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*Haematological and biochemical responses of red foxes (*Vulpes vulpes*) to different capture methods and shooting*

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Abstract

*This paper sought to determine whether common haematology and blood biochemistry values might assist in determining the relative welfare outcomes arising from the capture of red foxes (*Vulpes vulpes*) by treadle-snares, Victor Soft Catch® (VSC) #3 traps, cage traps, netting and sampling by shooting. Compared to all other capture methods and shooting, treadle-snared foxes had significantly higher mean albumin (ALB), creatine kinase (CK), red cell count (RCC), neutrophil to lymphocyte (N:L) ratio, sodium (Na), total protein (TP), white cell counts (WCC) and lower glucose (Gl). Treadle-snares were also associated with higher chloride (Cl), haemoglobin (Hb) and packed cell volume (PCV) than cage trapping and netting. Treadle-snares produced indicators of possible muscle damage, exertion and dehydration compared to cage and VSC traps. Cage trapping and netting produced lower indications of exertion, possible muscle damage and dehydration compared to both treadle-snares and VSC traps. These data do not support previous conclusions that due to similar injury scores, treadle-snares and VSC traps produced equivalent welfare outcomes. In restraining traps, injury and death sustained during capture are end-points of poor trapping welfare. Monitoring stress using physiological indicators allows the comparison of the relative potential for different capture techniques to cause pathological and pre-pathological states. As the response of physiological indicators to stress is not independent of time, accurate data on the duration of captivity and the relative intensity of struggling behaviour should be routinely collected when assessing the comparative humaneness of different trap devices.*

Keywords: *animal welfare, foot-hold traps, leg-hold traps, red fox, snares, stress*

Introduction

Leg-hold (or 'foot-hold') traps are widely used for the capture of red foxes (*Vulpes vulpes*; Saunders *et al* 1995; Sharp & Saunders 2005) and dingoes (*Canis lupus dingo*; Fleming *et al* 2001; Allen & Fleming 2004) in Australia. In the United States and Canada they are a common capture method for coyotes (*Canis latrans*), wolves (*Canis lupus*) and foxes (Fox & Papouchis 2004; Iossa *et al* 2007). The assessment of welfare outcomes arising from different leg-hold traps used for dogs and foxes (collectively referred to as 'canids') has mostly relied upon contrasting the extent of visible injuries assessed after capture (eg Tullar 1984; Van Ballenberghe 1984; Olsen *et al* 1986; Onderka *et al* 1990; Houben *et al* 1993; Phillips 1996; Hubert *et al* 1997; Fleming *et al* 1998). Analysis of trauma scores led past researchers to conclude that the treadle-snare (a leg-hold snare) was a more humane device for capturing canids (Stevens & Brown 1987; Fleming *et al* 1998) or that it delivered approximately equivalent welfare outcomes compared to Victor Soft Catch® #3 (VSC) traps (Meek *et al* 1995).

Physical injury caused by traps is only one indicator of potential stress and suffering (Iossa *et al* 2007). Trapping produces a wide range of stressors (Moberg 1985; Gregory 2005) and intense or prolonged stress can have negative impacts upon an animal's welfare (Jordan 2005). Anxiety may result from stressors such as abnormal light exposure, unfamiliar odours, aversive sounds and restricted movement (Morgan & Tromborg 2007). Limb oedema is frequently observed after trapping in leg-hold traps (Andelt *et al* 1999), yet its relationship to the onset of ischaemic injury cannot be easily predicted from gross examination, as necrotic tissue develops over many days or weeks (Walker 1991). Stress can produce pathology such as myocardial lesions, may affect tissue integrity in vital organs (Sanchez *et al* 2002) and increase the risk of infectious disease by reducing the effectiveness of the immune system (Raberg *et al* 1998). Capture myopathy can cause chronic debilitation in some species and predispose them to morbidity and death weeks after capture (Hulland 1993). Dehydration caused by prolonged confinement and/or intense activity during captivity (eg Powell 2005) is not frequently monitored as a potential welfare issue associated with trapping.

Welfare indicators are required to assist with monitoring welfare outcomes in conservation, agricultural (Bonacic

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et al 2006) and vertebrate pest control activities (Marks 2003; Littin *et al* 2004; Littin & Mellor 2005; Sharp & Saunders 2008). While many haematological and biochemical indicators are standardised, cost-effective and widely available, they have been seldom used to monitor the stress associated with different canid traps. The International Organisation for Standardisation (ISO) 'Technical Working Group on Traps' rejected the use of hormone and blood biochemistry to monitor welfare outcomes arising from canid trapping in favour of contrasting relative injury produced by different capture methods (Harrop 2000). Currently there are few data on physiological responses to different traps (Powell 2005).

A range of capture techniques and shooting were used during a study of urban red foxes in Melbourne (Australia) and their influence upon haematology and blood biochemistry values was contrasted with published normal values and those previously reported after known periods of confinement in traps or after shooting. This paper sought to determine whether common haematology and blood biochemistry values might assist in determining the potential welfare impacts of different fox capture techniques and whether the previous conclusions about welfare outcomes based upon injury scores alone for treadle-snares and VSC #3 traps were supported.

Materials and methods

Capture and shooting methods

All foxes were recovered from urban habitats within 20 km of central Melbourne, Australia and during previous studies (Marks & Bloomfield 1998, 1999a,b, 2006; Robinson & Marks 2001) they were routinely blood sampled after capture or shooting. The treadle-snare (Glenburn Motors, Yea, Victoria, Australia) and Victor® 'Soft Catch' #3 trap (VSC, Animal Capture Equipment and Services, Warrick, NSW, Australia) were set as described by Meek *et al* (1995), using fish-based cat-food as a lure. The treadle-snare is shaped like a small banjo and has a circular pan or 'treadle' similar to the Aldridge snare (see Skinner & Todd 1990). A wire-cable snare is placed around the pan and when triggered the snare is thrown up the animal's limb (by the 'thrower' spring), and tightened by another spring (Meek *et al* 1995; Fleming *et al* 1998). Cage traps measuring 1,200 × 450 × 450 mm (length × breadth × height) with a hook and modified floor press trigger (Wiretainers, Preston, Victoria, Australia) or 1,800 × 450 × 600 mm custom-made cage traps were baited with whole chicken carcases. Traps were set on or alongside known fox trails, fences, gates, culverts or outside diurnal shelter sites that were typically beneath houses or on the periphery of patches of blackberry (*Rubus fruiticosus* agg), wandering tradescantia (*Tradescantia albiflora*), African thistle (*Berkheya rigida*), fennel (*Foeniculum vulgare*) and introduced grasses (Marks & Bloomfield 2006). Traps were typically activated after 2000h and inspected at least every four hours during the night and were de-activated and covered before 0600h the next day. Blood samples were also opportunistically taken during fox control programmes that used terrier dogs to 'flush' foxes from shelter sites into 1-m high, 50-m long microfilament 'gill nets' that were set loosely surrounding diurnal shelter sites. Capture was usually completed within < 10 min of deploying the nets and releasing the dogs. Netting with dogs involved a relatively brief but moderately intense level of stress that was not associated with prolonged restraint and was not assumed to have potential for causing dehydration. A sample of shot foxes was taken at urban locations at the end of all research activities when this could be achieved safely. Sub-sonic .22 calibre ammunition was used with a Ruger 10/22 rifle that had been modified with a target-rifle barrel and fitted with a silencer and a telescopic sight. Foxes were head-shot from a distance of < 25 m after being illuminated with a 100 W spotlight. Shot foxes represented the sample of animals probably subjected to the least stress of all methods and were unlikely to be dehydrated due to shooting. Consequently, their blood values were compared against all capture methods (following Kreeger *et al* 1990a).

Sedation and blood sampling

Live captured foxes were covered with a hessian sack, restrained with a hand noose and dosed with an intramuscular injection of 4 mg kg^{-1} of a tiletamine/zolazepam combination (Zoletil®: Virbac, Sydney, NSW, Australia) to produce deep sedation and light anaesthesia. Tiletamine and zolazepam combinations have been used successfully for minor surgery in foxes without an indication of causing a significant alteration in haematology and blood biochemistry values (Kreeger *et al* 1990b). A 30 ml sample of blood was taken from the jugular vein with a 1 × 30 mm (19 G) needle and apportioned into 10-ml lithium heparin, EDTA and clot vacutainer tubes (Becton-Dickenson, Melbourne, Australia). Blood samples were taken close to the point of capture usually within the first hour and before the anaesthetic had fully abated. If anaesthesia was insufficient, an hour was allowed to elapse before administering the full dose again. After shooting, blood samples were taken within two minutes by cardiac puncture immediately after death had been confirmed by the loss of corneal reflex. Vacutainers were transported to Dorevitch Pathology (Camberwell, Victoria, Australia) at 0600h the following morning for haematology and biochemistry analysis with reference to: red cell count (RCC); packed cell volume (PCV); mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); platelets (PLT); white cell count (WCC); haemoglobin (Hb); neutrophils (N); lymphocytes (L); eosinophils (E); urea (BUN); albumin (ALB); glucose (Gl); total protein (TP); creatine kinase (CK); alkaline phosphatase (ALP); sodium (Na); potassium (K); chloride (Cl); bicarbonate (HCO^{3-}); and triglyceride (TAG). Relative changes in blood profiles for capture method and shooting that were potentially indicative of exertion, muscle damage, dehydration and restraint stress in trapped foxes (Kreeger *et al* 1990a; White *et al* 1991) or in other canids (Thrall *et al* 2004; Wakshlag *et al* 2004) were compared (Table 1).

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Statistical analysis and comparison with published data

Foxes were deemed to be adults (or older sub-adults) if their weight exceeded 3 kg and they were at least 9 months old, based upon minimum estimated weights and age at the time of capture using August as the birth month in Melbourne (Robinson & Marks 2001; Marks & Bloomfield 2006). Data were transformed if non-normally distributed prior to analysis. Comparisons of capture method with haematological and blood biochemistry values were analysed with a general linear model (GLM) using the least significant difference (LSD) test for *post hoc* comparison. Relationships between adult fox gender, weight and capture method were tested using binary logistic regression (SPSS version 16: SPSS, Chicago, USA). Comparisons were made with published accounts of blood values following trapping in VSC traps, shooting (Kreeger *et al* 1990a), cage traps (White *et al* 1991) and normal blood data based upon sampling a mixed population of captive silver and red foxes (both *V. vulpes*; Benn *et al* 1986).

Results

A total of 125 foxes were recovered and no trap deaths were recorded, although two foxes were euthanased immediately upon inspection of the trap site as they had sustained major trapping injuries (broken leg caused by a treadle-snare and trauma to the scrotum caused by a VSC #3 trap). Blood sampling was not attempted given that it would have produced additional suffering from handling and samples would have been taken > 10 min post mortem. Another 35 juvenile foxes were excluded from the sample due to likely age-related differences in haematology and biochemistry that precluded pooling these data and the existence of incomplete blood profiles related to inadequate volumes of blood taken from cubs. Foxes held by either VSC traps or treadle-snares typically had mild oedematous swelling of the restrained limb two hours after capture but injuries in cage-trapped or netted foxes were uncommon and were restricted to a few cases of minor lacerations on the gums if foxes had bitten at the netting or steel mesh of the cage trap.

Blood was successfully analysed from a total of 88 adult foxes (38 females, 50 males) captured with cage traps (n = 8), netting (n = 17), treadle-snares (n = 45) and VSC traps (n = 7) and via shooting (n = 11). There was no significant relationship between the capture method and gender (ß = –0.28, Wald = 1.62, df = 1, $P = 0.760$) or the mean weight of males (5.2 [± 1] kg) and females (4.7 [± 1.44] kg) ($F = 1.9$, df = 1, $P < 0.174$). Inconsistent records for ALP and E values produced a small data set and precluded analysis. Insufficient data were available to test for sex and weight responses, so these were pooled for analyses. Capture methods had no significant effects upon HCO^{3-}, TAG, BUN, MCV, MCH or PLT. Significant effects were detected for RCC ($F = 17.7$, df = 4, $P < 0.001$), PCV ($F = 19.1$, df = 4, $P < 0.001$), WCC ($F = 15.5$, df = 4, $P < 0.001$), Hb ($F = 3.07$, df = 4, $P < 0.05$), neutrophil to lymphocyte ratio (N:L) ($F = 10.8$, df = 4, $P < 0.001$), ALB ($F = 21.8$, df = 4, $P < 0.001$), Gl ($F = 12.4$, df = 4, $P < 0.001$), TP ($P = 20.0$, df = 4, $P < 0.001$), CK ($F = 60.7$, df = 4, $P < 0.001$), Na ($F = 18.6$, df = 4, $P < 0.001$), K ($F = 15.5$, df = 4, $P < 0.001$) and Cl ($F = 3.3$, df = 4, $P < 0.05$).

Table 1 Haematology and biochemistry profiles indicative of exertion, muscle damage, dehydration and restraint stress, previously used as markers in trapped foxes and canids due to variations (increase or decrease) relative to normal or control values (after Wintrobe 1976; Kreeger *et al* 1990a; White *et al* 1991; Thrall *et al* 2004; Wakshlag *et al* 2004).

	Exertion	Muscle damage	Dehydration	Restraint stress
Increase	CK, PCV	CK	ALB, BUN, Cl, Na, TP, PCV	WCC, RCC, Hb, N, N:L, Gl, CK, ALP
Decrease	Gl			E, L

Foxes captured in treadle-snares had significantly higher mean ALB, CK, RCC, N:L ratio, Na, TP and WCC and lower Gl when compared to all other capture methods. Compared to cage trapping and netting, treadle-snares were also associated with higher Cl, Hb and PCV values. Foxes captured in VSC #3 traps had significantly higher mean ALB compared to shot foxes and higher mean CK values than observed in foxes that had been shot, netted or captured in cage traps. Compared to cage traps, VSC #3 traps also had significantly higher Na, PCV and TP. Shot foxes had higher K than any capture method and a significantly higher concentration of Na than those captured in a cage traps or by netting (Table 2).

Discussion

Comparison of capture and shooting data

Treadle-snares had a significantly greater effect upon blood values than VSC #3 traps and corresponded closely with those reported for foxes held in VSC #1½ traps for 8 h for WCC, ALB, TP, CK, N:L and RCC (Kreeger *et al* 1990a). Kreeger *et al* (1990a) concluded that leg-hold traps produced a classic stress response characterised in part by an increase in hypothalamic-pituitary-adrenal (HPA) axis hormones, neutrophilia and elevated CK. Stress may reduce the number of neutrophils held in marginal pools in some species and increase the number circulating, yet is contingent upon the nature and intensity of a stressor (Oishi *et al* 2003). The N:L ratio may not be immediately detectable after short periods of stress, yet was informative about trapping stress in foxes held in padded leg-hold traps for two hours (Kreeger *et al* 1990a). Foxes captured using VSC #3 traps for four hours in the Melbourne study revealed similar increases in ALB, CK, WCC and Na values that were intermediate between those found after foxes were held for 2 and 8 h in VSC # 1½ traps (Kreeger *et al* 1990a). In previous studies, foxes held in a cage trap for 8 h (White *et al* 1991) had higher mean

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Table 2 Mean haematology and blood biochemistry values with sample size (n), standard error (± SE) and standard deviation (± SD) for foxes recovered using cage traps (C), netting (N), shooting (S), treadle-snares (T) and Victor Soft Catch #3 traps (V). The level of significant difference from multiple comparisons using the Least Significant Difference (LSD) test is given at two probability (*P*) levels.

		Unit	Method	n	Mean	(± SE)	(± SD)	$P < 0.05$	$P < 0.01$
Haemoglobin	Hb	g L^{-1}	C	8	104	3.7	10	T	S
			N	17	116	6.4	26	S, T	
			S	11	125	7.4	25	N	C
			T	45	152	2.8	19	C, N	
			V	7	135	4.6	12		
Neutrophils: Lymphocytes	N:L	ratio	C	8	4.0	1.4	4.0		T
			N	17	2.3	0.4	1.6		T
			S	11	5.4	2.1	7.0		T
			T	45	22.0	2.8	18.8		V, C, N, S
			V	7	5.9	1.9	5.0		T
Packed cell volume	PCV	%	C	8	35.2	0.25	0.7	N	S, T, V
			N	17	37.3	1.6	6.6	C, V	T
			S	11	39.6	2.2	7.3		C, T
			T	45	48.9	0.87	5.8		C, N, S
			V	7	42.1	1.0	2.6	N	C
Red cell count	RCC	$\mu L^{-1} \times 10^{-6}$	C	8	8.3	0.31	0.9	N	S, T
			N	17	8.9	0.41	1.7	C	T
			S	11	9.0	0.54	1.8		C, T
			T	45	11.3	0.2	1.3		S, V, C, N
			V	7	10.13	0.24	0.6		T
White cell count	WCC	$\mu L^{-1} \times 10^{-3}$	C	8	9.03	1.5	4.2	S, T	
			N	17	6.1	0.9	3.7		T
			S	11	3.8	1.1	3.6		T
			T	45	12.3	0.8	5.4		C, N, S, V
			V	7	5.7	1.4	3.7		T
Albumin	ALB	g dL^{-1}	C	8	2.6	0.1	0.3		T
			N	17	2.7	0.1	0.4		T
			S	11	2.7	0.1	0.4	V	T
			T	45	3.4	0.7	0.5		V, C, N, S
			V	7	3.0	0.1	0.2	S	T
Chloride	Cl	mmol L^{-1}	C	8	109.3	1.4	4.0	T	
			N	17	114.3	0.8	3.3	V	T
			S	11	113.4	1.1	3.6		
			T	45	116.7	0.6	4.0	C	N
			V	7	116.0	2.1	5.6	N	
Creatine kinase	CK	log IU L^{-1}	C	8	6.3	0.33	0.9		T, V
			N	17	6.2	0.33	1.4		T, V
			S	11	6.3	0.21	0.7		V, T
			T	45	9.5	0.13	0.9		C, N, S, V
			V	7	7.7	0.76	2.0		C, S, N, T
Glucose	Gl	mmol L^{-1}	C	8	6.0	0.4	0.7		T
			N	17	7.6	0.8	2.7		T
			S	11	7.5	1.0	2.8		T
			T	45	3.5	0.3	2.1		C, N, S, V
			V	7	6.5	1.6	3.6		T

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Table 2 (cont)

		Unit	Method	n	Mean	(± SE)	(± SD)	$P < 0.05$	$P < 0.01$
Potassium	K	mmol L^{-1}	C	8	4.7	0.2	0.6		S
			N	17	4.4	0.1	0.4	T, V	S
			S	11	5.9	0.3	1.0		C, N, T, V
			T	45	4.7	0.1	0.7	N	S
			V	7	5.1	0.2	0.5	N	T
Protein (total)	TP	g dL^{-1}	C	8	5.0	0.2	0.5	V	T
			N	17	5.4	0.2	0.9		T
			S	11	5.3	0.2	0.7		T
			T	45	6.6	0.1	0.7	V	C, N, S
			V	7	5.9	0.3	0.7	C, T	
Sodium	Na	mmol L^{-1}	C	8	139	1.1	3.1		V, S, T
			N	17	141.6	1.0	4.1	V	S, T
			S	11	144.9	0.9	3.0	T	N, C
			T	45	149.0	0.7	4.7	V	S, C, N
			V	7	144.8	0.9	2.4		C, T

values for ALB, CK, Hb, RBC and N:L ratio compared to those held for < 4 h in cage traps in Melbourne.

Cage trapping, netting and shooting produced lower indications of intense activity or muscle damage as indicated by lower elevations of CK (Noakes 1987; Kreeger *et al* 1990a) compared to both treadle-snares and VSC traps. Treadle-snares and VSC traps produced mean elevations of CK that were 24.5 and 4.1 times greater than found in shot foxes, respectively. Activation of the HPA-axis following capture with leg-hold traps caused a period of vigorous struggling that is likely to greatly influence the degree of physical trauma experienced (Kreeger *et al* 1990a) and the onset of pre-pathological states. Intense struggling immediately following capture in VSC #1½ traps decreased in magnitude after the first two hours (Kreeger *et al* 1990a) and a similar pattern was observed for foxes captured in cage traps (White *et al* 1991) and dingoes captured in VSC #3 traps fitted with activity-monitoring devices (Marks *et al* 2004). Elevated CK, indicative of intense activity and/or muscle damage, was found in foxes captured in padded and unpadded leg-hold traps (Kreeger *et al* 1990a), but was not elevated significantly in those captured in cage traps (White *et al* 1991) and the current results are consistent with these previous studies. Elevated ALB, Hb, Cl, Na, PCV and TP values in treadle-snared foxes indicated a trend towards dehydration that was less evident in VSC traps when compared to cage-trapped or netted foxes. Significant decreases in Gl recorded for treadle-snared foxes contrast with increases in Gl in silver foxes as a marker of restraint stress (Moe & Bakken 1997). Hypoglycaemia has only typically been seen in normal dogs immediately after they were subjected to strenuous exercise, possibly corresponding to muscle glycogen depletion (Wakshlag *et al* 2004). This explanation for the observed hypoglycaemia is consistent with greatly elevated CK and a trend towards dehydration resulting from intense or more protracted struggling in foxes captured in treadle-snares, associated with greater stress indicated by a significantly elevated N:L ratio. Black bears (*Ursus americanus*) captured in Aldridge snares had higher CK and ALB values and this was attributed to greater exertion, muscle damage and dehydration compared to values generated from individuals captured by remote activated tranquilising collars (Powell 2005). Elevation of CK has also been reported for polar bears (*Ursus maritimus*) captured in snares (Lee *et al* 1977; Schroeder 1987; Hubert *et al* 1997). Grizzly bears (*Ursus arctos*) had higher N:L ratios, as well as increased concentrations of Na and Cl that were attributed to dehydration over 2–23 h of captivity in snares, probably aggravated by intense activity (Cattet *et al* 2003).

There may be difficulties in comparing the response of some blood parameters due to unavoidable inconsistency in the application of the different capture and sampling methods. As netting did not require a prolonged period of restraint, opposed to the other trapping methods used, it is unknown whether some blood parameters such as N:L ratio had sufficient time to fully respond to the relatively brief period of stress encountered before blood sampling. Mean N:L ratios after capture in treadle-snares were 4.1 times greater than found in shot foxes, but only 0.43 of this value in netted animals. While Moe and Bakken (1997) concluded that the HPA and the sympathoadrenal medullary (SAM) system in silver foxes was activated within 5 min of handling and restraint, the time-period from the onset of netting stressors to blood sampling was much shorter than other capture methods used in the Melbourne study. The

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Table 3 Published mean haematology and blood biochemistry values with sample size (n), standard error (± SE) and standard deviation (± SD) taken after red foxes were held in cage (C) or Victor Soft Catch #1½ (VSC) traps for known times in hours (h) or where samples were taken from shot (S) foxes, captive populations for blood normals (Norm) and immediately prior to surgery (PRS) and eight hours post-surgery (POS).

		Unit	Group	n	h	Mean	(± SE)	(± SD)
Haemoglobin	Hb	g L^{-1}	C[1]	10	8	136	7.0	22.1
			Norm[3]	30	–	170	2.6	14.2
			PRS[4]	20	–	155	2.0	8.9
Neutrophil: Lymphocytes	N:L	ratio	C[1]	10	8	10.4	0.7	2.2
			S[2]	19	–	2.1	0.6	2.6
			VSC[2]	6	2	10.5	1.5	3.7
			VSC[2]	4	8	25.1	1.8	3.6
			Norm[3]	30	–	0.9	0.2	1.1
Packed cell volume	PCV	%	C[1]	10	8	42.8	2.6	8.2
			S[2]	20	–	50.2	1.5	6.7
			VSC[2]	6	–	44.2	2.9	7.1
			VSC[2]	9	–	46.7	5.3	15.9
			Norm[3]	30	–	48.0	0.7	4.0
			PRS[4]	10	–	48.1	0.4	1.3
Red cell count	RCC	$\mu L^{-1} \times 10^{-6}$	C[1]	10	8	9.4	0.6	1.9
			S[2]	20	–	11.6	0.3	1.3
			VSC[2]	6	2	10.9	0.6	1.5
			VSC[2]	4	8	11.8	0.9	1.8
			Norm[3]	30	–	10.8	0.1	0.5
			PRS[4]	20	–	11.6	0.1	0.4
White cell count	WCC	$\mu L^{-1} \times 10^{-3}$	C[1]	10	8	7.1	1.1	3.5
			S[2]	20	–	3.4	0.4	1.8
			VSC[2]	6	2	4.2	1.0	2.4
			VSC[2]	4	8	7.8	1.9	3.8
			Norm[3]	30	–	9.3	0.4	2.2
			PSR[4]	10	–	7.6	0.6	1.9
			POS[4]	10	8	11.7	0.7	2.2
Albumin	ALB	g dL^{-1}	C[1]	10	8	3.0	0.1	0.3
			S[2]	6	–	3.1	0.1	0.2
			VSC[2]	5	2	3.1	0.1	0.2
			VSC[2]	23	8	2.9	0.1	0.5
			Norm[3]	30	–	2.9	0.7	3.8
			PRS[4]	20	–	3.4	0.1	0.4
Creatine kinase	CK	log IU L^{-1}	C[1]	10	8	7.3	0.2	0.6
			S[2]	23	–	6.6	0.3	1.4
			VSC[2]	6	2	6.9	0.4	1.0
			VSC[2]	5	8	10.8	0.3	0.7
			Norm[3]	30	–	1.9	0.2	1.1
			PRS[4]	10	–	2.6	2.0	6.3
			POS[4]	10	8	3.6	2.8	8.9
Glucose	Gl	mmol L^{-1}	Norm[3]	30	–	7.6		1.1

[1] White *et al* (1991); [2] Kreeger *et al* (1990a); [3] Benn *et al* (1986); [4] Kreeger (1990b).

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Table 3 (cont)

		Unit	Group	n	h	Mean	(± SE)	(± SD)
Protein (total)	TP	g dL^{-1}	C[1]	10	8	4.6	0.1	0.3
			S[2]	23	–	4.8	0.2	1.0
			VSC[2]	6	2	5.3	0.3	0.7
			VSC[2]	5	8	5.1	0.2	0.4
			Norm[3]	30	–	6.5	0.1	0.5
			PSR[4]	10	–	5.4	0.1	0.3
Sodium	Na	mmol L^{-1}	C[1]	10	8	150.4	1.4	4.4
			S[2]	23	–	144.4	2.3	11.0
			VSC[2]	6	2	157.3	1.6	3.9
			VSC[2]	5	8	138.6	4.6	10.3
			Norm[3]	30	–	156	0.8	4.4

PCV taken from shot foxes by Kreeger *et al* (1990a) were higher than normal values reported by Benn *et al* (1986) or those from cage-trapped foxes (White *et al* 1991) and this may be an artefact of blood sedimentation post mortem. Similarly, elevated K values (hypokalaemia) are known to be a consequence of severe trauma, shock and release of intracellular K (Baekhyo *et al* 1986) that may be consistent with gunshot wounding, but also due to red cell haemolysis (Thrall *et al* 2004) and it is possibly linked to the blood collection by cardiac puncture post mortem. Moreover, CK values were reported to be substantially lower in captive wild red foxes before and after surgery (Kreeger *et al* 1990b) compared to shot fox samples in the United States (Kreeger *et al* 1990a) and the Melbourne data. This is possibly because shooting trauma also elevates CK values, as seen in shot pigs (Münster *et al* 2001) and after brain gunshot trauma (Kaste *et al* 1981). However, the standard errors observed for the mean blood values obtained from shot foxes in Melbourne overlap with those reported by Kreeger *et al* (1990a) and White *et al* (1991) for CK, Na, TP and WCC, and closely approximated the ALB and N:L values suggesting some consistency for blood data collected by shooting. Nonetheless, blood profiles from shot foxes may not be representative of normal values and they should be used with caution unless various parameters are confidently known to be unaffected by shooting and post mortem sampling. Excitement and strenuous exercise can cause contraction of the spleen and expulsion of erythrocytes into circulation (Wintrobe 1976) and this may alter normal RBC, Hb and PCV (Hajduk *et al* 1992). Blood normals for captive-bred foxes had higher Hb and RCC and were attributed to splenic contraction as a stress response during blood sampling in manually restrained and unsedated foxes (Benn *et al* 1986). To prevent this, some studies have used transponder collars to remotely anaesthetise free-ranging animals prior to blood sampling (eg Powell 2005) and this appears to provide less equivocal blood normals for free-ranging animals (Table 3).

Although significant stress due to intense struggling, potential muscle damage and a trend towards dehydration was suggested after four hours of restraint in treadle-snares and to a lesser degree VSC traps, no deaths or debilitation following their release was associated with trapping injury in radio-collared adult foxes (Marks & Bloomfield 2006) and cubs (Robinson & Marks 2001), which were frequently observed for up to two years. Of these, 13/20 adults had been captured by treadle-snares and no obvious diminished mobility was seen after release (CA Marks unpublished data) nor were injuries related to prior trapping observed upon later capture (Marks & Bloomfield 1999b). Bubella *et al* (1998) radio-collared and observed 40 red foxes that had been captured with treadle-snares in an alpine habitat. Treadle-snares were inspected during early morning and periods of captivity of 12 h or less may be assumed as captures predominantly occurring at night. Recovered foxes had signs of oedema and skin abrasions, yet no deaths or debilitation, deformation of limbs or limping was observed in the two years of the study. Nine foxes that were later recaptured showed no sign of having been trapped previously (Bubella *et al* 1998). Therefore, foxes appear to recover from the stress associated with treadle-snare captures of approximately 12 h in duration and their survival does not appear to be compromised. However, there are no data published indicating the physiological impact of either treadle-snares or VSC traps upon foxes held for 24 h, which represents a commonly accepted minimum inspection period for traps (Andelt *et al* 1999). Longer periods confined to leg-hold traps are thought to be associated with correspondingly larger exertion, struggling, injury and death (Powell & Proulx 2003) and the level of physiological response corresponding to morbidity and chronic debilitation in foxes after capture remains speculative.

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Why do treadle-snares cause a greater physiological response?

Treadle-snares require adequate clearance from obstacles to allow the mechanism to function without obstruction, whereas VSC traps can be placed closer to or beneath overhanging vegetation. In this study, treadle-snares were tethered to a solid fixture by 2-m lengths of snare cable and chain, in contrast to 0.5-m long chains that were used to anchor the VSC traps. The snaring mechanism allows the foxes' foot to remain in contact with the ground, so that they have the ability to run or leap to the end of the snare tether where they are brought to a sudden stop, while in VSC traps their co-ordinated movement appears to be impaired (CA Marks, personal observation 1995). Many predators have evolved an ability to accelerate at greater rates than prey species, so that a short and efficient chase allows the predator to capture the prey without reaching top speed (McNeil-Alexander 2006). For example, racing greyhounds reach maximal horizontal acceleration of 15 m s^{-1} and can do so from a standing start in the first two strides (Williams *et al* 2007). Being pulled to a sudden stop at higher speed may be associated with greater muscle damage, similar to the case hypothesised for Aldridge snares (Powell 2005) where constant tugging by bears captured in snares caused fractures, muscle, tendon, nerve and joint injury (Lemieux & Czetwertynski 2006). By reducing the length of snare-anchoring cables used for bears, it was suggested that dehydration and muscle injury could be reduced (Cattet *et al* 2003). Traps and snares can also be attached to a movable object that produces less resistance than pulling at a fixed cable and this may also permit animals to seek shelter (Independent Working Group on Snares 2005). However, Englund (1982) reported that 13% of foxes held in leg-hold snares attached to drags moved more than 500 m from the original point of capture and some escaped detection with the snare and drag attached to their leg. Canids and a range of other species may become tangled in snares and trap cables more easily when drags are used and this may be responsible for increased incidence of fractures and dislocations (Linhart *et al* 1988; Logan *et al* 1999; Powell 2005).

Traps have a wide range of moving parts with attachments, chains and mechanisms that produce a varying amount of sound when activated and resisted by captive animals. Loud noises were shown to be aversive to domestic dogs and affected gastric motility and hormone release (Gue & Peters 1989), activity and behaviour (King *et al* 2003). Noise is an important stressor that affects the welfare of captive laboratory animals (Jain & Baldwin 2003). In a forest habitat, ambient noise levels ranged from 40–70 dB and in savannah habitats it was 20–36 dB (Waser *et al* 1986). However, the sound of metal on metal during cage cleaning in a laboratory was measured to be 80 dB and had a wide spectrum of harmonics that were rich in different frequencies (Morgan & Tromborg 2007). Noise made by the capture device may compound stress experienced by the captured animal and contribute to the initial startle responses. When inspecting fox trap lines that also used Victor Soft Catch #3 traps, treadle-snares holding foxes were heard up to 50 m away by a characteristic 'metal against metal' sound of the moving treadle plate, the chain moving through the eye of the main spring and the sound of the device hitting hard surfaces. In contrast, Victor Soft Catch #3 traps made far less sound if they were tethered on a short chain and fox captures could not be heard until a close approach to the trap site (CA Marks, personal observations 1995). Post-capture noise is a possible stressor and contributing reason why comparative blood biochemistry values for foxes trapped in treadle-snares and Victor Soft Catch traps differed significantly. The welfare outcomes resulting from treadle-snares might be improved if the restraining chain can be greatly reduced in length and if the snare is set to fully detach from the snare 'thrower' in order to reduce ongoing noise stressors.

What are some appropriate physiological indicators of trapping stress?

A range of physiological responses to different capture techniques have proved to be useful indicators for assessing welfare outcomes of trapping and restraint for red foxes (Kreeger *et al* 1990a; White *et al* 1991), kit foxes (*Vulpes macrotis mutica*: McCue & O'Farrell 1987), African wild dogs (*Lycaon pictus*: De Villiers *et al* 1995), grizzly bears (Cattet *et al* 2003), black bears (Powell 2005), river otters (*Lontra canadensis*: Kimber & Kollias 2005), Eurasian otters (*Lutra lutra*: Fernández-Morán *et al* 2004), brushtail possums (*Trichosurus vulpecular*: Warburton *et al* 1999) and koalas (*Phascolarctos cinereus*: Hajduk *et al* 1992).

Physiological measures that provide a generalised indication of the cumulative physiological and pathological impact of trapping must have sufficient persistence to be meaningful many hours after initial capture. While cortisol has been commonly used to investigate stressors (Carstens & Moberg 2000) and capture stress in canids (Kreeger *et al* 1990a; De Villiers *et al* 1995) and foxes, sequential sampling may be required if stress response changes substantially during the period of capture. This is difficult to achieve in the field without introducing additional stressors from restraint, venipuncture or human presence (Beerda *et al* 1996; Hennessy *et al* 1998) and may be influenced by strong diurnal changes in cortisol as identified in dogs (Castillo *et al* 2009) but as yet unqualified in red foxes. Moreover, as the duration of a canid's captivity is rarely known with accuracy, the magnitude of the cortisol response at capture of an animal is of limited value as an indicator of overall stress, given that peak cortisol is usually achieved in minutes and may decline within an hour (Beerda *et al* 1998).

Injection of corticosteroids or adrenocorticotrophic hormones in dogs was reported to cause an increase in neutrophils (N) and a decrease in lymphocytes (L) within 2–4 h (Jasper & Jain 1965). Even short-term mental stressors have also been shown to cause a significant increase in neutrophil activation (Ellard *et al* 2001). Neutrophil counts were significantly increased while lymphocytes decreased in dogs subjected to air transport (Bergeron *et al* 2002) and in coyotes following capture and restraint (Gates & Goering 1976). Monitoring neutrophil

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activation due to transport stress was found to be a useful welfare indicator in European badgers (*Meles meles*: Montes *et al* 2004). Leukocyte counts are subject to diurnal variation, with neutrophils typically peaking in dogs during the day, corresponding to a decline in lymphocytes which tend to peak during the mid evening (Lilliehöök 1997; Bergeron *et al* 2002) and this may be significant if small changes in N:L ratios are being monitored. The use of N:L ratios is likely to be far less affected by handling and blood sampling in the field and in this current study appeared to provide a useful general indicator of capture stress.

After capture, the degree of struggling by the captured animal is probably one of the most significant welfare issues associated with trapping given the potential for injury, exhaustion and dehydration as suggested by these Melbourne data. Shock, surgery or disease affecting the skeletal (Prudhomme *et al* 1999) and myocardial muscle (Moss *et al* 1987), stressful exercise (Noakes 1987) and manual or mechanical restraint associated with muscle trauma (rhabdomyolysis) is known to cause elevated CK values (Goode *et al* 1977). Creatine kinase concentrations are used for diagnosing skeletal muscle damage (Aktas *et al* 1993). In rats, the concentration of serum CK correlated strongly with the volume of muscle traumatised by crushing injury (Akimau *et al* 2005), yet CK elevation is not regarded by other authors a correlate with the degree of trauma (Thrall *et al* 2004). In humans, tourniquet ischaemia of the arm produced with the application of a pneumatic cuff for one hour caused elevations in CK and TP that could be detected for three days after its removal (Rupiński 1989). Some stressors do not produce a significant increase in CK in some species or breeds (probably due to genotypic differences). In Alaskan sled dogs there was little indication of increases in serum CK after days of strenuous racing (Hinchcliff *et al* 1996), yet elevation of CK is associated with physical exertion in most domestic dog breeds (Aktas *et al* 1993). The reliability of CK as a specific marker for diagnosis of muscle disease (Auguste 1992, in Aktas *et al* 1993) is also influenced by myocardial disease associated with parvovirus, dirofilariasis, haemolysis and venipuncture and interaction with some therapeutic agents (reviewed in Aktas *et al* 1993). For example, the progressive evaluation of captured river otters (*Lontra canadensis*) showed that CK was not a good indicator of musculoskeletal injury due to likely interactions with existing pathology independent of capture injury (Kimber & Kollias 2005). In flying foxes (*Pteropus hypomelanus*), anaesthesia with isoflurane reduced the intensity of CK changes (Heard & Huft 1998). Creatine kinase values should also be interpreted carefully as struggling associated with restraint will peak within 4–6 h after muscle damage and decline within 24–48 hours (Thrall *et al* 2004) suggesting that the period of captivity should ideally be known with some accuracy. Much greater CK values observed in foxes restrained by a limb, compared to cage trapping (White *et al* 1991) or shooting (Kreeger *et al* 1990a; White *et al* 1991), were confirmed by the Melbourne study. Relative variation in the previously reported mean values of CK obtained using different capture methods and shooting were consistent with our current study, yet the degree to which CK values scale with the magnitude of injury and exertion requires clarification.

Kreeger *et al* (1990a) used other serum chemicals such as lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) that can be elevated by intense exertion to assist in the diagnosis of rhabdomyolysis in concert with CK values. Alkaline phosphatase (ALP) elevation in trapped foxes was likely to be initiated by stress-related cortisol release (Kreeger *et al* 1990a). Other authors have used elevation in ALB, ALB: globulin ratio, Gl, Na, TP (Powell 2005) as well as Cl and BUN values (Cattet *et al* 2003) to indicate dehydration caused by prolonged confinement and/or intense activity. The hydration status of animals as a result of captivity cannot easily be determined without the use of physiological indicators unless it is severe (Cattet *et al* 2003). A decline in mean Gl seen in treadle-snared foxes upon their recovery may be due to strenuous activity and muscle glycogen depletion, although there is little published research on stress-induced hypoglycaemia in canids (Wakshlag *et al* 2004) and its significance as a welfare marker for intense exertion in foxes requires verification.

Few studies have sought to monitor the duration or captivity and changing intensity of struggling after trapping and then related this to welfare indicators. Marks *et al* (2004) used activity monitoring data loggers on Victor Soft Catch traps used to capture dingoes and were able to contrast the intensity of struggling and duration of captivity with injury scores. However, a wide range of variation in trap inspection times suggests that different injury scores could be highly influenced by the duration of captivity. Trap inspection is required every 8 h in Sweden (Englund 1982), every 24 h in 33 states of the United States (in 1995; Andelt *et al* 1999), yet in Victoria (Australia) some trappers are required to inspect leg-hold traps only every 48 h. Comparisons of physiological indicators and injury data from different traps will only be valid if the mean period of captivity for any experimental group is not significantly different between or within studies that are compared.

Animal welfare implications

Although former studies concluded that due to similar injury scores, treadle-snares and VSC traps produced equivalent welfare outcomes, this study did not support this finding. Physiological data suggest that restraint by the treadle-snare is more stressful for foxes than produced by the VSC #3 over a similar period. Exertion and possible muscle damage is implied by the larger increases in CK, N:L ratios and a trend towards dehydration that is most likely caused by greater exertion after capture. While the VSC #3 trap probably provides some improved welfare outcomes compared to the treadle-snare, it produces greater indications of stress than cage traps and shooting as found in previous studies. Foxes held in either treadle-snares or VSC traps for 24 h, which represents a commonly accepted inspection period for traps and snares, are likely to be subjected to stress that is substantially higher than this study determined over a < 4 hour period of captivity. A tran-

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quiliser trap device (TTD) that can be attached to a trap and used to deliver sedative and anxiolytic agents or a rapid acting and humane toxicant may be one of the few practical approaches to significantly reduce the potential welfare impacts on canids caused by leg-hold (or 'foot-hold') traps and snares (Marks *et al* 2004). Scoring trap-related injuries and monitoring survival may reveal some relative differences in extreme welfare outcomes, yet trap injury and reduced survival caused by the capture device are endpoints of poor trapping welfare. Monitoring N:L ratios, CK and profiles that indicate dehydration gave insights into comparative capture stress that cannot be known from gross examination and injury scores. While these commonly used parameters have limitations, they appear to provide a useful general indicator of comparative capture stress that is less affected by handling and sampling than HPA hormones. Establishing ranges of blood normals unaffected by restraint stress and/or post mortem influences for 'free-ranging' canids will provide a benchmark for quantifying the influence of various stressors upon blood parameters and assist the use of common physiological indicators to investigate comparative welfare states arising from trap use. As the response of physiological indicators to stress is not independent of time, accurate monitoring of the duration of captivity and the relative intensity of struggling behaviour should be routinely collected when assessing the welfare outcomes associated with different trap devices.

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