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Stress in wild-caught Eurasian otters (Lutra lutra): effects of a long-acting neuroleptic and time in captivity

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Abstract

*As part of a translocation project, 28 Eurasian otters (*Lutra lutra*) were captured from the wild and transported to the Barcelona Zoo for veterinary evaluation, quarantine and intraperitoneal implantation of telemetry devices. Eleven animals were injected with the long-acting neuroleptic (LAN) perphenazine enanthate at the time of capture and the remaining animals served as a control group. During their time in captivity, which averaged 23 days, all of the animals were bled three times. Haematological and biochemical parameters were evaluated, including red blood cell count (RBC), haemoglobin (Hb), white blood cell count (WBC), blood urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), creatine kinase (CK), albumin, and serum cortisol. No significant differences were found between treated and control otters except for monocyte count, which was higher in treated animals. Time after capture had an effect on many parameters. RBC and Hb decreased at first and then increased, while WBC and segmented neutrophils decreased over time. Most of the biochemical parameters considered to vary in relation to stress, including AST, ALT, CK, AP and LDH, decreased over time, suggesting that the stress responses of the animals decreased throughout the period of captivity. However, no significant change in serum cortisol levels was noted. The lack of effect of perphenazine treatment on haematological parameters should encourage further research on other stress indicators applicable to wild animals, such as behaviour or faecal cortisol concentration. Finally, the results obtained in this study suggest that, when captive conditions are adequate, keeping wild-caught animals in human care for a period of time prior to their release into the wild can be beneficial. However, further studies taking into account other welfare indicators would be useful.*

Keywords: *animal welfare, Eurasian otter, long-acting neuroleptic,* Lutra lutra*, perphenazine, stress*

Introduction

The translocation of wild animals is an important tool in wildlife management and conservation. Among others, the Arabian oryx (*Oryx leucoryx*), golden lion tamarin (*Leontopithecus rosalia*), red wolf (*Canis rufus*), black-footed ferret (*Mustela nigripes*), and river otter (*Lutra canadensis*) have been reintroduced into the wild as a part of conservation programs (Clark *et al* 1994; Serfass *et al* 1996).

The capture, handling, transport, and confinement inherent to these projects inflict a substantial amount of anxiety and fear on animals, particularly when free-ranging wild or semi-wild individuals that have had little previous exposure to humans are to be translocated. Being pursued, caught, and physically manipulated constitute stressful events for animals (Nielsen 1999). Some species are particularly susceptible to stress induced by capture and by adaptation to captive conditions. This may lead to high levels of anxiety, which in turn may result in the refusal of food and water, and in self-injury and exhaustion; sometimes with fatal consequences. Interestingly, few researchers have focused on the animal welfare implications for the individuals to be translocated during such programs, and most attention has focused on social implications and the spreading of infectious diseases (Seal & Wolf 1992). Prevention of exertional myopathy (a condition characterised by damage to skeletal and cardiac muscles and associated with physiological imbalances following extreme exertion, struggle and stress [Williams & Thorne 1996]) should be one of the most important considerations when planning and executing operations that require handling wild animals (Williams & Thorne 1996). This condition has occurred in a wide range of species, but primates, birds, and ungulates appear particularly susceptible. Recently, exertional myopathy has been recorded in North American river otters during a translocation program (Hartup *et al* 1999). This condition can be identified by elevated levels of the intracellular serum enzymes, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK), in the blood of the affected animal (Nielsen 1999). Data from evaluations of serum enzymes may not be of direct management use in the field, but they are useful in later evaluation of the trapping or holding operation (Williams & Thorne 1996).

Exertional myopathy is important not only because it may cause the death of reintroduced animals, but also because it may have a negative impact on the success of the project as well as on the welfare of the affected animals.

There are several techniques to reduce stress-related problems during translocation programs. Firstly, reducing or extending time spent in captivity could result in a reduction in stress level and/or an improvement in the animal's general condition, which would improve its chances of survival after release. However, there are differing opinions regarding when to release wild-caught animals.

Secondly, in recent years, long-acting neuroleptics (LANs) have been used with increasing frequency in newly caught wild animals to relieve anxiety and facilitate transportation or adaptation (Ebedes 1993; Holz & Barnett 1996; McCoy *et al* 1997). The currently used LANs are derived from the phenothiazines or thioxanthenes, and, depending on the product and the dose given, effects can be maintained for up to 28 days (Ebedes 1993). LANs have been more traditionally used for the treatment of human psychosis, especially for the maintenance therapy of acute and chronic schizophrenia. Recently captured wild animals or animals being translocated may show alarm symptoms similar to those shown by schizophrenic patients, such as anxiety, agitation, psychomotor excitement, and aggressiveness, and these need to be controlled (Ebedes 1993). For veterinary use, the following LANs have been suggested: perphenazine enanthate, pipothiazine palmitate, fluphenazine decanoate, zuclopenthixol decanoate, flupenthixol decanoate, and zuclopenthixol acetate. Among the LANs available, the use of perphenazine has been extensively reported in wild animals during recent years. The onset of effect is slow, with sedation and calming effects in wild animals first occurring about 12–16 h after injection. Maximum effect is usually observed on the third day, with the duration of effect being up to seven days (Ebedes 1993).

Effects observed in wild antelopes treated with LANs have included alteration of mood, indifference to surroundings, and loss of fear of humans (Ebedes 1993). Although LANs have been used for more than 40 years (Morris & Jarris 1960), most records refer to their use in ungulates, especially in South Africa, and little data are available for any species of carnivore (Winterer & Wiesner 1998). We did not find any reference to the use of LANs in otters.

The Eurasian otter (*Lutra lutra*) is one of 13 species of the family Lutrinae. Although its former distribution range was larger than that of any other species of otter (Kruuk 1995), numbers of Eurasian otters began to diminish at an increasing rate at the beginning of the 20th century, until the species became restricted to the most isolated and wild areas where nature was still well-conserved (Foster-Turley *et al* 1990; Ruiz-Olmo 2001). In Spain, the Eurasian otter still thrives in the western half of the country, whereas in the eastern part most populations have been severely decimated (Delibes & Rodriguez 1990). Results obtained in the last Spanish otter survey could indicate the recovery of the species in some areas (Ruiz-Olmo & Delibes 1999). Translocation projects for the otter have been carried out in several countries (Serfass *et al* 1996; Sjöåsen 1997), including Spain (Saavedra & Sargatal 1998), where a reinforcement program is currently underway to strengthen the eastern populations with animals from the western part of the country.

Several blood parameters (haematological and biochemical), together with other physiological parameters, are sensitive indicators of alterations in animal homeostasis during capture and periods of stress (Kock *et al* 1987; Rietkerk *et al* 1994), and have been proposed to be reliable indicators of stress levels during wild animal management (Morton *et al* 1995; Whittington & Grant 1995; Marco *et al* 1997). Some of the blood parameters cited as stress indicators are haematologic values such as haemoglobin (Hb), red blood cell count (RBC), and white blood cell count (WBC), and others are biochemical values such as blood urea, albumin, AST, CK, LDH, alkaline phosphatase (AP), alanine aminotransferase (ALT), and cortisol (Kock *et al* 1987; Morton *et al* 1995; Marco *et al* 1997). Different studies have revealed significant differences in these variables in relation to the methods used for capturing and handling animals (Hatting *et al* 1988).

The aims of this study were: 1) to evaluate the influence of stress on several haematological and biochemical parameters in wild-caught Eurasian otters during a reintroduction project, and 2) to assess stress levels in relation to the use of long-acting neuroleptics and the time that animals spend in human care.

Materials and methods

Twenty-eight adult Eurasian otters were live-trapped in South Western (Extremadura: 39º30'N; 6º30'W) and Northern (Asturias: 43º30'N; 6º30'W) Spain between November 1995 and May 1998. Padded leg-hold traps (#1–1.5 Soft Catch, Woodstream Corp, Lititz, Pennsylvania, USA) were placed at night and recovered the following morning as described elsewhere (Serfass *et al* 1996). The time that otters remained in the traps ranged between 1–8 h.

Once the animals were located at the trap sites, they were covered with a net (see Fernandez-Moran *et al* 2001b) and chemically immobilised by a manual (i.m.) injection of 5 mg/kg body weight of ketamine hydrochloride (100 mg/ml, Imalgene 1.000®, Rhône Merieux, Lyon, France) plus 50 mg/kg body weight of medetomidine (1 mg/ml, Domtor®, Orion Corporation, Finland).

Eleven otters, ie the treatment group, were injected (i.m.) with the LAN, perphenazine enanthate (100 mg/ml, Trilafon® enantat, Schering-Plough BV, Maarssen, The Netherlands), at a dosage of 2.9–5.4 mg/kg (average: 4.4 mg/kg). The remaining 17 otters were untreated and formed the control group. All of the otters were transported to Barcelona Zoo (BZ), where they were individually housed indoors in wire-mesh cages (2.44 m long × 1.22 m wide × 1.22 m high) suspended above the ground, with attached wooden nest boxes (0.91 m long × 0.61 m wide × 0.51 m high). Food and water were offered

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ad libitum and the diet consisted of a mixture of fresh or thawed trout, chicks, fresh eels, and crayfish for the first 3–5 days, and later only fresh trout. Otters remained at BZ for a period of 20–30 days (average: 23 days), during which they were subjected to clinical examinations, quarantine, and surgery for intraperitoneal radiotransmitter implantation. No human contact occurred during this period apart from visual inspection at feeding and cleaning times.

All of the animals were blood-sampled three times during their time in captivity. The majority of the otters began to eat in captivity after between 2–5 days at BZ. Eating in captivity was taken to indicate sufficient adaptation to the new environment to permit safe chemical immobilisation. At that time the otters were immobilised, treated for capture-related injures, and blood-sampled (sample A). Once they were considered free of disease (according to clinical studies that usually took place 5–10 days post-capture [see Fernandez-Moran *et al* 2002]), they were immobilised again for intraperitoneal implantation of the radiotransmitter device (Hoover 1984; Arnemo 1991). Otters showing signs of illness other than superficial wounds, lacerations, or missing nails, were not operated upon and thus were not included in the study. Blood was obtained before performing the surgical procedure (sample B). Following surgery, otters were given a penicillin–streptomycin combination (100,000 IU-100 mg s.c., Dipenisol Retard, Bayer, Bayer, S.A. Barcelona, Spain). Animals were allowed to recover from surgery for a period of between 10–12 days. After that, the otters were immobilised in order to carry out a post-surgery check-up prior to their release into the wild. At that time (20–30 days after capture) the otters were blood-sampled again (sample C).

Prior to each immobilisation, the animals were fasted for at least 5 h. After anaesthesia (following the methods described elsewhere [Fernandez-Moran *et al* 2001b]), otters were positioned on their backs and 10 ml of blood was obtained from the jugular vein using a 20 gauge needle. Seven ml of blood were deposited into Vacutainer® tubes (Becton-Dickinson, Rutherford, New Jersey, USA) for preparation of serum, and 3 ml into tubes coated with ethylene diamine tetracetic acid (EDTA) for haematology. The blood collected for serum chemistry determinations was allowed to clot at 20ºC and was then centrifuged, and the serum was separated and kept at 4ºC until the determinations were made. The following haematological parameters were measured (as described by Fernandez-Moran *et al* 2001a): RBC, Hb, and WBC. Biochemical profiles were measured (as described by Fernandez-Moran *et al* 2001a) and included: AST, ALT, AP, LDH, CK, blood urea, total bilirubin, albumin, and serum cortisol.

Each otter was monitored during anaesthesia for pulse, respiration rate, oxygen saturation (N-20P, Nellcor Inc, Hayward, California, USA), and rectal temperature. Thereafter, and at least 30 mins after the induction, anaesthesia was reversed by injection (i.m.) of 5 mg atipamezole (5 mg/ml, Antisedan®, Orion Corporation, Espoo, Finland) per mg of medetomidine hydrochloride previously administered.

Haematology and biochemical parameters were analysed with repeated measures analysis of variance (ANOVA) using the SPSS statistical program (SPSS, Chicago, USA).

Results

The values of 17 haematological and serum chemistry parameters measured on three occasions in the 28 treated and untreated wild-caught Eurasian otters are shown in Table 1. Statistically significant differences were observed between perphenazine-treated animals and untreated animals only for monocyte count (means: 0.4 versus 1.2; 0.4 versus 0.7; and 0.3 versus 0.6 [10^9/litre] for untreated versus treated animals, samples A, B, and C respectively; $P < 0.05$ for all tests). Consequently, results were combined for the entire sample of 28 otters. Values for RBC, Hb, WBC, segmented neutrophils, blood urea, ALT, AST, CK, LDH, AP, and albumin were significantly affected by time ($P < 0.05$ for all tests). RBC and Hb increased over time (Figures 1a & 1b), while the WBC and segmented neutrophil counts decreased (Figures 1c & 1d). Most biochemical parameters did not change significantly, but blood urea, ALT, AST, CK, LDH, and AP all decreased significantly over time (Figures 1e–j). Contrary to this, albumin increased significantly (Figure 1k). Although not statistically studied, treated otters could be approached more easily and were calmer than those in the control group.

Discussion

We will discuss first the effects of time. Two types of stress reactions have been described in newly captured animals: the primary short-term traumatic stress inflicted upon an animal during the acts of pursuit, capture, and initial physical manipulation, and the secondary long-term fatiguing stress imposed upon the animal during transport, confinement, and adaptation to captivity (Nielsen 1999). For the otters in the current study, it is difficult to separate these two kinds of stress response because each time the otters were manipulated they were stressed in some way (although the methodology followed was the same for each animal). The statistical differences observed for RBC and Hb over time are difficult to explain. These two parameters started high, fell in the second sample and then increased in the third (see Figures 1a & 1b). In domestic animals, factors described as producing a reduction in these parameters are anaemia, end of gestation, tranquillisation and anaesthesia, and haemolysis (Bush 1991). Although it is possible that transport, capture, and adaptation to captivity caused mild anaemia, the otters recovered quickly; furthermore, the RBC and Hb values in the three samples were within the normal range published by Lewis *et al* (1998). On the other hand, we observed higher WBC and neutrophil counts in the first samples, which then decreased constantly throughout the study. The effect of stress on WBC count varies with species and depends upon the normal relative leukocyte distribution. Dogs, cats, and possibly otters, having relatively low lymphocyte counts, respond to stress with an increase in leukocytes (Bush 1991). Leukocytosis and neutrophilia in other carnivores and in ungulates have been

Table 1 Mean values (and standard deviations) of 17 haematological and serum chemistry parameters measured on three occasions (A, B, and C) for the 28 treated and untreated wild-caught Eurasian otters. A: 2–5 days post-capture, B: 5–10 days post-capture, C: 20–30 days post-capture.

Parameter	n	A		B		C	
		Mean	SD	Mean	SD	Mean	SD
White blood cells (10^9/litre)	25	12.31	7.11	10.60	4.40	7.06	3.28
Red blood cells (10^9/litre)	25	6.01	1.09	5.80	0.99	6.52	0.58
Haemoglobin (g/dl)	25	14.7	2.7	13.9	2.4	15.4	1.6
Segmented neutrophils (10^9/litre)	25	8.97	5.54	7.34	3.62	4.5	2.67
Band neutrophils (10^9/litre)	25	0.29	0.48	0.27	0.35	<0.1	<0.1
Lymphocytes (10^9/litre)	25	1.85	1.18	1.78	0.72	0.1	0.25
Monocytes (10^9/litre)	25	0.78	1.14	0.7	0.51	0.44	0.47
Eosinophils (10^9/litre)	25	0.44	0.53	0.45	0.3	0.53	0.33
Basophils (10^9/litre)	25	1.04	2.9	2.16	0.11	1.2	2.76
CK (iu/litre)	27	10056.3	17947.5	911.4	641.2	723.4	435
AP (iu/litre)	26	80.5	45	62.7	30.8	56.3	33.9
ALT (iu/litre)	28	484	390	168.3	132.7	86.2	26.7
AST (iu/litre)	27	764.5	972.9	215.8	106.3	172.2	63.7
LDH (iu/litre)	26	3523.3	2173	1982.7	1039.6	1889.3	1007.2
Albumin (g/litre)	23	27.0	3.1	28.1	3.8	30.7	4.3
Blood urea (mmol/litre)	28	16.8	10.7	13.8	4.0	12.0	3.8
Cortisol (mmol/litre)	21	361.6	1139.8	41.4	30.4	35.9	27.6

attributed to capture stress (Kreeger *et al* 1990; Rietkerk *et al* 1994; Weaber & Johnson 1995), and this would suggest that the stress response decreased with time in captivity in our otters. Also, the otters may have suffered infected wounds or lesions during capture that would have improved over time, thus decreasing the leukocyte number. Treatment of injured otters with antibiotics upon arrival at BZ, as well as prophylactic treatment during surgery on all otters, may also have led to the reduction of the leukocyte count (Fernandez-Moran *et al* 2002). Values obtained in sample C were also within the range of those previously published (Lewis *et al* 1998).

Evaluations of serum CK are especially useful and, although the dynamics of CK in the serum of wild-caught otters have not been determined, high CK levels seem to reflect active or very recent muscle degeneration and/or myonecrosis. Recently captured otters in this study presented high values of these enzymes, suggesting them to be highly stressed or approaching this condition. As a consequence of an increase in protein catabolism, stress may induce hypoalbuminemia. In our case, the albumin fraction increased during captivity, possibly because of a reduction in stress levels. Blood urea also decreased while the otters were in captivity, but this parameter may be more closely related to diet than to stress.

When otters were maintained in quiet places without human contact for many days, the abnormal values for parameters advocated as stress indicators, ie high leukocytes, neutrophils, AST, ALT, LDH, CK, and low albumin, were gradually reduced (and, in the case of albumin, increased), and stabilised over the two last samples. Sample C reflects normal values for these parameters, similar to those reported for this species (Lewis *et al* 1998).

Plasma cortisol level has been extensively used as a stress indicator (Harlow *et al* 1987; Parrott *et al* 1994; Morton *et al* 1995), and its determination together with other variables has been suggested to be the best method for assessing stress in wild animals. In our case, no statistical difference in cortisol level was found between the three blood samples, probably because cortisol levels were consistently high as a result of the animals' short-term stress responses to being injected.

There are differing opinions regarding when to release wild-caught animals. The American Society of Mammalogists, in its guidelines for the capture, handling, and care of mammals, recommends that translocated animals should be released as soon as possible after capture to minimise behavioural or physiological stress resulting from conditions in captivity (American Society of Mammalogists 1998). In the same way, Arnemo (1991) opted for immediate surgery and early release (0–5 days post-surgery) to avoid further stress and to minimise the risk of abnormal behaviour in the capture and translocation of 5 wild otters in Norway (although incomplete healing has been reported in North American river otters following implantation [Woolf *et al* 1984]). Hoover and colleagues (Hoover 1984; Hoover *et al* 1984, 1985) kept all otters intended for reintroduction for 5 days of post-operative daily clinical assessment before release. Spelman (1998) recommended that after capture, otters should be allowed to adjust to their temporary captive

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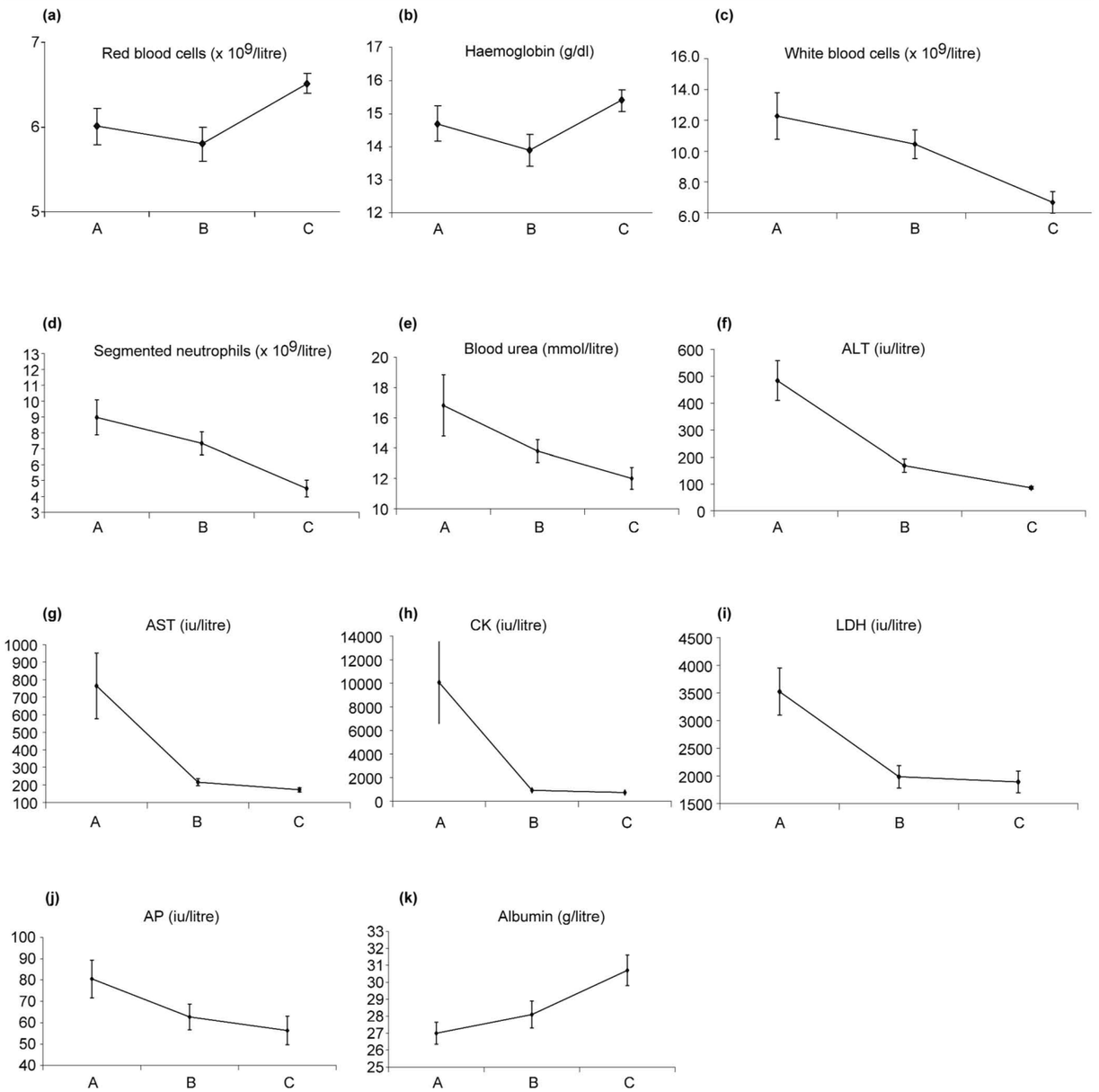
Figure 1



Mean (± standard error) levels of haemotological and biochemical parameters in 28 wild-caught Eurasian otters measured on three occassions in captivity: A: 2–5 days post-capture, B: 5–10 days post-capture, and C: 20–30 days post-capture. (a) red blood cells, (b) haemoglobin (c) white blood cells, (d) segmented neutrophils, (e) blood urea, (f) alanine aminotransferase, (g) aspartate aminotrasferase, (h) creatine kinase, (i) lactate dehydrogenase, (j) alkaline phosphate, and (k) albumin.

environment for several days before anaesthesia. In a North American river otter translocation project carried out in the State of New York (Kolias 1998), otters were held for 10–15 days for assessment, treatment, and pre-release improvement of body condition. In the current study, we followed similar methods to those described by Serfass *et al* (1996). Although these authors did not evaluate stress levels, they assumed that animals would benefit from being in human care before being released (Serfass *et al* 1996). The data we present here suggest that this occurred with our translocated animals. Our results show that the wild-caught otters were in better general homeostatic condition upon release than when recently captured. The same seems to have been true of the otters studied by Kollias (1998), which increased their admission body weight on average by 20%. In similar circumstances to those of the current study, a rest period before release might be beneficial for other reintroduced animals.

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Now we will focus on the effect of LANs. In our study, with the exception of monocyte count, no differences were found when comparing the haematological and biochemical values of the treatment and control groups. As mentioned by Ebedes (1993), in wild animals it is impossible to assess, control, and individualise the dosage of tranquillisers, and the safest alternative is to use the lowest possible effective dose. We found reference dosages only for wild ungulates (ranging between 20–200 mg depending on the size of the animals [Ebedes 1993]), and for zoo felids (ranging between 0.5–0.6 mg/kg [Winterer & Wiesner 1998]). We used a total dose of between 2.9–5.4 mg/kg, which is relatively high compared with dosages reported for use in other species. However, Blumer (1991) pointed out that in ungulates there appears to be an inverse relationship between the dosage of perphenazine enanthate and the average size of the species, with larger species requiring lower doses per unit weight. To our knowledge this is the first time LANs have been used in otters, therefore the dosage used was selected based on the authors' previous unpublished experiences.

Although we do not know whether our dosage reached the adequate therapeutic level, we did not observe adverse effects, such as extrapyramidal symptoms, which have previously been reported with the use of these drugs. Perphenazine enanthate, at the dosage used in this study, was ineffective in suppressing physiological responses to capture stress. However, this does not mean that the administration of this neuroleptic was not beneficial for the otters. They could be approached easily without becoming alarmed, and were calm and unresponsive to human presence. This failure of perphenazine enanthate to reduce the physiological response to capture, while still inducing apparent sedation in undisturbed animals, is consistent with the effects of phenothiazines in human patients and has been previously reported in other species (Knox *et al* 1992). These authors recommend the use of perphenazine enanthate to produce reliable sedation of impala (*Aepycerod melampus*) under circumstances in which the animals are not exposed to handling.

This study confirms previous reports of changes in haematological and serum biochemical values associated with the capture and housing of wild animals, and indicates an improvement in the homeostasis of wild-caught otters while in captivity under proper care. Based on the results obtained in this research, perphenazine enanthate at the dosages used here does not seem to significantly alter the haematological and biochemical parameters involved in the stress response. However, this does not mean that the use of LANs is not valuable in controlling or decreasing the stress suffered by captured wild animals. Indeed, there are many other factors indicative of stress that were not looked at in this paper but are worthy of study, such as body weight, food ingestion, daily faecal cortisol level, behavioural changes, and so on. Further research of these factors should be conducted in the future. Also, we suggest that further work to assess the effects of different dose rates of LANs on stress would be of interest.

Animal welfare implications

In reintroduction programs, animals may suffer stress due to their capture, transport and release into the wild. Until now, most of the research conducted during such programs has focused on social implications and the spreading of infectious diseases. In the present study, levels of stress in wild-caught otters were measured, and variation in stress related to time in captivity and the use of a long-acting neuroleptic drug was studied. These data will help others to improve the welfare of animals involved in reintroduction projects.

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