), reared in cages and thus showing a reduced fear of man, were sampled (control group). In each area the hares were captured by coursing with 3-4 dogs (greyhounds or lurches). The dogs were released by the different hunter teams to find and drive into trammel nets any hare that was seen running. After capture, the hares remained inside darkened, wooden capture-boxes for a variable period of time before blood drawing. For blood sample collection all the hares were physically restrained and their eyes immediately covered. Blood, always collected within 1-2 minutes, was drawn from the auricular vein. Blood samples (plasma) were analysed for glucose, AST, ALT, CPK and cortisol concentrations. Body temperature, heart and respiratory rate, sex, and age were evaluated in each hare. The effect of origin, sex and age on haematic and physiological parameters was analysed by ANOVA. Every measured parameter of the hares bearing to the capture group or the control group (reared) was then subjected to stepwise and to discriminant analysis, in order to select the groups of stressed (discriminated by the controls) and non-stressed hares.
CPK, AST and glucose were found to be the best parameters for distinguishing stressed from non-stressed hares. The intensive exercise suffered by the wild hares induced a depletion of energetic reserves, so that most of the captured hares showed lower glucose and higher CPK activity in the plasma, probably due to muscle damage ($P< 0.05$). After reclassifying the hares in the two groups of stressed and non stressed hares, the reference values (means ± SE) resulted as follows: estimated non-stressed hares, glucose 234 ± 9 .4 mg/dl, AST 112 ± 22.2 U/l, CPK 1334 ± 734 U/l; estimated stressed hares, glucose 128 ± 7 mg/dl, AST 164 ± 13 U/l, CPK 4658 ± 454 U/l.
These three cheap and quickly analysable analytes can be useful to the game manager in detecting stressed and non stressed hares, in order to improve the capturing techniques by the evaluation of the following relationship: (number of stressed hares + number of the dead hares during the capture)/number of total captured hares.

Key words: Lepus europaeus Pallas, Blood samples, Capture, Stress.

RIASSUNTO

VALUTAZIONE DELLO STRESS IN LEPRI CATTURATE PER LA TRASLOCAZIONE

Sono stati studiati alcuni parametri ematici e fisiologici nella lepre allo scopo di valutare le tecniche di cattura discriminando i soggetti stressati da quelli non stressati.
A tale scopo sono state utilizzate 66 lepri prelevate in 14 zone di ripopolamento e cattura della Provincia di Firenze (gruppo sperimentale) e 30 lepri nate ed allevate in gabbia (gruppo di controllo). Per la cattura è stato utilizzato il metodo in

battuta con i cani liberi da squadre diverse di cacciatori. Dopo la cattura, le lepri sono state poste in cassette di contenimento in legno dove erano mantenute per un periodo variabile (da 10' a 1h). Dopo essere state prelevate dalla cassetta di contenimento o dalla gabbia le lepri erano contenute manualmente da un operatore esperto e immediatamente bendate. Entro 1-2 minuti venivano sottoposte ai seguenti rilievi: prelievo di sangue dalla vena auricolare, controllo della temperatura corporea, della frequenza respiratoria e cardiaca, determinazione dell'età e del sesso. Sui campioni di sangue sono stati analizzati: glucosio, ALT, AST, CPK e cortisolo. Gli effetti dell'origine, sesso ed età sono stati analizzati sui parametri ematici tramite l'ANOVA. I parametri relativi allo stress da cattura sono stati quindi sottoposti alla regressione multipla, selezionati con il metodo stepwise, quindi utilizzati nell'analisi discriminante per l'individuazione dei soggetti stressati e non-stressati.

L'analisi effettuata ha permesso di individuare nei parametri ematici CPK, AST e glucosio gli indicatori più validi per poter distinguere in tali condizioni i soggetti stressati da quelli non-stressati. L'intenso esercizio fisico sopportato dai soggetti selvatici ha portato infatti ad un esaurimento delle riserve energetiche in molti animali, come dimostrano i bassi valori di glicemia registrati a carico delle lepri di cattura (P< 0,05). L'intensa attività ed i probabili danni muscolari subiti sono stati confermati in molte lepri catturate dai più alti livelli ematici di CPK. Dopo la nuova classificazione effettuata dall'analisi discriminante, che ha individuato i soggetti stressati e non stressati, i valori di riferimento (media ± SE) dei due gruppi sono risultati i seguenti: gruppo non-stressato, glicemia 234 ± 9,4 mg/dl, AST 112 ± 22,2 U/l, CPK 1334 ± 734 U/l; gruppo stressato, glicemia 128 ± 7 mg/dl, AST 164 ± 13 U/l, CPK 4658 ± 454 U/l.

I parametri individuati, di rapida ed economica determinazione, sono risultati in grado di distinguere i soggetti stressati da quelli non stressati e potrebbero essere favorevolmente impiegati per migliorare le tecniche di cattura.

Parole chiave: Lepus europaeus *Pallas, Parametri ematici, Cattura, Stress.*

Introduction

Capture for traslocation (transport and release in different areas, presenting more or less similar habitats) is normally adopted in Italy to control the status and density of the hare populations. During these operations direct death may occur and stress can play a role in the development of future complications in the released hares. The stress may be light, with no physiological consequence; it may be heavy and cause physiological alterations; or it may be extremely heavy and also cause cell deaths. Stress which alters the "normal" physiology of the animals reduces the immune response and consequently resistance to disease (Spraker, 1993; Ranucci *et al*., 1996; Williams and Thorne, 1996; Diverio *et al*., 1998; Montané *et al*. 2003).

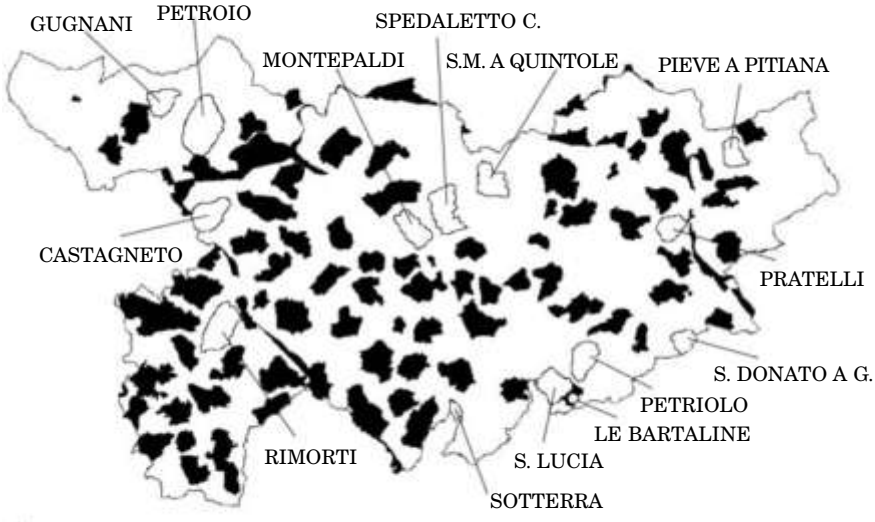
Various physiological welfare indicators are used to evaluate stress. Most of these indicators are not yet fully defined; in addition, the "damage level" must be determined in each species subjected to capture and handling for traslocation. For this reason, we performed a study to discriminate stressed hares from non stressed hares for the evaluation of the techniques employed in the capture.

Material and methods

The study was carried out on hares from non-hunting areas in Central Italy (10° 50' to 11° 40' East, 43° 20' to 43° 50' North) and on animals born and reared in cages. A total of 66 hares (experimental group) were sampled in 14 different non-hunting areas (Figure 1) where hares are usually captured for subsequent release in free hunting low-density areas (Paci and Bagliacca, 2003). In each area the hares were captured by coursing with 3-4 dogs (greyhounds or lurches). Different teams of hunters released the dogs to find and drive into trammel nets any hare that was seen running. The team of hunters was composed of game and wildlife officers, volunteers, and game wardens. The hares ran different distances and for varying periods of time in relation to the size of the area. After capture, all the hares remained inside the darkened, wooden capture-boxes for a variable period of time (10' to 3 h) before the blood drawing. For sample collection the hares were removed from the boxes, physically restrained and their eyes immediately covered (blood was always collected within 1 - 2 minutes after the animals were removed from their capture-boxes).

Blood was drawn from the auricular vein using disposable syringes and 0.5x16 mm needles. Body temperature (by digital thermometer introduced in the rectum), as well as heart (mediated auscultation by phonendoscope) and respiratory rate (by visual observation), were measured. Sex, age (radius and ulna ossification stage, examined with

Figure 1. Geographic location of the different protected areas for the wild reproduction of the hares.



the unaided eye and palpation, Strohl-tubercle Broekhuizen and Maaskamp, 1979), were evaluated. In relationship to the presence of Strohl-tubercle the hares were consequently classified in two groups, young (<1 yr old) and adult (> 1 yr old).

The same parameters were measured in a control group of 30 captive hares (about 1 year old) in the same season. The animals were chosen from a group of individuals showing a reduced fear of man; their eyes were covered immediately after removal from their rearing cage, and blood drawing operations were carried out quickly and with no evidence of discomfort. The reared animals were monitored for the following month to control the absence of any resulting consequence.

The blood samples (~1.5 ml of plasma, Lieparine) were analysed for Glucose by colorimetric method (SCLAVO, Italy), for CPK (Creatin Phosfo Kinase), ALT (ALanine amino Transferase), and AST (ASpartate amino Transferase) by kinetic methods (SCLAVO, Italy). Cortisol concentrations were determined by radioimmunoassay kit (SEAC, Radim Group, UK). The effect of origin, sex and age on haematic and physiological parameters was analysed by ANOVA.

Every measured parameter of the hares bearing to the capture group or the control group (reared) was then subjected to multiple regression; the most important parameters were chosen in the final model by the forward (probability to enter = 0.25) and backward (probability to leave = 0.10) methods. The parameters selected in the final model were then subjected to discriminant analysis and the wild animals, which were not discriminated by the control group, were considered "non-stressed" or with a stress level similar to the control/captive-reared hares and consequently assigned to the category of non-stressed animals. The two different groups (non-stressed and stressed) thus consisted of every captive hare plus the non-discriminated wild hares, and of the remaining wild hares (SAS, 2002).

Results and discussion

Haematic parameters

The effects of the different origin, sex, and age on haematic parameters are presented in Table 1.

Table 1. Haematic concentration of the different parameters observed in the hares.

		Captive reared		Wild Captured			
		Females	Males	Females		Males	
		Young	Young	Young	Adult	Young	Adult
Glucose	n.	15	15	10	9	16	14
	mg/dl	240 [a]	211 [ab]	138 [bc]	163 [bc]	176 [abc]	134 [c]
	SE	21.8	21.8	19.0	18.0	15.0	15.1
AST	n.	15	15	10	9	16	13
	U/l	109	90	224	197	124	178
	SE	40.9	40.9	32.5	31.3	25.7	26.0
ALT	n.	15	15	10	9	16	14
	U/l	65.56	56.84	83.60	85.91	69.56	77.20
	SE	8.6	8.6	7.5	7.1	5.9	5.9
CPK	n.	15	15	10	9	16	14
	U/l	796 [b]	1010 [ab]	5930 [a]	4151 [a]	2650 [a]	4364 [a]
	SE	1115.4	1115.4	973.3	922.9	769.4	770.5
Cortisol	n.	15	15	12	10	16	14
	μg/l	12.6	10.8	15.3	11.1	12.5	9.3
	SE	2.53	2.53	2.03	2.02	1.76	1.76

Note: means with different superscripts differ for P<0.05.
AST = ASpartate amino Transferase; ALT = ALanine amino Transferase; CPK = Creatin Phosfo Kinase.

Young females, reared in cages, presented the highest glucose values and adult wild captured males showed the lowest (240 mg/dl vs. 134 mg/dl, P< 0.05). In the captive-reared animals, males and females presented similar values; analogously no difference was found between male and female wild hares. Since running and escape attempts during capture caused a considerable depletion of carbohydrate resources in several hares (Bateson and Bradshaw, 1997), differences were found between captive and wild animals. In particular the glucose level of wild males was lower than in captive hares and the glucose level of young captive females was higher than that found in wild hares, except the young wild males. Also ALT and especially AST, which is an indicator of muscle damage (myopathy), tended to differ between captive and captured hares, although the differences are not statistically significant. AST activity is always very high in wild mammals that have been running (such as hunted deer) and tends to continue rising even after the end of the action of the stressing factor (Bateson and Bradshaw, 1997). The lack of significant differences is probably due to the great variability of values that characterises this parameter in the hares. The most sensitive enzyme that shows muscle damage and even intense muscle activity is CPK. In spite of its enormous variability, this value differed between hares of captive and wild origin. The wild captured young and adult males and females presented higher values than captive-reared females, and only the captive-reared males did not reach the minimum statistical difference from the wild hares.

The concentration of cortisol, which is a measure of the adrenal cortex response, always higher in coursed animals than in non-coursed animals, is usually a useful indicator for measuring stress (Del Giudice *et al.*, 1990; Hastings *et al.*, 1992; Morton et al., 1995). Cortisol is commonly referred to as the "stress hormone" because its secretion has been associated with many types of physical and emotional challenges, such as exercise, social

Table 2. Physiological parameters of hares of different origin.

		Captive reared		Wild Captured			
		Females	Males	Females		Males	
		Young	Young	Young	Adult	Young	Adult
	n.	15	15	16	12	20	18
Rectal temperature	°C	38.3	38.4	38.0	38.6	38.2	38.3
	SE	0.34	0.35	0.15	0.17	0.14	0.14
Respiratory rate	n./min	74	66	71	84	67	68
	SE	6.0	6.3	4.6	5.3	4.1	4.3
Heart rate	n./min	96	99	100	125	95	101
	SE	8.7	8.3	8.2	9.5	7.3	7.7

isolation, loss of social status and hypoglycaemia (Broom and Johnson, 1993). In our experience cortisol did not show any difference between the two groups and seems a less useful indicator in evaluating hare capture stress (Paci *et. al*., 2004). Broom and Johnson (1993) reported that some situations, which we would expect to be painful, are sometimes, but not always, associated with increased plasma glucocorticoid levels. Within glucocorticoids we measured only cortisol, since blood can be collected from the hares within 1 to 2 minutes after the animals are removed from their cages or capture-boxes, and cortisol is characterised by a sufficient delay in its release into the bloodstream. Catecolamines (adrenaline and noradrenaline), released from the adrenal medulla and more sensitive, were not measured since handling during the blood drawing influences their level. The measured cortisol levels are not surely influenced by stress related to handling during the blood drawing but, since Cortisol can return quite rapidly (within 2 to 3 hours) to basal values after the stress, it could have already disappeared from the bloodstream at the moment of blood drawing in most of the wild captured hares. The hares, in fact, after the capture operations by tramnel nets, remain in darkened places inside the wooden capture-boxes before examination and blood drawing for a variable period of time (10' to 3h).

Physiological parameters

Regarding respiratory rate, body temperature and heart rate, which can also be easily measured by the hunters with little disturbance to the animals (Table 2), no difference was shown between the two groups. Since increased respiratory rate and heart rate can be a response to difficult situations perceived by individuals (Broom and Johnson, 1993; Diverio et al., 1996), the absence of a trend of differentiation shows that the captured animals did not perceive constriction in the capture boxes to be a dangerous situation and are able to rest in these boxes.

Stepwise selection and Discriminant analysis

Only three parameters entered into the variable set of the discriminant model, selected by the stepwise analysis. Glucose, CPK and AST were the selected parameters which best distinguished the two groups (Table 3). Every captive hare was classified within the captive group, 48 wild hares were classified within the captured group, and 11 wild hares were not discriminated from the captive group (7 animals were lost by the analysis, in relationship to the fault of one or more of the required parameters). The reared hares were assigned to the non stressed group, the discriminated wild hares were assigned to the stressed group, and the undiscriminated captured hares were assigned to the non stressed group again. The reference values of the stressed hares and non stressed hares, useful for the evaluation of the capturing techniques, were consequently estimated on the two popula-

Table 3. Results of the stepwise selection.

	F Ratio	Prob>F	Entered
T °C	0.36	0.55	
Respiratory rate	0.11	0.74	
Heart rate	0.78	0.38	
Glucose	7.31	0.01	*
CPK	6.24	0.02	*
AST	3.34	0.07	*
ALT	2.27	0.14	
Cortisol	0.01	0.94	

AST = ASpartate amino Transferase; ALT = ALanine amino Transferase; CPK = Creatin Phosfo Kinase.
** Significant parameters entered in the final model*

Table 4. Reference values for stressed and non stressed hares.

	Non - stressed hares			Stressed hares		
	Glucose mg/dl	AST U/l	CPK U/l	Glucose mg/dl	AST U/l	CPK U/l
Max.	352	230	4235	210	760	18,968
90%	306	179	2842	184	296	9890
Upper quartile	256	144	1619	155	182	6526
Median	228	93	1068	135	135	3184
Lower quartile	197	78	785	97	103	1985
10%	183	65	609	69	82.6	1201
Min.	160	60	484	44	60	637
n.	41	41	41	48	48	48
Mean	234	112	1334	128	164	4658
SD	46.0	45.8	907.9	41.5	108.4	3898
upper 95% Mean	255	134	1747	142	193	5712
lower 95% Mean	213	90	921	115	134	3605

Median = 50th percentile
Lower quartile = 25th percentiles. Upper quartile = 75th percentiles
AST = ASpartate amino Transferase; CPK = Creatin Phosfo Kinase.

tions that were statistically thus determined (Table 4).

Conclusions

The obtained results showed that most captured hares are stressed, and pain-related stress can probably also cause a certain degree of "muscle damage" as shown by the observed very high values of CPK and AST (Montané *et al.*, 2003). Glucose, CPK and AST, three cheap and quickly analysable parameters, should be routinely inserted into the

hare monitoring procedure to evaluate the degree of stress suffered by animals and, consequently, the ability of the different groups of hunters (Morton *et al.*, 1995; Montané *et al*., 2003). The reference values obtained with our study can be used as a reference to distinguish "badly- captured hares" from "well-captured hares" (stressed hares and non stressed hares or hares with a stress level similar to that suffered by the captive-reared animals). The relationship between the number of the stressed hares plus the number of dead hares during capture with the number of all captured hares, can be then used to score the different courses and/or hunting teams.

Part of the results were presented to the 2nd World Lagomorph Conference, Vairao, Portugal, July 26-31, 2004.

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