© 2011 Universities Federation for Animal Welfare
The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2011, 20: 211-224
ISSN 0962-7286

Comparison of effects of different methods of culling red deer (Cervus elaphus) by shooting on behaviour and post mortem measurements of blood chemistry, muscle glycogen and carcase characteristics

MS Cockram*[†], DJ Shaw[‡], E Milne[‡], R Bryce[§#], C McClean[§¶] and MJ Daniels[§¥]

[†] Sir James Dunn Animal Welfare Centre, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, PEI, C1A 4P3, Canada
[‡] The Royal (Dick) School of Veterinary Studies and The Roslin Institute, The University of Edinburgh, Roslin, Midlothian EH25 9RG, UK
[§] Formerly Deer Commission for Scotland, Great Glen House, Leachkin Road, Inverness IV3 8NW, UK
[#] Aberdeen Centre for Environmental Sustainability (ACES), Institute of Biological and Environmental Sciences, University of Aberdeen, 23 St Machar Drive, Aberdeen AB24 3UU, UK
[¶] Glen Tanar Estate, Estate Office, Glen Tanar, Aboyne, Aberdeenshire AB34 5EU, UK
[¥] John Muir Trust, Tower House, Station Road, Pitlochry PH16 5AN, UK
* Contact for correspondence and requests for reprints: mcockram@upei.ca

Abstract

*Methods for culling wild red deer (*Cervus elaphus*) were compared by observing behaviour and collecting post mortem samples from wild deer shot: (i) by a single stalker during daytime; (ii) by more than one stalker during daytime; (iii) by using a helicopter for the deployment of stalkers and carcase extraction; or (iv) by a single stalker at night, and compared with farmed red deer shot in a field or killed at a slaughterhouse. Culling by a single stalker during the day and shooting in a field were the most accurate in achieving placement of a shot in a target area, but when compared across all methods, there were no significant differences in the percentages of deer that were either wounded or appeared to have died immediately after the first shot. Plasma cortisol concentrations in deer shot using helicopter assistance were similar to those in deer at the slaughterhouse, but higher than deer shot at night or during the day by a single stalker, or in a field. Deer shot using helicopter assistance and also deer culled by a collaborative and single stalking during the day had lower muscle glycogen concentrations than those culled by a single stalker at night. There was no evidence that a particular culling method was associated with an increased risk of accidental or pre-culling injury. If a helicopter is used to assist culling, the deer are more likely to be disturbed before they are shot and therefore, measures should be taken to minimise the disturbance to the deer.*

Keywords: *animal welfare, blood chemistry, culling, meat quality, red deer, stalking*

Introduction

In Scotland, management culling to control the number of wild red deer (*Cervus elaphus*) is undertaken to maintain a healthy population and prevent extensive damage to vegetation. During management culling, hinds are shot preferentially to control population size, whereas stags are preferentially shot for sport (Reynolds & Staines 1997). In this context, the term 'management culling' refers to the selection, often on the basis of inferior quality, and subsequent killing of surplus animals from the deer population. Stalking is the British term for the traditional method of culling wild red deer that is undertaken by pursuing deer by stealth and then shooting them with a rifle (the rifleman is known as a stalker). In some countries, an equivalent term would be deer hunting and the rifleman would be described as a deer hunter. However, in the United Kingdom (UK), the term deer hunting is normally restricted to the pursuit of deer using hounds.

The objectives when shooting a wild red deer should be to render it insensible as soon as possible after the first shot and not cause it injury or undue stress before it is shot. A stalker normally aims for the chest or neck as they represent an achievable target for stopping and ultimately killing the deer (Gracey *et al* 1999; Thomas & Allen 2003). The pathophysiological effects of a bullet entering various parts of the body of a deer shot during stalking on the time to loss of consciousness and death have not been documented in detail, but a bullet that causes extensive damage to the heart and/or major blood vessels will cause extensive blood loss. This haemorrhage is likely to result in a rapid fall in blood pressure that will reduce the flow of blood to the brain and this is likely to result in unconsciousness, followed by death (Gracey *et al* 1999; Gregory 2005). The Deer Commission for Scotland (2008) best practice guide on culling suggests that "a well-placed shot, with an appropriate bullet in the recommended target areas, will result in death in less than five minutes in most cases". Deer stalking codes of practice (British Association for Shooting and Conservation 2009) and best practice guidance (Deer Commission for Scotland 2008) recommend a broadside shot through the heart or lungs. Although shots aimed to sever the cervical spinal cord are used, there is a risk of missing the spinal cord and causing a wound (an injury in which the skin is cut or broken) that is not fatal or results in a delayed death (Urquhart & McKendrick 2003a). Shooting deer from a distance does not always result in immediate death and some deer can be wounded (ie injured and suffering from wounds) (Bradshaw & Bateson 2000). Bradshaw and Bateson (2000) considered that the main potential welfare issue with stalking was the number of deer that were wounded and, in particular, the escape of wounded deer. Bradshaw and Bateson (2000) reported that deer shot more than once during single rifle stalking had a greater plasma cortisol concentration than those killed with one shot. Other concerns during stalking are the risk of injury when deer try to escape and any disruption of social groups (Bradshaw & Bateson 2000).

The methods used to cull deer in Scotland range from single rifle stalking and shooting the deer with minimum disturbance prior to the shot, through night shooting with a spotlight, to 'collaborative culling' involving multiple rifles, sometimes utilising helicopters to deploy stalkers. The aim of this study was to consider the potential welfare implications associated with different culling methods that had not been compared with traditional methods of stalking, but are used to increase the number of wild red deer that are culled. In addition, comparisons are made between the responses of wild deer culled by these various methods and those of farmed deer either shot in a field or killed at a slaughterhouse. These comparisons provide some relative information on the occurrence of unwanted side-effects, such as stress and injury, and some information on the effectiveness of each culling method for killing wild deer under conditions that are less well controlled, compared with those used to kill farmed deer under more controlled and regulated conditions. It is possible that some aspects of one or more of the methods of culling deer could be less effective in killing the deer, result in more wounds that might cause suffering, cause more disturbance and stress to the deer, increase the risk of accidental injury (Littin & Mellor 2005) and reduce the quality of the venison compared with other methods.

To evaluate the welfare implications of the various culling methods under field conditions, a range of measurements similar to those used by Bradshaw and Bateson (2000), were used. Some of the limitations and interpretation of these measurements are discussed by Mason (1998) and Urquhart and McKendrick (2003a,b). The location of entry wounds in the carcase were used to assess whether the rifleman hit a preferred target area. The response of the deer after it was shot and the number of deer shot more than once were used to provide information on the effectiveness of the culling methods in apparently killing the deer as soon as possible. The time from the start of culling to the time when a deer was first shot, the time between a first shot and a second shot, and the time from a first shot to apparent death were used to assess the potential duration of any stress and suffering that the deer may have experienced. The degree of disturbance to the deer was assessed by observing their behaviour and measuring blood lactate concentration and muscle glycogen concentration to assess the degree and type of exercise. The potential implications of this disturbance and the effectiveness of killing on (i) the stress experienced by the deer, was assessed using plasma cortisol and free fatty acids concentrations and (ii) the occurrence of accidental injury, was assessed using plasma activities of creatine kinase and aspartate aminotransferase, and by visual appraisal of the carcase for signs of fractures and/or bruising. The potential implication of reduced muscle glycogen concentration at the time of death on venison quality was assessed by measuring muscle pH, 24 h post mortem.

Materials and methods

Culling procedures

Management culling of wild red deer was observed at 13 locations in upland Scotland between January and February 2006 and between December 2006 and March 2007. Most observations and samples were undertaken on open moorland with the remainder in forestry or fields. Hinds were the main target for management culling and were therefore preferentially selected for sampling. However, samples were taken from a smaller number of calves and stags (shot under relevant authorisation of the Deer Scotland [1996] Act). No deer shot by guests (ie non-professional stalkers that pay to shoot deer during the 'stag season') were included, only deer shot by professional stalkers, as part of normal management culling, were included in this study. Other than for the helicopter-assisted method, when a helicopter was used, carcases were transferred to the larder (cold storage area) using a vehicle. Observations were also made and samples collected from farmed deer shot in fields at one location in March 2007 and killed at one slaughterhouse in March 2006. The farmed deer killed at the slaughterhouse were mainly hinds and those shot in the field were composed of approximately equal numbers of hinds and stags.

Culling methods were studied by comparing behaviour and post mortem samples from wild deer shot: (i) by a single stalker during daytime (single-rifle culling); (ii) by more than one stalker during daytime (collaborative culling); (iii) by using the assistance of a helicopter for the deployment of stalkers (ie the movement of stalkers to strategic locations where they could start to identify a target) and carcase extraction during daytime (helicopter-assisted culling); or (iv) at night (night shooting). Samples were also collected from farmed red deer shot in the field during daytime (field) or killed at a slaughterhouse using a captive bolt and exsanguination during daytime (slaughterhouse). A summary per culling method, of the number of deer observed, the number of locations, the number of culling events and the number of deer culled per event is shown in Table 1.

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press

Table 1 Summary of culling events per culling method.

Culling method	Number of deer observed	Number of locations	Number of culling events	Number of culling events per day	Number of deer culled per event	Number of culling events when only one deer was culled
Single rifle	31	6	16	1	1–4	6
Collaborative	17	4	9	1	1–3	2
Helicopter-assisted	45	7	17	1	1–5	3
Night	21	5	14	1–2	1–3	8
Field	20	1	4	1	1–8	1
Slaughterhouse	30	1	1	1	30	0

Single-rifle stalking

Groups of wild red deer were located and stalked (hunted with the use of guns, but without the use of dogs) on each occasion by a single rifleman. Ninety percent of the culled deer were shot using a 0.270-inch calibre rifle using 130-grain bullets and 10% were shot using a 6.5-mm calibre rifle using 120-grain bullets. A sound moderator was used on the rifles. After several shots had been fired, the group of deer normally moved away and the culling event ended.

Collaborative stalking

Groups of wild red deer were located and stalked by two or more riflemen. Stalkers were strategically positioned near deer groups using radio communication. Deer moving in response to shooting by one rifleman would often move into the range of another rifleman where more deer could be shot. This was repeated until the target deer were shot or the deer moved to a different area. Fifty-three percent of the culled deer were shot using a 6.5-mm calibre rifle using 120-grain bullets and 47% were shot with a 0.270-inch calibre rifle using 130-grain bullets. A sound moderator was used on the rifles.

Helicopter-assisted stalking

A helicopter was used to locate groups of wild red deer and then strategically deploy one or multiple riflemen using radio communication. The number of riflemen deployed depended on the area covered, but normally between four and six riflemen were used for each group of deer. Fifty-seven percent of the culled deer were shot using a 0.270-inch calibre rifle using 130-grain bullets, 23% were shot with a 6.5-mm calibre rifle using 120-grain bullets, 18% were shot using a 0.243-inch calibre rifle using 100-grain bullets and 2% were shot with a 0.243-inch calibre rifle using 130-grain bullets. A sound moderator was used on the rifles. The helicopter moved out of the sight of the deer and shooting was conducted as for collaborative stalking. To increase efficiency, the helicopter was used to transport riflemen between areas containing groups of deer and to remove the carcases to the larder.

Night shooting

Groups of wild red deer were located in darkness with the use of a spotlight from a vehicle travelling along roads and tracks. When deer were located, shooting was carried out by a single rifleman either inside or immediately outside of the vehicle. Between one and three shots were normally fired before the group moved away and the culling event ended. Sixty-seven percent of the culled deer were shot using a 0.270-inch calibre rifle using 130-grain bullets and 33% were shot with a 6.5-mm calibre rifle using 120-grain bullets. A sound moderator was used on the rifles.

Field shooting

Groups of farmed red deer were shot by a single rifleman in a field at a deer farm. Sixty-five percent of the culled deer were shot using a 0.270-inch calibre rifle using 130-grain bullets, 30% were shot with a 0.270-inch calibre rifle using 150-grain bullets and 5% were shot with a 0.270-inch calibre rifle using 120-grain bullets.

Slaughterhouse

A single group of farmed red deer was transported to a slaughterhouse, lairaged for 24 h, stunned in the head with a captive bolt and then exsanguinated.

Methods of data and sample collection

Behavioural observations

One or two observers closely followed the stalkers and the times of events were recorded using a stopwatch. A culling event was defined as the period, on one day, at one location, between the time when, in the case of collaborative or helicopter-assisted culling, the first rifleman was deployed (the stalker was positioned at an appropriate strategic location to enable them to start to identify a target) or in the case of single-rifle culling, night or field shooting, the rifleman started stalking the deer (ie had identified a target deer and started to position themself, without disturbing the deer, by moving into an appropriate location to shoot the deer), until all of the deer that had been selected for culling had been shot or the group moved to another area. Where possible, the behaviour of the individual deer that was shot and if part of a group, the behaviour of the group, before the first shot was fired, was recorded as standing alert or idling, lying down, walking, running or trotting. The apparent time of death was recorded as the time when the deer collapsed to the ground and showed no further signs of life. If the deer was subsequently confirmed as dead when the stalker examined the deer, the apparent time of death, ie when the deer collapsed to the ground and showed no further signs of life was used as the time of death. If the deer was still alive after shooting, the time that it was dispatched by the stalker using alternative methods (usually by exsanguination) was recorded as the time of death. The response of the deer after the first shot was recorded as: (i) collapsed without moving location, (ii) ran 5–50 m before collapsing or (iii) walked or ran away without collapsing within 50 m of their location at the time of the shot. If the deer was part of a group, the behaviour of the group after the first deer was shot was noted as: no response, increased activity, walked away or ran away. Where it was not possible for the observer to record all details, as much information as possible was gathered from the stalkers after the event.

On-site sample collection and handling

An observer attempted to reach the carcase as soon as possible after death. However, there was a delay in sample collection from field-shot deer. The timings of sample handling in relation to time of death were recorded using a stopwatch. When wild red deer were bled from a knife wound made immediately above the sternum and farmed deer were bled from the jugular vein and carotid artery in the neck, blood samples were collected into a 10 ml tube containing heparin. The median (or second quartile) time of blood collection after death was 5 min, first quartile (Q_1) 2 min and third quartile (Q_3) 9 min, n = 164. The blood lactate concentration was measured on-site using a Lactate Pro meter (Arkray Inc, Kyoto, Japan) and test strips. A muscle sample was collected from immediately below the skin on the left shoulder (*m triceps brachii*) (median time after death was 9 min, Q_1 4 min and Q_3 27 min, n = 163). It was snap frozen in a mixture of isopentane and dry ice (median time after death was 10 min, Q_1 6 min and Q_3 35 min, n = 164), stored frozen using dry ice, transported to the laboratory and kept frozen until analysis. The blood samples were stored in a cool container; the temperature near the samples was recorded at 15-min intervals using a temperature logger (Tinytag Explorer, Gemini Data Loggers Ltd, Chichester, UK) (median temperature 3.5°C, at the time of collection, 84% of the samples were kept at a temperature of < 10°C and 16% < 18°C) and transported to the nearest location for centrifugation. The samples were then centrifuged at 3,000 rpm for 5 min; the plasma extracted, stored frozen using dry ice (median time after death was 227 min, Q_1 131 min and Q_3 328 min, n = 164), transported to the laboratory and kept frozen until analysis.

Carcase identification and examination

Unique tags were attached to a hind leg of the carcase for identification at the larder. After death, the deer were categorised as an adult or a calf and the sex of adult deer was recorded. The locations of entry and exit wounds in the carcase caused by bullets were noted using methodology similar to that described by Urquhart and McKendrick (2003a). Any signs of skin injury and any bone fractures were recorded. The carcases were transferred to a larder within 360 min of death. After skin removal, any obvious signs of bruising were recorded. At approximately 24 h after death (median time after death was 1,526 min, Q_1 1,447 min and Q_3 1,746 min, n = 150), the pH (pH_{24}) in the muscle of the hind leg (*m biceps femoris*) was recorded using a meat pH meter (Hanna Instruments 99163, Hanna Instruments Ltd, Leighton Buzzard, UK).

Laboratory analytical methods for plasma and muscle samples

Plasma haemoglobin concentration was measured on an ABX Pentra 60 haematology analyser (Horiba ABX-UK, Shefford, UK) using a modified Drabkins method. The following were measured in plasma on an Instrumentation Laboratory IL600 analyser (Instrumentation Laboratory UK, Warrington, UK) using Instrumentation Laboratory Kits: creatine kinase activity (kit 18482100), aspartate aminotransferase activity (kit 18257540) and total bilirubin concentration (kit 18254640). Plasma cortisol concentration was measured on a DPC Immulite Analyser using Immulite kit LKCO1 (Siemens Healthcare Diagnostics, Deerfield, USA). The muscle glycogen concentration was analysed using an adapted enzymatic method (Bergmeyer 1974; Adamo & Graham 1998).

Statistical analysis

The data presented a number of difficulties with respect to the analysis — it was not possible to collect balanced numbers of samples from different culling events for each culling method. One hundred and sixty-four samples were obtained, the majority of these (78%) were from adult hinds, and the remainder were from adult stags (15%) and calves (7%). Thirty-four per cent of culled deer were not culled singly. As measurements were only made at the slaughterhouse on one occasion, this method was excluded from detailed analyses to ensure that the results were not being overly influenced by the one event. In addition, some of the events only involved a single deer, yet others involved multiple deer, and some locations were used more than others, making standard analyses difficult. Therefore, the following analytical approach was adopted. Where possible, an initial simple univariate general linear model (GLM) analysis of the dependent variable and culling method, which ignored multiple deer per event/location, was conducted. For those data that consisted of binary outcomes (yes/no), GLM with binomial errors were used. Where univariate analyses were not possible, data were converted into binary outcomes and Fisher exact tests were used. Table 2 summarises the different statistical procedures used for the various variables. For the continuous variables, residuals were checked, and if not normally distributed, transformation of this parameter was carried out until normalised residuals were obtained — see Table 2 for transformation. If the univariate comparison was statistically

Table 2 Summary of data transformations, type of statistical procedure used, and the category level associated and additional individual variable covariates inserted into multivariate models.

Category/*Variable*	**Data transformation**	**Statistical procedure**	**Multivariate group level covariates**	**Multivariate additional individual covariates**
Shooting			Sex + Age + Site of entry wound	
Cull start: first shot ≥ 1 min (Y/N)	–	Binomial errors GLM		–
First shot: death ≥ 1 min (Y/N)	–	Binomial errors GLM		–
First–second shot (t)	Square root	GLM		–
Entry/exit wound head/neck/lower chest (Y/N)	–	Binomial errors GLM		–
Entry wound not head/neck/ chest (Y/N)	–	Binomial errors GLM		–
Behaviour			Sex + Age + Site of entry wound	
Standing alert prior to shot (Y/N)	–	Binomial errors GLM		–
Deer active prior to shot (Y/N)	–	Fisher's exact		[a]
Ran away after shot (Y/N	–	Fisher's exact		[a]
Muscle exercise (time, glycogen and PH)			Sex + Age + Site of entry wound + First shot-death (t) + Start of cull-death (t)	
Death to muscle freezing (t)	Log_{10}	GLM		–
Death to muscle pH sampling (t)	Reciprocal	GLM		–
Muscle glycogen	Log_{10}	GLM		+ Death-muscle freezing (t)
Muscle pH		GLM		+ Death-muscle pH sampling (t)
Death to blood sampling (t) (blood/plasma)	Square root	GLM		
			Sex + Age + Site of entry wound + First shot-death (t) + Start of cull-death (t) + Death-blood sampling (t) + Death-bleeding (t) + Blood sampling to plasma separation and storage (t)	
Blood lactate concentration	Log_{10}	GLM		–
Plasma total bilirubin	Square root	GLM		–
Plasma creatine kinase	Box-Cox (λ =–0.166)	GLM		–
Plasma aspartate aminotransferase	Log_{10}	GLM		–
Stress			Sex + Age + Site of entry wound + First shot-death (t) + Start of cull-death (t) + Death-blood sampling (t) + Death-bleeding (t) + Blood sampling to plasma separation and storage (t)	
Plasma cortisol	Log_{10}	GLM		+ Number of deer previously culled
Plasma free fatty acids	Log_{10}	GLM		+ Body condition score + Kidneys fat coverage

[a] No multivariate analyses performed for these variables; t: time.

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press

Table 3 Time from the start of a culling event to the first shot, time from the first shot to apparent death and number of deer for which a second shot was recorded.

Culling method	Time from start of culling event to first shot (min)				Time from first shot to apparent death (min)				Number of deer for which a second shot was recorded
	Median	Q_1	Q_3	n	Median	Q_1	Q_3	n	
Single rifle	0	0	0	31	0	0	3	31	6
Collaborative	0	0	16	17	0	0	2	17	2
Helicopter-assisted	10	0	53	45	0	0	2	45	11
Night	0	0	0	21	0	0	0	21	2
Field	5	0	10	7	0	0	1	7	1

Table 4 Responses of shot deer.

Culling method	Number of deer observed	% of deer in which time to apparent death from the first shot was recorded as > 0 min	Number of deer observed	Behaviour of individual deer after first shot		
				Collapsed (%)	Ran 5–50 m before collapsing (%)	Ran/walked away without collapsing within 50 m of their location at the time of the shot (%)
Single rifle	31	32	31	71	10	19
Collaborative	17	29	15	67	33	0
Helicopter-assisted	45	38	44	43	48	9
Night	21	19	21	52	43	5
Field	7	29	0			

significant, *post hoc* multiple comparisons of the mean values using Tukey contrasts were undertaken.

As many parameters not related to the culling method could have influenced the values observed, the next stage was to carry out a multivariate mixed-effect model (hereafter 'multivariate'). This model had a series of random effects (location and event nested within location) and covariates, which are summarised by variable group and individual additional covariates in Table 2. The results of the multivariate were compared to that of the univariate model, and if there were differences, contrasts were used to examine the differences in the multivariate model between the individual variable levels.

However, even with the above approach, the potential influence of the collection of multiple samples from an individual event had not been thoroughly considered. Therefore, in the final part of the analyses, a bootstrap approach was also undertaken in which a single deer per event was randomly sampled to generate a sample of 60 upon which analyses were carried out. This sample creation and subsequent statistical testing was performed 10,000 times to ensure generation of results that were robust in any conclusions from the analyses. These latter results are reported as a percentage of the 10,000 iterations in which statistical differences (at the 5% level) were obtained, with the greater the percentage, the greater the robustness of any analyses. The frequency with which the bootstrap results differed from the entire data set in terms of statistical/non-statistical significance ($P < 0.05$) was considered.

Results

Shooting

For 58% of the deer, the time between the start of the cull and the time of the first shot was < 1 min, but for those shot in the helicopter-assisted group, the median time was 10 min (Table 3). This was reflected in the analysis, with a greater number of deer shot ≥ 1 min after the start of the cull for helicopter-assisted culling than for night, collaborative and single-rifle methods ($P < 0.001$). For 54% of the wild deer, the time between the first shot and death (apparent death) was recorded as < 1 min and the median time between the first shot and death for all culling methods was < 1 min (Table 3). It was therefore not surprising that

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press

Table 5 Total number of entry wounds and percentage (%) of the total number of entry wounds for each method by location of entry wound (ie number of entry wounds at a particular location for all deer culled by a particular method/total number of entry wounds for all deer culled by a particular method × 100).

Culling method	Number of carcases examined	Total number of entry wounds	Location of entry wounds for all carcases for each method (% of entry wounds)					
			Head	Neck	Lower chest	Upper chest	Abdomen	Leg
Single rifle	28	32	13	19	48	10	3	7
Collaborative	15	18	0	6	65	18	6	6
Helicopter-assisted	43	53	8	12	46	12	21	2
Night	18	20	0	0	45	40	15	0
Field	20	20	100	0	0	0	0	0
Slaughterhouse	30	30	100	0	0	0	0	0

Table 6 Percentages (%) of deer with a wound (entry or exit) in the head, neck or lower chest/heart area, in the head, upper or lower chest/heart area and those with at least one entry wound in the leg and/or abdomen.

Culling method	% of deer with at least one entry or exit wound in either the head, neck or lower chest/heart area	% of deer with at least one entry or exit wound in either the head, upper or lower chest/heart area	% of deer with at least one entry wound in the leg or abdomen	% of deer with at least one entry wound in the leg	% of deer with at least one entry wound in the abdomen
Single rifle	89	83	7	7	4
Collaborative	67	93	13	7	7
Helicopter-assisted	74	84	19	0	19
Night	50	94	17	0	17
Field	100	100	0	0	0

there were no statistically significant differences between culling methods in whether the time from the first shot to death was < or ≥ 1 min ($P = 0.641$; Table 4).

Thirteen percent of the deer were shot at twice with the median time between the two shots 7 min (Q_1 2 min, Q_3 28 min). A second shot was only recorded once for the deer shot in the field and twice for deer culled at night or by collaborative culling — the majority of deer shot at twice were via helicopter-assisted (n = 11) and single-rifle culls (n = 6). However, a comparison of these two methods showed no statistically significant difference in the time between the first and second shot (univariate: $P = 0.437$, multivariate: $P = 0.960$). Thirty-four percent of the wild deer did not collapse immediately after the first shot but moved a short distance before collapsing and 10% either ran or walked away without collapsing (Table 4). Nine of these eleven deer were recorded as having been shot at a second time. Seven of the eleven deer might have been wounded after the first shot, ie there was an entry wound in either the abdomen or leg (in addition to an entry wound in a target area that was likely to have resulted in the death of the deer after the first or second shot). The median time between the first and second shot in these deer was 26 min (range 3 to 50 min). Field shooting was 100% accurate in achieving a shot in the head and the data from this method have been excluded from subsequent analyses of wound sites. Table 5 provides descriptive information on the percentage of the total number of entry wounds for each method by location of entry wound. Table 6 shows the percentages of deer with an entry or exit wound at various locations on the body.

There was a statistically significant difference between the other four culling methods in the percentage of deer with an entry or exit wound in either the head, neck or lower chest/heart area ($P = 0.019$). Deer culled at night had a lower percentage (50%) compared to single-rifle stalking (89%; Table 6). This difference remained in the multivariate analyses ($P = 0.007$). However, if a wound in the neck is excluded and a wound in the upper chest is included, there was no statistically significant difference between the four culling methods in the percentage of deer with at least one entry or exit wound in either the head, chest (upper or lower)/heart area ($P > 0.228$; Table 6). There was also no statistically significant difference between the four culling methods in the percentage of deer with at least one entry wound not in either the head, neck or chest (univariate: $P = 0.702$, multivariate: $P = 0.780$). Finally, no significant differences were found between culling methods in the percentages of deer that had at least one entry wound in the leg or in the abdomen ($P > 0.108$; Table 6).

Table 7 Behaviour of individual deer before being shot (percent of deer).

Culling method	Number of deer observed	Lying down	Standing alert	Standing idling	Walking	Running	Trotting
Single rifle	31	6	81	13	0	0	0
Collaborative	14	0	71	7	7	14	0
Helicopter-assisted	41	0	73	0	5	2	20
Night	21	0	100	0	0	0	0

Table 8 Response of a group of deer after the first shot was fired (percent of responses).

Culling method	Number of times groups were observed	No response	Increased activity	Walked away	Ran away
Single rifle	30	7	7	7	80
Collaborative	16	0	13	0	87
Helicopter-assisted	37	5	11	0	84
Night	18	0	22	0	78

Table 9 Muscle glycogen concentration (mmol glucosyl units kg^{-1} dry muscle) and muscle (*m biceps femoris*) pH_{24}.

Culling method	Muscle glycogen				Muscle pH			
	Median	Q_1	Q_3	n	Median	Q_1	Q_3	n
Single rifle	502	382	613	31	5.49	5.41	5.56	29
Collaborative	475	345	638	17	5.55	5.46	5.64	17
Helicopter-assisted	407	257	543	43	5.51	5.44	5.58	44
Night	802	645	975	21	5.44	4.96	5.47	19
Field	714	411	1,126	20	5.74	5.53	5.87	12
Slaughterhouse	834	578	990	30	5.69	5.73	5.77	29

Behaviour

Field-shot deer were excluded from the behaviour analysis due to insufficient data. Overall, 81% of the deer were standing alert before they were shot, with a statistically significant difference between culling methods ($P = 0.030$). All the night-shot deer were standing alert, compared to only 71–81% of the deer in the other culling methods (Table 7). Whether the deer were active or inactive before the first shot also differed between culling methods ($P < 0.001$). Helicopter-assisted culling resulted in the greatest activity (27% of the deer were active) and it was the only method where the deer were observed trotting before they were shot. The only other method in which deer were active before they were shot was the collaborative culling method where 21% of the deer were active (Table 7). Eighty-two percent of groups of deer ran away after the first shot. There was no statistically significant difference between the four culling methods in whether the behaviour of the group was to run away or not after the first shot ($P = 0.891$; Table 8).

Muscle exercise

Muscle glycogen

There was a statistically significant difference between the culling methods in the muscle glycogen concentration (univariate: $P < 0.001$ and multivariate: $P = 0.043$; Table 9). Deer shot at night had a greater concentration than those culled via helicopter-assisted, collaborative, and single rifle; whereas those shot in the field had a greater concentration than those culled with the assistance of a helicopter (Table 9). The statistical significance observed for the univariate and multivariate models were mirrored in the bootstrap analyses with statistically significant differences between culling methods in 85% of the univariate bootstraps, but only 40% of the multivariate bootstrap analyses.

There was also a statistically significant difference between the culling methods in the time from death to freezing of the muscle sample (univariate and multivariate: $P < 0.001$). It was significantly longer for deer shot in a field compared with the other methods, and

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press

Table 10 Plasma cortisol, plasma free fatty acids and blood lactate concentrations.

Culling method	Plasma cortisol (nmol L^{-1})				Plasma free fatty acids (mmol L^{-1})				Blood lactate (mmol L^{-1})			
	Median	Q_1	Q_3	n	Median	Q_1	Q_3	n	Median	Q_1	Q_3	n
Single rifle	16	11	27	30	0.210	0.180	0.280	30	3.1	1.9	6.8	29
Collaborative	49	27	83	17	0.240	0.195	0.420	17	6.0	2.7	7.9	17
Helicopter-assisted	61	31	122	45	0.420	0.295	0.605	45	6.8	3.7	10.9	41
Night	13	9	23	21	0.200	0.160	0.270	21	2.9	2.4	4.8	19
Field	23	10	38	20	0.225	0.173	0.315	20	10.7	7.7	12.2	19
Slaughterhouse	89	72	113	29	0.410	0.255	0.555	29	6.8	4.6	8.1	30

longer in those culled with the assistance of a helicopter or collaborative culling compared with those culled by the single-rifle method. The statistical analyses for muscle glycogen concentration were therefore repeated with muscle samples frozen > 60 min after death removed, however qualitatively the results remained the same.

Muscle pH

There was a statistically significant difference between the culling methods in the muscle pH_{24} ($P < 0.001$). The pH was lower in deer shot at night than those culled via helicopter-assisted, collaborative, and single rifle (Table 9). However, this difference was lost in the multivariate analyses ($P = 0.060$). The main apparent reason for this was the impact of time between death and muscle pH sampling ($P = 0.041$). Excluding this variable in the multivariate analyses resulted in a statistically significant difference between culling methods ($P = 0.033$), with muscle pH from helicopter-assisted and single-rifle culling greater than that of night-shot deer. However, the bootstrap analyses only revealed statistically significant differences between culling methods in 48% of the univariate, and 38% of the multivariate samples. There was a univariate statistically significant difference between the culling methods in the time from death to the muscle pH reading ($P < 0.031$). Deer shot in a field were measured later than those culled: via helicopter-assisted, collaborative and single rifle. However, these differences disappeared in the multivariate model ($P = 0.719$).

Blood lactate concentration

There was a statistically significant difference in blood lactate concentration between the culling methods ($P < 0.001$). The blood lactate concentration was greater in deer shot in the field than in those culled at night, collaborative, and single rifle and was greater in those culled with the assistance of a helicopter than in single-rifle-culled deer (Table 10). However, these differences were lost in the multivariate analyses ($P = 0.056$).

Potential artefacts affecting blood chemistry

There was a statistically significant difference between the five culling methods in the time from death to blood sampling (univariate: $P < 0.001$ and multivariate: $P = 0.004$). This time was longer for deer shot in a field compared to the other four methods. Therefore, all of the statistical analyses for each of the plasma variables were repeated (i) with three blood samples obtained > 30 min after death removed and (ii) with blood samples not stored between 0 and 10°C at the time of collection removed; however, this made no qualitative difference to any univariate or multivariate analyses. The potential influence of haemolysis on the plasma samples was also examined — however, only seven samples had a plasma haemoglobin concentration greater than 0.6 g L^{-1}. In the plasma samples from collaborative culling, one sample had a haemoglobin concentration of 1.3 g L^{-1} and in helicopter-assisted culling there were six samples between 0.7 and 1.7 g L^{-1}. While there was a statistically significant difference in the plasma total bilirubin concentrations between the five culling methods ($P < 0.001$), this difference was lost in the multivariate analysis ($P = 0.202$). The median plasma bilirubin concentration was 2.1 µmol L^{-1} (Q_1 1.1, Q_3 4.1).

Injury

Only one occurrence of accidental or pre-culling injury was recorded. This one injury was observed in the hind leg muscle of one deer culled with the assistance of a helicopter. No fractures, bruising or other injuries were recorded. There were no statistically significant differences between the culling methods in either plasma creatine kinase activity (median 1,019, Q_1 291, Q_3 5,545 IU L^{-1}, n = 162) (univariate: $P = 0.408$ and multivariate: $P = 0.364$) or plasma aspartate aminotransferase activity (median 103 Q_1 70, Q_3 320 IU L^{-1}, n = 161) (univariate: $P = 0.594$ and multivariate: $P = 0.701$).

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press

Stress

Cortisol

Twenty-two percent of the plasma cortisol measurements were apparently positive, ie above 5.5 nmol L^{-1} but below the level of 13.5 nmol L^{-1} above which the instrument was able to give reliable readings. As these values were not distributed randomly between culling methods, these very low plasma cortisol concentrations were reallocated using a random value generated from a uniform distribution with a minimum of 5.5 and a maximum of 13.5. After this adjustment, there was a statistically significant difference in the plasma cortisol concentration between the culling methods (univariate: $P < 0.001$ and multivariate: $P = 0.002$). Deer culled with the assistance of a helicopter had a greater plasma cortisol concentration than those culled at night, in the field, and by single rifle; and deer culled via collaborative stalking had a greater plasma cortisol concentration than those shot at night (Table 10). The bootstrap analyses showed that statistically significant differences between culling methods were observed for 99.8% of the univariate, but only 3% of the overall multivariate comparisons.

Free fatty acid concentration

There was a statistically significant difference in the plasma free fatty acid concentrations between the culling methods (univariate and multivariate $P < 0.001$). Deer culled with the assistance of a helicopter had greater plasma free fatty acid concentrations than those culled at night, in the field, and by collaborative and single-rifle methods (Table 10). The bootstrap results revealed that statistically significant differences between culling methods were observed for 99.5% of the univariate, but only 34% of the overall multivariate comparisons.

Discussion

The difficulties in obtaining sufficient numbers of separate culling events for each culling method meant that the statistical analyses were problematic. The results might also have been influenced by factors other than differences between culling methods. In addition, the observations, the sampling, storage and transportation of samples were performed under difficult environmental conditions. The effects of some of the culling methods studied might have been greater if the daily culling rate was higher than that obtained during this study. Although it has been possible to come to some tentative conclusions, it should be recognised that because of these practical problems and because this was not a controlled experiment, some results with reported significant differences between culling methods might not have been due to the effect of the culling method. These factors affect the confidence with which conclusions can be drawn regarding the welfare implications of the various culling methods.

The times between death and blood sampling and between death and centrifugation of the blood samples were similar to that reported by Bradshaw and Bateson (2000) in their paper on culling of red deer. Blood lactate concentration can increase with time after death (Ferrante & Kronfeld 1994; Bradshaw & Bateson 2000). In the case of field-shot farmed deer, due to the farmers' request for sampling to take place after the carcases had been brought inside for preparation, there was a 20-min median delay in blood sampling. Therefore, the blood lactate concentrations for field-shot deer might have been unreliable. A major concern was the possibility of haemolysis of red blood cells after death and during blood handling that might (i) have affected some biochemical analyses, eg plasma creatine kinase activity or (ii) be confused with pre-mortem haemolysis. However, few plasma samples had detectable haemoglobin concentrations and the plasma total bilirubin concentration was not indicative of major pre-mortem haemolysis. In red deer shot during single-rifle stalking, Bradshaw and Bateson (2000) reported a median plasma haemoglobin concentration of 0.07 g L^{-1} (Q_1 0.05, Q_3 0.14) and a median plasma bilirubin concentration of 6.1 µmol L^{-1} (Q_1 5.4, Q_3 7.4).

Shooting

An important welfare issue is the efficiency of the rifle bullet in killing the deer quickly and with minimal suffering. The locations of the entry wounds provide one method of assessing whether there was any influence of culling method on whether the rifleman hit a preferred target area. The close range and more controlled conditions when either stunning farmed deer with a captive bolt in the slaughterhouse or shooting farmed deer in the field, permitted 100% success in causing an entry wound in the head. However, shooting wild deer from a distance is more difficult and headshots are not recommended because of the small target area and the high risk of a badly injured deer that is likely to escape. Baker (2003) noted that when stalking wild deer there were three recommended target areas: (i) the thorax, "the largest-sized area and therefore with more margin for error, but with the main disadvantage that the animal will often run some distance before dropping"; (ii) "the posterior cervical area, which is smaller in lethal area, but will provide an instant knock down"; and (iii) "the shoulder area, breaking the spinal cord again, with instant effect" (presumably collapse). If a wound in either the head, neck or lower chest (ie near the heart) is considered to be an acceptable location in which the deer are likely to be killed relatively quickly, 89% of single-rifle culls, 67% of collaborative culls and 74% of helicopter-assisted culls had a wound in an acceptable location. Only 50% of deer shot at night had a wound in the head, neck or lower chest, but an additional 45% had a wound in the upper chest. Alternatively, if a wound in either the head, chest (upper or lower)/heart area is considered to be an acceptable location in which the deer are likely to be killed relatively quickly, then 83% of the single-rifle culls, 93% of the collaborative culls, 84% of the helicopter-assisted culls and 95% of the night-shot deer had a wound in an acceptable location. The shoulder area is a recommended site for night shooting (Deer Commission for Scotland 2010).

Urquhart and McKendrick (2003a) examined carcases of red deer culled in Scotland and reported that 33% of the plucks (heart and lungs) examined had a wound tract involving the heart and 48% had a wound tract through only the lungs. Although a wound in the heart is likely to have quickly killed the deer, a wound in the upper chest might also have killed the deer quickly by massive haemorrhage (Thomas & Allen 2003). In 17 deer that were recorded with just an entry wound in the upper chest, 13 were recorded as collapsing immediately and four collapsed after they had moved a short distance. Gregory (2005) could not find any direct information on time to loss of consciousness and death during haemorrhage in deer, but considered that this time would depend on which tissues are damaged and, in particular, on the rate of blood loss and thereby the rate of induction of cerebral hypoxaemia. Based on experiments in rats, Gregory estimated that a deer would need to lose at least 2 L of blood to ensure rapid death. Urquhart and McKendrick (2003b) rejected an approach based on the anatomical distribution of individual entry/exit wounds to assess, retrospectively, the welfare consequences for culled deer. The trajectory of the bullet and the outcome of the complex pathological processes that occur when a deer is struck by a high-velocity bullet were considered too difficult to predict from entry wounds alone. Without considering whether a vital structure was injured, eg viscera, major blood vessels or the central nervous system, they considered that it was not possible to assess the immediacy or otherwise of death. Consideration of just the entry wound and the likely trajectory of the bullet could underestimate the effect that a bullet may have caused to the surrounding tissues, ie even if the bullet does not directly penetrate a vital structure, the extensive peripheral damage might have been sufficient to kill the deer effectively. Tissue in the immediate path of a bullet fired from a high-velocity rifle is lacerated and crushed and those in the vicinity are damaged by shock waves released by the bullet when it penetrates the body. Soft tissue and bone balloon outward from the path of the bullet and this creates a temporary cavity that crushes and compresses to cause extensive trauma to tissue and the regional circulation (Pavletic 1996).

The responses of a deer after it is shot also provide useful information on the effectiveness of the culling methods in killing the deer as soon as possible. At the time that the deer were shot, the observers were often located 100–200 m from the deer and not in a position to examine the deer for signs of life. Therefore, the apparent time of death was taken as the time at which the deer collapsed and showed no further signs of life visible from a distance. It is possible that the 'actual' time of death was later than the apparent time of death. Immediate and permanent collapse of the deer after they were shot does not necessarily indicate that the deer had been killed instantly (eg collapse might occur following paralysis without loss of consciousness). However, it is one measurement to indicate whether the deer had been shot in an area that resulted in either immediate death or immobility (permitting close killing by the stalker), as compared with a deer with a non-fatal or non-paralysing wound that did not collapse immediately and permanently. The prevalence of wounded deer and especially the effect of these wounds on the suffering experienced by the deer are important welfare issues arising from shooting (Baker & Harris 2005). A deer with a wound in the abdomen or leg without a lethal wound in the head, neck or chest might remain alive for a considerable time. Rifle-shot wounds to the abdomen can cause peritonitis secondary to bowel perforation. Traumatised tissue, vascular compromise, foreign debris and the inoculation of bacteria into the wound increases the risk of infection (Pavletic 1996). Thomas *et al* (2001) considered that the small number of deer that escape after they are shot either die through starvation and sepsis or will adapt to their wounds. There was no significant difference between the culling methods for wild deer in the percentage of deer with at least one entry wound not in the head, neck or chest. In other studies, between 5 and 7% of the wounds in carcases from wild deer culled in Scotland were recorded in the abdomen and between 3 and 4% were recorded in the leg (Urquhart & McKendrick 2003a, 2006). In the current study, 3% of the entry wounds in deer shot by a single stalker were in the abdomen and 7% were in the leg, whereas the percentage of entry wounds in the abdomen was 15% for deer culled at night and 21% for deer culled with helicopter assistance. However, no significant differences were found between culling methods in the percentages of deer that had at least one entry wound in the leg, in the abdomen or in the leg or the abdomen.

When deer are wounded, the time between the first shot and a subsequent fatal shot is important. However, as Baker (2003) suggests, it is not correct to assume that a second shot was the lethal one. A second shot may be taken 'to make sure' that the deer is shot effectively, even though the first shot may have been lethal. In many circumstances, eg if the deer is moving, it can be more difficult to be accurate with the second shot than the first. The time between the first shot and any second shot required to kill a deer should obviously be as short as possible. For all deer observed, over all culling events, a second shot was fired on 22 occasions and the median time between the first and second shot was 7 min (Q_1 2 and Q_3 29 min). A second shot was recorded as having been fired in 19% of the deer culled with a single rifle and for 24% of the deer culled with the assistance of a helicopter. In other studies of single stalking of wild deer that used a variety of methods to collect the data, more than one shot was recorded in 12% (Bradshaw & Bateson 2000) and 14% of the deer (Urquhart & McKendrick 2003a, 2006).

Behaviour

Observations of the behaviour of the deer before it was shot and the behaviour of the group of deer after the first deer was shot, provide information on the relative disturbance to the deer by the various culling methods. The median time between the start of the cull and the first shot was longer during the helicopter-assisted cull (about 10 min) than for other culling methods (between 0 and 5 min). This might have provided a longer opportunity for the deer to be disturbed before the first shot. Farmed red deer on moorland spend most of their daylight hours grazing (46%), lying down (46%) and only about 4% moving

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press

(Hester *et al* 1999). However, in this study, most deer were standing alert before they were shot, and the only culling method in which any deer were lying down before the first shot was with the single-rifle method. The deer were active during the helicopter-assisted cull and the collaborative cull. Helicopter-assisted culling resulted in the greatest activity (27% of the deer were active) and it was the only method where the deer were observed trotting before they were shot. The only other method in which deer were active before they were shot was the collaborative culling method, where 21% of the deer were active. The British Association for Shooting and Conservation code of practice for deer stalking recommends that except when a second shot is required to kill a wounded deer, shots should never be taken at a deer that is running, as this will increase the risk of a non-fatal wound (British Association for Shooting and Conservation 2009). Although the intention of using a helicopter was limited to the deployment of personnel and the removal of carcases, deer can be disturbed by the presence of a helicopter and when chased will walk with occasional bursts of running (Cattet *et al* 2004). Deer behaviour is affected by human disturbance and this is shown by increased time spent alert with the head raised (Bullock *et al* 1993). The response of farmed red deer to visual (human in bright clothing) or auditory stimuli (loud music) is to run away from the stimulus and then to remain in an alert posture for about 10–15 min after the stimulus has been removed (Hodgetts *et al* 1998).

Injury

There was no evidence that a particular culling method was associated with an increased risk of accidental or pre-culling injury. Only one occurrence of injury as a result of trauma was recorded, but no fractures or carcase bruising was recorded. This one injury might have occurred before the start of culling. The biochemical measurements of plasma creatine kinase activity as an indicator of muscle damage and plasma aspartate aminotransferase activity as a non-specific indicator of soft tissue damage (Kramer & Hoffman 1997) were not affected by culling method. However, other than for the samples collected at the slaughterhouse, the plasma creatine kinase activity in each of the culling groups was high. Whether this is a true result that indicates that muscular exertion or damage occurred before death or more likely an artefact arising from the difficulty of blood sampling, handling and storage is unclear. Even though the median plasma creatine kinase activity of 816 IU L^{-1} in deer shot by single-rifle culling was the lowest numerical value amongst the culling methods, it was higher than that reported in studies by Bateson and Bradshaw (1997) (270 IU L^{-1}) and Bradshaw and Bateson (2000) (266 IU L^{-1}).

Muscle glycogen and pH

The time between death and muscle sampling (median time 10 min) was too long to ensure that the muscle glycogen concentration at the time of death was measured accurately. In an ideal situation, the muscle sample should be snap frozen immediately after death. The glycogen in the muscle starts to be metabolised from death and therefore the concentration of glycogen in a muscle sample frozen 10 min after the death of the animal will be less than would have been present at death. By 24 h after death, almost all muscle glycogen would have been metabolised. If the muscle glycogen content was normal at the time of death, the muscle pH_{24} would be low (approximately 5.7; Wiklund *et al* 2001), but if lower than normal muscle glycogen was present, eg due to prolonged exercise (Hoppeler & Billeter 1991), the muscle pH_{24} would be higher than normal. The rate of energy expended by deer during running is 2–3 times that during walking (Parker *et al* 1984) and therefore the greater activity of the deer in the helicopter-assisted culling method might have been responsible for the reduced muscle glycogen concentration and the slightly high muscle pH_{24} in this group. A raised muscle pH_{24} is consistent with the utilisation of muscle glycogen before death and decreased lactic acid formation during post mortem glycogen metabolism. However, the influence of different methods of carcase extraction on the muscle pH_{24} is not known. The relatively high muscle glycogen concentrations in deer shot in the field and at night suggests that they experienced the least prolonged exercise before death. This was consistent with the greater muscle glycogen concentration found by Pollard *et al* (2002) in farmed stags shot in a paddock compared with those transported and held overnight at a slaughterhouse lairage before stunning with a captive bolt. Although it is possible that factors other than exercise, such as nutritional differences between farmed and wild red deer, could have affected the muscle glycogen concentration, Wiklund *et al* (2003) did not find any significant effect of diet on muscle glycogen concentration in farmed red deer.

Lactate

Raised blood lactate concentrations can occur following breakdown of muscle glycogen after extreme muscular exertion and from catecholamine-induced glycogenolysis (Shaw & Tume 1992). Although there were statistically significant differences between deer shot in a field and those culled at night, collaborative and single-rifle-culled deer, this was probably an artefact caused by the delay in blood sampling the deer after they had been shot in the field. The difference in the blood lactate concentration between those culled with the assistance of a helicopter and single-rifle culled deer might have been a consequence of greater muscular exertion in those culled with the assistance of a helicopter.

Stress

Measurement of plasma cortisol concentration is a standard approach in studies of stress and welfare (Mormède *et al* 2007). The relationship between the concepts of stress and welfare is complex, but both are thought to reflect an animal's cognitive evaluation of their environment. If an animal perceives a situation as aversive, a stress response is likely to occur (Veissier & Boissy 2007). Although raised plasma cortisol concentration is thought to be associated with psychological stress, a similar rise in plasma cortisol concentration can follow excitement. Therefore, it is not appropriate to infer that a raised plasma cortisol concentra-

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press

tion necessarily indicates poor welfare (Webster 1998). That said, in Great Britain, the Welfare of Animals (Slaughter or Killing) Regulations (1995) that regulates the slaughter of farmed animals in a slaughterhouse, contains a provision to prohibit avoidable excitement, pain or suffering during the movement, lairaging, restraint, stunning, slaughter or killing of animals. Therefore, it is reasonable to suggest that a preferred culling method for red deer would be one that avoids a raised plasma cortisol concentration, regardless of whether it is due to excitement or distress. A cortisol response can follow exercise (Marc *et al* 2000) and deer culled by some methods appeared to have experienced more exercise than those culled by other methods. However, a greater cortisol response in deer culled by some methods may not necessarily be just an indication of a physiological response to exercise associated with the mobilisation of body energy reserves (Hyyppä 2005). It is possible that deer with a raised cortisol response perceived their situation as aversive. There is some indication in humans, that the cortisol response to exercise is greater when the exercise is perceived negatively and associated with negative emotional states (Harte & Eifert 1995). Marc *et al* (2000) found differences in the cortisol response of trained and untrained horses to exercise and suggested that the decreased responsiveness of the adrenal cortex in trained compared with untrained horses might indicate that trained horses experienced less stress during physical exercise.

For wild deer, culling involving the assistance of a helicopter was the most stressful method. This conclusion is based on the raised plasma cortisol concentration and the raised free fatty acid concentration that can occur following release of catecholamines during stress. The plasma concentrations of cortisol and free fatty acids in single-rifle-culled, night-culled and field-shot deer were lower than those found in deer culled using the assistance of a helicopter. Deer culled using the assistance of a helicopter had a greater mean plasma cortisol concentration (91 nmol L^{-1}) than those culled at night (21 nmol L^{-1}), by single-rifle methods (47 nmol L^{-1}) or shot in a field (36 nmol L^{-1}), and those culled via collaborative methods (82 nmol L^{-1}) had a greater plasma cortisol concentration than those shot at night. Slaughterhouse slaughtered deer had a mean plasma cortisol concentration of 92 nmol L^{-1}. Based on plasma cortisol concentrations, shooting farmed deer in the field or wild deer on open moorland using single-rifle stalking methods is apparently less stressful than the handling, transport and lairage involved with slaughter at a slaughterhouse. This conclusion is supported by other studies that have reported lower plasma cortisol concentrations in farmed red deer shot in a field compared with those transported, lairaged and then stunned at a slaughterhouse (Smith & Dobson 1990; Pollard *et al* 2002). Bateson and Bradshaw (1997, 2000) and Bradshaw and Bateson (2000) also reported relatively low plasma cortisol concentrations in deer shot during single-rifle stalking.

Animal welfare implications

Within the limitations of this one study that used professional stalkers and a low rate of culling, the plasma cortisol concentrations suggest that culling wild red deer by rifle and using the assistance of a helicopter for the deployment of stalkers and carcase extraction was as stressful as slaughtering farmed red deer at a slaughterhouse. It was more stressful than culling wild deer by rifle with a single stalker at night or during the day or shooting farmed deer by rifle in a field, but it was not more stressful than culling wild deer by rifle when more than one stalker was deployed at a time. Helicopter-assisted culling appeared to have been associated with exercise of the deer before death, but there was no evidence that this was associated with an increased risk of injury. Single-rifle culling and field shooting were the most accurate in achieving at least one wound in the head, neck or lower chest of the deer. However, there was no significant difference between the culling methods in the percentage of deer that appeared to have died immediately after the first shot and no significant difference between culling methods in the percentage of deer that were wounded in the abdomen or leg. However, between 7% (single stalking) and 19% (helicopter-assisted stalking) of the deer were wounded in the abdomen or leg. Further studies are recommended on factors, such as movement by deer as they are shot, that may affect the number of deer that are wounded. If a helicopter is used to assist culling, the deer are more likely to be disturbed before they are shot. Whether this was due to disturbance from the helicopter directly or due to the use of the helicopter to deploy stalkers rapidly so that they could re-stalk the same group of deer after one or more of the deer had been shot and they had moved to a new location is not clear. Therefore, if a helicopter is used to assist culling, measures should be taken to minimise the disturbance to the deer.

Acknowledgements

The Deer Commission for Scotland funded this work. We are grateful to Linda Norrie and Sandra Grant for performing the muscle glycogen analysis, the staff of the R(D)SVS Veterinary Pathology Unit for carrying out the other biochemical assays and to Brian Kelly and Rod Else for assistance with setting up the project. We gratefully acknowledge the staff at Macaulay Institute, Reediehill and Round Green deer farms for access to farmed deer. We also thank all of the stalkers who assisted in the study and all the landowners who gave permission for it to take place on their land.

References

Adamo KB and Graham TB 1998 Comparison of traditional measurements with macroglycogen and proglycogen analysis of muscle glycogen. *Journal of Applied Physiology 84*: 908-913

Baker AE 2003 Culling of wild red deer. *Veterinary Record 153*: 31-32

Baker PJ and Harris S 2005 Shooting in the dark. *Animal Welfare 14*: 275-276

Bateson P and Bradshaw EL 1997 Physiological effects of hunting red deer (*Cervus elaphus*). *Proceedings of The Royal Society of London Series B-Biological Sciences 264*: 1707-1714

Bateson P and Bradshaw EL 2000 The effects of wound site and blood collection method on biochemical measures obtained from wild, free-ranging red deer (*Cervus elaphus*) shot by rifle. *Journal of Zoology 252*: 285-292

Bergmeyer HU 1974 *Methods of Enzymatic Analysis, Volume VI*. Academic Press: New York, USA

Bradshaw EL and Bateson P 2000 Welfare implications of culling red deer (*Cervus elaphus*). *Animal Welfare 9*: 3-24

British Association for Shooting and Conservation 2009 *The BASC Deer Stalking Code of Practice*. http://www.basc.org.uk/en/codes-of-practice/deer-stalking.cfm

Bullock DJ, Kerridge FJ, Hanlon A and Arnold RW 1993 Short-term responses of deer to recreational disturbances in two deer parks. *Journal of Zoology 230*: 327-332

Cattet MRL, Caulkett NA, Wilson C, Van den Brink T and Brook RK 2004 Intranasal administration of xylazine to reduce stress in elk captured by net gun. *Journal of Wildlife Diseases 40*: 562-565

Deer Commission for Scotland 2008 *Best Practice Guides. Culling: Shot Placement*. http://www.bestpracticeguides.org.uk /culling_shotplace.aspx

Deer Commission for Scotland 2010 *DCS Guide: Night shooting*. http://www.bestpracticeguides.org.uk/reference/dcs_night.aspx

Ferrante PL and Kronfeld DS 1994 Effect of sample handling on measurement of plasma-glucose and blood lactate concentrations in horses before and after exercise. *American Journal of Veterinary Research 55*: 1497-1500

Gracey JF, Collins DS and Huey RJ 1999 *Meat Hygiene*. WB Saunders Company Ltd: London, UK

Gregory NG 2005 Bowhunting deer. *Animal Welfare 14*: 111-116

Harte JL and Eifert GH 1995 The effects of running, environment, and attentional focus on athletes' catecholamine and cortisol levels and mood. *Psychophysiology 32*: 49-54

Hester AJ, Gordon IJ, Baillie GJ and Tappin E 1999 Foraging behaviour of sheep and red deer within natural heather grass mosaics. *Journal of Applied Ecology 36*: 133-146

Hodgetts BV, Waas J and Matthews L 1998 The effects of visual and auditory disturbance on the behaviour of red deer (*Cervus elaphus*) at pasture with and without shelter. *Applied Animal Behaviour Science 55*: 337-351

Hoppeler H and Billeter R 1991 Conditions for oxygen and substrate transport in muscles in exercising mammals. *Journal of Experimental Biology 160*: 263-283

Hyyppä S 2005 Endocrinal responses in exercising horses. *Livestock Production Science* 92: 113-121

Kramer JW and Hoffman WE 1997 Clinical enzymology. In: Kaneko JJ, Harvey JW and Bruss ML (eds) *Clinical Biochemistry of Domestic Animals* pp 303 -325. Academic Press: San Diego, USA

Littin KE and Mellor DJ 2005 Strategic animal welfare issues: ethical and animal welfare issues arising from the killing of wildlife for disease control and environmental reasons. *Revue Scientifique et Technique-Office International Des Epizooties 24*: 767-782

Marc M, Parvizi N, Ellendorff F, Kallweit E and Elsaesser F 2000 Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized treadmill exercise test as physiological markers for evaluation of training status. *Journal of Animal Science 78*: 1936-1946

Mason G 1998 Animal welfare: the physiology of the hunted deer. *Nature 391*: 22

Mormède P, Andanson S, Auperin B, Beerda B, Guemene D, Malnikvist J, Manteca X, Manteuffel G, Prunet P, van Reenen, CG, Richard S and Veissier I 2007 Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiology & Behavior 92*: 317-339

Parker KL, Robbins CT and Hanley TA 1984 Energy expenditures for locomotion by mule deer and elk. *Journal of Wildlife Management 48*: 474-488

Pavletic MM 1996 Gunshot wound management. *Compendium on Continuing Education for the Practicing Veterinarian 18*: 1285-1299

Pollard JC, Littlejohn RP, Asher GW, Pearse AJT, Stevenson-Barry JM, McGregor SK, Manley TR, Duncan SJ, Sutton CM, Pollock KL and Prescott J 2002 A comparison of biochemical and meat quality variables in red deer (*Cervus elaphus*) following either slaughter at pasture or killing at a deer slaughter plant. *Meat Science 60*: 85-94

Reynolds P and Staines B 1997 Deer management in Scotland. In: Bolton M (ed) *Conservation and the Use of Wildlife Resources* pp 171-190. Chapman and Hall: London, UK

Shaw FD and Tume RK 1992 The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents: a review of recent work. *Meat Science 32*: 311-329

Smith RF and Dobson H 1990 Effect of preslaughter experience on behaviour, plasma cortisol and muscle pH in farmed red deer. *Veterinary Record 126*: 155-158

Thomas L and Allen T 2003 Culling of wild red deer. *Veterinary Record 152*: 815

Thomas LH, Wise DR, Denny DR and Allen WR 2001 Deer hunting and welfare. *Animal Welfare 10*: 346

Urquhart KA and McKendrick IJ 2003a Survey of permanent wound tracts in the carcases of culled wild red deer in Scotland. *Veterinary Record 152*: 497-501

Urquhart KA and McKendrick IJ 2003b Culling of wild red deer: comment. *Veterinary Record 152*: 815-816

Urquhart KA and McKendrick IJ 2006 Prevalence of 'head shooting' and the characteristics of the wounds in culled wild Scottish red deer. *Veterinary Record 159*: 75-79

Veissier I and Boissy A 2007 Stress and welfare: two complementary concepts that are intrinsically related to the animal's point of view. *Physiology & Behavior 92*: 429-433

Webster AJF 1998 What use is science to animal welfare? *Naturwissenschaften 85*: 262-269

Welfare of Animals (Slaughter or Killing) Regulations 1995 The Welfare of Animals (Slaughter or Killing) Regulations. *Statutory Instrument 1995 No. 731 ISBN 011052778X*. The Stationery Office Limited. www.opsi.gov.uk/si/si1995/uksi_19950731_en_1.htm

Wiklund E, Manley TR, Littlejohn RP and Stevenson-Barry JM 2003 Fatty acid composition and sensory quality of Musculus longissimus and carcass parameters in red deer (*Cervus elaphus*) grazed on natural pasture or fed a commercial feed mixture. *Journal of the Science of Food and Agriculture 83*: 419-424

Wiklund E, Stevenson-Barry JM, Duncan SJ and Littlejohn RP 2001 Electrical stimulation of red deer (*Cervus elaphus*) carcasses, effects on rate of pH-decline, meat tenderness, colour stability and water-holding capacity. *Meat Science 59*: 211-220

https://doi.org/10.1017/S0962728600002700 Published online by Cambridge University Press