

Project Reference RP58b: The Impact of Different Culling Methods on Physiological and Physical Carcase Variables of Red Deer

Report from The Royal (Dick) School of Veterinary Studies, The University of Edinburgh to the Deer Commission for Scotland

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Summary

1. The potential welfare implications associated with methods for increasing the number of wild red deer that are culled were studied by comparing behaviour and post-mortem samples from wild deer shot (a) using the assistance of a helicopter for the deployment of stalkers and carcase extraction, (b) by more than one stalker at the same time, (c) by one stalker during the day or (d) by one stalker at night. Samples were also collected from farmed red deer shot in the field or killed at a slaughterhouse using a captive bolt and exsanguination.
2. Observations were made and 164 samples (78% from adult hinds, 15% adult stags and 7% calves) were collected at 13 locations during 56 events over two culling seasons for wild red deer and during the killing of farmed deer in the field on five occasions and at a slaughterhouse on one occasion. After death, blood and muscle samples were collected, the blood lactate concentration was measured, the locations of bullet wounds and other signs of injury were noted and muscle pH_u measured. The plasma activities of creatine kinase and aspartate aminotransferase, the plasma concentrations of haemoglobin, total bilirubin, urea, glucose, free fatty acid, β -hydroxybutyrate and cortisol and the muscle glycogen concentration were measured. A simple univariate analysis of the dependent variable and culling method (excluding the one slaughterhouse event), was conducted and if statistically significant, post-hoc multiple comparisons were undertaken. A multivariate mixed-effect model was then constructed with a series of random effects (location and event nested within location) and covariates (sex and age; time between start of cull and death; time between first shot and death; site of entry wound). For specific dependent variables, other covariates thought likely to have influenced the measurement were added. If differences between culling methods were obtained contrasts were used to examine the differences between culling methods. As 34% of the deer were not culled singularly, a bootstrap approach was also undertaken in which a single deer per event was randomly sampled to generate a sample of 60 for statistical comparisons and this was repeated 10,000 times.
3. The difficulties in obtaining sufficient numbers of separate culling events for each culling method (rather than total number of deer culled per method) and potential issues with sample handling meant that the statistical analyses were problematic. Although the reliability with which the results can be reported is less than the authors would have liked, it has been possible to come to some tentative conclusions regarding the various culling methods. It should be cautioned that there are other feasible, but less likely, explanations for the results obtained and it should be noted that not all of the conclusions suggested below are supported by the most conservative statistical analyses. Some of the apparent differences might have been due to factors other than those related to a particular culling method.
4. Plasma cortisol concentrations in wild deer shot using the assistance of a helicopter for the deployment of stalkers and carcase extraction (91 nmol/l), were similar to those found in

farmed red deer killed at a slaughterhouse (92 nmol/l). They were higher than in wild deer shot at night by a single stalker (21 nmol/l) or shot during the day by one stalker (47 nmol/l), or in farmed deer shot in a field (36 nmol/l). Wild deer shot when more than one stalker was deployed during the day (82 nmol/l) had greater plasma cortisol concentrations than those shot at night by one stalker. It is concluded on the basis of these plasma cortisol concentrations that the culling of wild deer by rifle, using the assistance of a helicopter for the deployment of stalkers and carcass extraction, was more stressful than the culling of wild deer by rifle with one stalker at night or during the day, or the culling of farmed deer by rifle in a field but it was not more stressful than the culling of wild deer by rifle when more than one stalker was deployed at a time.

5. The time between the start of the cull and the first shot in wild deer shot using the assistance of a helicopter for the deployment of stalkers and carcass extraction was longer than for other methods and, during this time, the deer were more active and were observed trotting before they were shot. In some deer, this method of culling appeared to have been associated with haemolysis of red blood cells before death. The haemolysis could have occurred due to exercise (some deer had detectable concentrations of plasma haemoglobin from the breakdown of red blood cells and raised plasma total bilirubin concentration, caused by the breakdown of the haem in haemoglobin in the liver before death). The lower muscle glycogen concentration in wild deer shot using the assistance of a helicopter for the deployment of stalkers and carcass extraction (and also in wild deer in culled by one stalker during the day) compared to those culled by one stalker at night or in farmed deer culled in the field suggests that glycogen was utilised as an energy source in the muscle during exercise. The raised muscle pH_u in wild deer shot using the assistance of a helicopter for the deployment of stalkers and carcass extraction and in deer culled by one stalker during the day was consistent with the utilisation of muscle glycogen before death and decreased lactic acid formation during post-mortem glycogen metabolism. As the blood lactate concentration was higher in wild deer shot, using the assistance of a helicopter for the deployment of stalkers and carcass extraction, than in wild deer shot by one stalker during the day, this suggests that intense anaerobic muscular exercise might have occurred shortly before death. The muscle glycogen concentration and the muscle pH_u suggest that farmed deer culled in the field experienced the least exercise before death. It is concluded that culling of wild deer shot using the assistance of a helicopter for the deployment of stalkers and carcass extraction appeared to have been associated with more exercise by the deer before death than in those culled by the other methods.
6. There were no differences between the culling methods in plasma creatine kinase and aspartate aminotransferase activities that would have indicated muscle damage or injury. With all the methods there was an absence of bruising and a very low occurrence of skin injury. It is concluded that there was no evidence that a particular culling method was associated with an increased risk of injury before death.
7. Culling of wild deer with a rifle by one stalker during the day and culling by rifle of farmed deer in a field were the most accurate and culling by rifle at night by one stalker was the least accurate in achieving placement of a shot likely to have quickly killed the deer. However, there was no significant difference between the culling methods in the number of deer that appeared to have died immediately after the first shot. There was no significant difference between culling methods in the percentage of deer that were wounded in the abdomen or leg. The highest percentage of deer that after the first shot, ran or walked away without collapsing were in wild deer shot using the assistance of a helicopter for the deployment of stalkers and carcass extraction and those shot by rifle at night by one stalker.
8. There was no effect of culling method on the pH of neck/shoulder muscle (*M. Triceps brachii*) or back muscle (*M. Longissimus dorsi*). Wild deer culled at night by rifle by one stalker had a lower pH of back-leg muscle (*M. Biceps femoris*) than those culled using the assistance of a helicopter for the deployment of stalkers and carcass extraction, with one

and upper-quartile values were less than 6.0, which is considered acceptable in relation to meat quality. It is concluded that there was no consistent evidence that the muscle pH of the carcass was influenced by culling method.

9. Farmed deer shot in the field had greater plasma concentrations of urea and glucose and a lower plasma concentration of β -hydroxybutyrate than wild deer from the different culling methods. It was concluded that the farmed deer shot appeared to have been in a better nutritional state than the wild deer shot.
10. If wild deer are shot using the assistance of a helicopter for the deployment of stalkers and carcass extraction they are more likely to be disturbed before they are shot than with the other methods and measures should be taken to minimise the disturbance to the wild deer. Culling of wild deer by rifle at night appeared to be associated with little disturbance to the deer, but the effectiveness of this method is dependent on whether the high proportion of deer shot in the upper rather than in the lower chest in this study is a significant factor in achieving a quick and humane death. The effects of location of wounds when shooting wild deer on the effectiveness of the kill merits further research.

Introduction

Different culling methods are employed to cull deer in different situations in Scotland. These range from single rifle 'stalking' deer and shooting them 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 objectives when shooting a wild deer are to render it instantaneously or almost instantaneously insensible from the first shot and not to cause it injury or undue stress before it is shot. It is more difficult for a stalker shooting a deer to achieve an instantaneous death, than it is in a slaughterhouse where the head is a close and controlled target. A stalker normally aims for the thorax or neck as they represent an achievable target for stopping and ultimately killing the deer (Thomas and Allen, 2003). Although shots aimed to kill red deer by severing the cervical spinal cord are used, the risk of missing the spinal cord and causing a non-fatal wound or a delayed death is high (Urquhart and McKendrick, 2003a). Shooting deer from a distance does not always result in an effective kill and some deer can be wounded. The likelihood of wounding deer might be greater in situations where guests on hunting estates shoot stags as compared with the culling of deer by professional stalkers. Bradshaw and Bateson (2000) studied wild stags and hinds shot in the head or neck by stalkers in the southwest of England and in Scotland and assessed behaviour, wounding rates, body condition and blood composition. They considered that the main potential welfare issue with stalking related to wounding rates and in particular, when deer escaped, but were wounded. Other concerns were the risk of injury when the deer tried to escape from the stalkers and disruption of social groups. It was estimated that 2% of deer escaped wounded and 11% of those shot during single rifle stalking were not killed with the first shot. Bateson and Bradshaw (2000) reported that deer shot more than once during single rifle stalking had a greater plasma cortisol concentration than those killed with one shot. Deer shot in the chest ran a short distance before collapsing whereas those shot in the head/neck did not run after they were shot. In deer killed after a prolonged chase by hounds, Bateson and Bradshaw (1977) found evidence of utilisation of carbohydrate in the muscles, muscle damage, increased plasma cortisol concentration and haemolysis. In comparison with death after hunting with hounds, Bateson and Bradshaw (1977) and Bradshaw and Bateson (2000) reported that single rifle stalking resulted in a less severe metabolic response with a significantly lower plasma activity of creatine kinase, less evidence of haemolysis and lower plasma concentrations of cortisol, free fatty acids and lactate.

The intention of this study is to consider the potential welfare implications associated with methods for increasing the number of wild red deer that are culled. However, as discussed by Fraser (2003), animal welfare cannot be assessed in a scientific way that is independent of ethical considerations. Although it is possible to undertake measurements that can be considered to be relevant to animal welfare and report them in a scientific manner, the choice of which variables to measure is dependent on what can be objectively measured and the perspective that a particular researcher brings to the animal welfare assessment. In some circumstances, a comprehensive welfare assessment may not be possible and many important aspects of welfare cannot be measured in an empirical manner. In the course of an animal welfare assessment, the same set of variables may be interpreted differently by different people depending on their value related judgments and they can legitimately come to divergent conclusions. This does not mean that it is not possible to undertake animal welfare assessments, but it does mean that the basis for the assessment must be explicit and the limitations of the methodology recognized.

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 sampling was undertaken on open moorland with the remainder in forestry or fields. Hinds were the main target for culling and were therefore preferentially selected for sampling. However, additional 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 (as is common during the 'stag season') were included, only deer shot by professional stalkers, as part of normal management culling, were included in this study. Observations were also made and samples collected from farmed deer in fields at one location in March 2007 and 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.

Deer that were culled were studied by comparing behaviour and post-mortem samples from wild deer shot (a) using the assistance of a helicopter for the deployment of stalkers and carcass extraction (helicopter assisted culling), (b) by more than one stalker (collaborative culling), (c) by one stalker (single rifle culling) or (d) at night (night culling). Samples were also collected from farmed red deer shot in the field (field) or killed at a slaughterhouse using a captive bolt and exsanguination (slaughterhouse).

The following methods used to cull red deer were compared.

Single rifle stalking

At six sites, on 16 days, groups of wild red deer were located and stalked on each occasion by one rifleman. After several shots had been fired, the group of deer normally moved away and the culling event ended.

Collaborative stalking

At four sites, on 9 days, 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 on to a different area.

Helicopter-assisted stalking

At seven sites, on 17 days, 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. 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 and groups of deer and to remove the carcasses to the larder.

Night shooting

At five sites, on 13 days, 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.

Field shooting

On 4 days, farmed red deer were shot by a single rifleman in a field at a deer farm. Between one and eight deer were shot in each culling event.

Slaughterhouse

A single group of farmed red deer were transported to a slaughterhouse, lairaged for 48 hours, stunned in the head with a captive bolt and then exsanguinated.

The types of rifle cartridge used to shoot the deer are shown in Table 1.

Table 1. Type of rifle cartridge used to shoot deer (% of deer shot per culling method)

Culling method	Type of rifle cartridge						Captive bolt
	6.5 x 55 mm 120 grain	0.243 inch 100 grain	0.243 inch 130 grain	0.270 inch 120 grain	0.270 inch 130 grain	0.270 inch 150 grain	
Helicopter assisted	23	18	2	0	57	0	0
Collaborative	53	0	0	0	47	0	0
Single rifle	10	0	0	0	90	0	0
Night	33	0	0	0	67	0	0
Field (farmed deer)	0	0	0	5	65	30	0
Slaughterhouse (farmed deer)	0	0	0	0	0	0	100

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, between the time when the first shot was fired on a group of deer at one location, until all of the deer had been shot or the group moved to another area and were stalked again before shooting restarted. Where possible, the behaviour of the individual deer before and after 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 or if the deer was still alive after shooting, the time that it was dispatched by alternative methods (usually by exsanguination). It was noted whether the deer (a) collapsed without moving location, (b) ran <5m, 5-20m or 20-50m before collapsing or (c) walked or ran away without collapsing. If the deer was part of a group, the behaviour of the deer 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 carcass as soon as possible after death. The timings of sample handling in relation to time of death were recorded using a stop watch. When wild red deer were bled from a knife wound in front of the sternum and farmed deer were bled from the neck, blood samples were collected into one 10ml tube contained heparin and two 2.5ml tubes containing fluoride oxalate. The blood lactate concentration was measured on-site using a Lactate Pro meter and test strips. A muscle sample was collected from immediately below the skin on the left shoulder (*M. Triceps brachii*), snap frozen in a mixture of isopentane and dry ice, 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 minute intervals using a temperature logger (Tinytag Explorer) and transported to the nearest location for centrifugation. The samples were centrifuged at 3000rpm for 5 minutes; the plasma extracted, stored frozen using dry ice, transported to the laboratory and kept frozen until analysis.

The temperature profile for storage of blood samples in Figure 1 shows that most samples were stored at an ideal temperature (the median temperature was 3.5°C). At the time of collection 84% of the samples were stored at a temperature of <10°C. Twenty-six of the samples were kept above this temperature and even then, the maximum temperature was 18°C. After 0.5h of storage, the number of samples that were kept at a temperature above 10°C was reduced to 18. However, ten samples were stored at a temperature of <0°C.

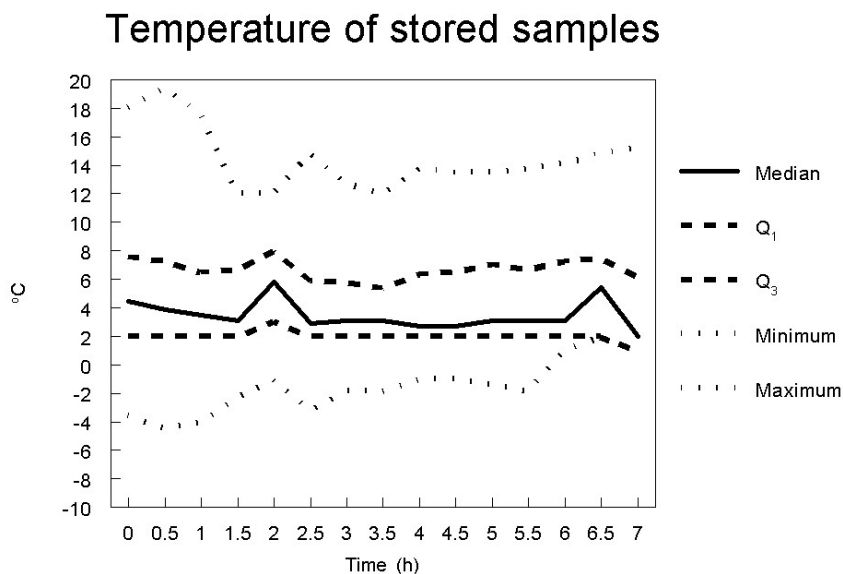


Figure 1. Temperature of stored blood samples

The time between death and blood sampling was short, and on average less than 9 minutes (Table 2). The longer time (4h) between blood sampling and plasma separation should not have caused any great difficulty, as the storage temperature of the blood was low.

Table 2. Sampling times (hours) for blood and muscle samples

Time between(hours)	Mean	SE	N	Min	Q1	Median	Q3	Max
Death and blood sampling	0.14	0.002	159	0.00	0.08	0.08	0.15	2.12
Blood sampling and separation of plasma after centrifugation	3.57	0.015	164	0.00	3.30	3.30	5.10	11.30
Blood sampling and plasma storage	3.97	0.015	164	0.00	3.69	3.69	5.42	11.80
Death and plasma storage	4.19	0.015	159	0.02	4.17	4.17	5.47	11.97
Death and muscle sampling	0.42	0.005	159	0.00	0.13	0.13	0.41	4.05
Death and muscle freezing	0.47	0.005	159	0.02	0.17	0.17	0.52	4.05
Death and muscle pH reading	27.61	0.065	145	10.75	25.32	25.32	28.63	85.90

Carcass identification and examination

Unique tags were attached to a back leg of the carcass for identification at the larder. After death, the deer were categorised as an adult or a calf, the sex of the deer was recorded, hinds were recorded as in milk or yield. The locations of entry and exit wounds in the carcass caused by bullets were noted (Urquhart and McKendrick, 2003a). Any signs of skin injury and any bone fractures were recorded. The carcasses were transferred to a larder within 6h of death. Body condition was assessed as thin, normal or fat and the percentage of fat on the kidneys was recorded as <25%, 25-50%, 51-75% or >75%. After skin removal, any obvious signs of bruising were recorded. After

approximately 24h after death, the pH in the muscle of the neck/shoulder (*M. Triceps brachii*), back (*M. Longissimus dorsi*) and back-leg (*M. Biceps femoris*) was recorded using a meat pH meter (Hanna Instruments 99163).

Laboratory analytical methods for plasma and muscle

Plasma haemoglobin concentration was measured on a 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), total bilirubin concentration (kit 18254640), urea concentration (kit 18255440) and glucose concentration (kit 18250740); free fatty acid concentration using WAKO Chemicals (WAKO Chemicals Neuss, Germany) NEFA-C kit and plasma β -hydroxybutyrate concentration using a Randox kit (Ranbut Rb1008) (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). 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, H., 1974; Adamo and Graham, 1998).

Number of samples, number of culling events and statistical methodology

The number and profile of samples collected are presented in Table 3, with 164 deer culled in 62 culling events. There was marked variation both in the number of culling events per culling methodology (1-16), and in how many deer were culled (8-37). This resulted in the average number of deer culled per culling event varying from 1.5-30, with 34% (21/62) of the culling events only involving a single deer (Table 3 and Figure 2).

Table 3. Number of samples and number of culling events by culling method

Culling method	No. of samples	No. of culling events	Mean no of samples / culling event	Min	Max
Helicopter assisted / single rifle [†]	8	3	2.7	1	5
Helicopter assisted / multiple rifles [†]	37	14	2.6	1	5
Collaborative	17	9	1.9	1	3
Single rifle	31	16	1.9	1	4
Night	21	14	1.5	1	3
Field (farmed deer)	20	5	4.0	1	8
Slaughterhouse (farmed deer)	30	1	30.0	30	30
Total	164	62	2.6		

[†] Combined into single category ‘*Helicopter assisted*’ for the statistical comparisons between culling methods.

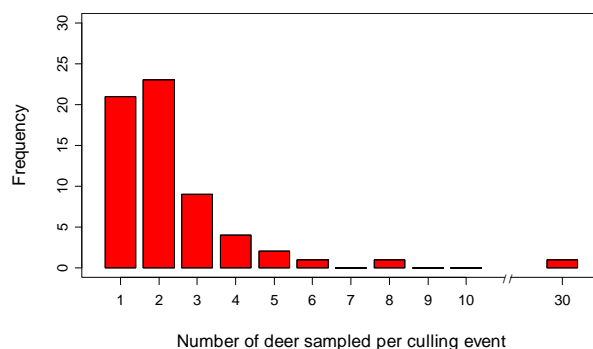


Figure 2. Frequency distribution of number of samples per culling event

This distribution of samples per culling events greatly complicated the statistical analyses. For the 66% of culled deer that were not culled singularly, taking into account the culling event in any analyses is important, as there could be factors that are related to the actual culling event and affect any measured parameters for all the deer culled in that event. Multiple samples per event would normally be accounted for in any statistical analyses using mixed-effect models; however, such models are not appropriate in this case because of the 34% of events, which only involved a single deer. However, the multiple samples per event cannot be completely ignored. As a consequence, two analytical approaches have been adopted. First, a set of analyses was undertaken where the number of samples collected per event was entered as a covariate in all statistical analyses. This approach partly accounts for the influence of event on any values measured. However, in order to be more confident of any results obtained a bootstrap approach was also undertaken. Here a random sample per culling event was selected generating a sample of 62 upon which analyses were carried out. This sample creation and subsequent statistical testing was carried 10,000 times in order 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.

Finally, there was one culling method in which only one event occurred (slaughterhouse), and this further confounds any potential analyses - the culling cannot be disentangled from the method. In other words despite the fact that samples were collected from 30 deer at an slaughterhouse – the results obtained could be a result of that particular visit to that slaughterhouse as opposed to anything specific to all slaughterhouses. Therefore, all analyses that compared culling methods excluded the slaughterhouse samples in order to ensure the results were not being overly influenced by the one culling event.

Table 4 shows the breakdown of samples collected by type of deer. The majority of samples were from female deer, with 87% of these yeld hinds. Relatively few calves were sampled; therefore, in terms of the analysis on sex and age the following groups were investigated: yeld hinds, milk hinds and adult males. In addition, all the analysis on the impact of culling method on various parameters were also carried out on just the adult female deer data ($128/164 = 78\%$), in order to rule out any disproportionate influence of the minority of deer that were not adult females.

Table 4. Number of samples by type of deer (Sex/age)

Sex/age	No. of samples
Yeld hind (pregnant)	58
Yeld hind (not pregnant)	53
Milk hind (pregnant)	13
Milk hind (not pregnant)	4
Female calf	5
Male calf	6
Stag	25
Total no.	164

Ideally, the role of type of deer and culling method would have been explored in depth, but as Table 5 shows, the data is very unbalanced in terms of representation of deer group and culling method, thereby precluding such analyses.

Table 5. Number of samples in each culling method by type of deer (Sex/age)

	Helicopter assisted / single rifle	Helicopter assisted / multiple rifles	Field	Collaborative	Single rifle	Night	Slaughterhouse
Yeld hind (pregnant)	5	19	5	9	14	6	0
Yeld hind (not pregnant)	2	9	5	4	3	2	28
Milk hind (pregnant)	0	2	0	1	7	3	0
Milk hind (not pregnant)	0	2	0	0	2	0	0
Female calf	1	1	0	1	1	1	0
Male calf	0	2	0	0	4	0	0
Stag	0	2	10	2	0	9	2

A whole series of parameters that are not related to the culling method could have influenced the values observed (Table 6). Ideally, a multivariate analysis would be constructed with all of the above relevant factors included as either covariates (*e.g.* Sex) or as random factors to be taken account of (*e.g.* some events have more than one deer culled, so there may be an event effect). However, this is not possible as there are extremely unbalanced categories within factors.

Table 6. Uncontrolled factors and the variables most likely to have been influenced by them

Uncontrolled factors	Variables most likely to have been influenced
Date † Location Event Observer	Potentially all variables
Stalker † Type of weapon † Type of rifle cartridge † Site of entry wound Site of exit wound † No. of entry wound sites † Skin injury † Fractures †	Variables related to injury and stress
Sex Age (calf / adult) Pregnancy status † Whether lactating † % of kidneys covered by fat Body condition score	Potentially all variables Variables related to nutritional state
No. of deer previously culled Time from.... .. first to second shot .. first shot to time of death .. start of cull to first shot .. start of cull to death	Variables related to injury and stress
.. death to bleeding .. death to blood sampling .. blood sampling to plasma separation .. blood sampling to plasma storage .. death to plasma storage	Blood Chemistry
.. death to muscle sampling .. death to muscle freezing	Muscle glycogen
.. death to muscle pH reading	Muscle pH
Sample temperature at various time points	Blood chemistry

† factor not taken into consideration in the statistical analyses

As stated above of the 62 unique culling events, 21 (34%) only have one deer culled, and so it was not possible to disentangle an “event” effect from a particular “deer” effect.

There were also some issues with respect to unbalanced data for:

(a) Location:

There were two locations where only one deer was culled. This makes taking into account the impact of location difficult. There was also a single slaughterhouse studied.

(b) Stalker:

There is marked variation in the stalkers – with 10 stalkers only culling 1 deer. This implies that the impact of stalkers cannot be examined.

(c) Observer:

The vast majority of data was observed by two people. There are stalkers that only operated at one location, this makes disentangling location and stalker more complicated. If location and observer are considered, there are locations in which a single observer was present. However, the two main observers went to more than one location.

It was also difficult to take into account the time between the first shot and death, the time between the start of cull and death and the site of the entry wound:

- no time between first shot and death was recorded for 13 of the deer from the field group. This only leaves seven field shot deer, which is likely to impact on any analyses.
- no time between the start of cull and death was recorded for five deer (all field shot).
- no entry wound site was recorded for 10 deer, but these were spread across different culling methods and so their impact is considered to be minimal.

The following approach was therefore adopted for the statistical analyses:

(i) The slaughterhouse samples were not included as the 30 deer were culled at a single event. This means that it was not possible to distinguish between an “*event*” effect and a “*slaughterhouse*” effect.

(ii) A simple univariate analysis of the dependent variable and culling method which ignored the multiple deer/event/location was conducted. If this was statistically significant, post-hoc multiple comparisons of the mean values using Tukey contrasts were undertaken.

(iii) A multivariate mixed-effect model was then constructed. This model had a series of random effects (factors to take account of because of non-independence between deer) and covariates (factors to take account of as they may influence the results observed for the culling method) :

a) Random effects :

- *Location*
- *Event* nested within location

For some locations, this was the same as event. Therefore, the analysis was repeated just including those locations where more than one event occurred.

b) Covariates :

- *Sex* and *Age* of animal
- *Time between first shot and death* - unless was considered a dependent variable
- *Time between start of cull and death*
- *Site of entry wound*

The impact of *stalker* as a covariate was ignored.

All models were re-run with just the main two *observer*’s data.

Date as a covariate was ignored– “*Event*” should account for any date effect.

For specific dependent variables, other covariates were added into the multivariate model (see *Table 6*) for these covariates.

If differences between culling methods were obtained contrasts were used to examine the differences between the individual variable levels

- (iv) The problem with this analysis was that some of the events only involve a single deer, and yet others involved multiple deer. In order to ascertain what influence these differences had on the overall patterns, bootstrap analyses were also performed. For these analyses :
- a. A single deer per event was randomly sampled.
 - b. Univariate analysis as described above was then repeated
 - c. The multivariate analyses were then run.
 - i. The only random effect was location;
 - ii. The analyses was also repeated for only those data for which more than event occurred in a location;
 - iii. All models were also re-run with just the 2 main observer's data;
 - iv. The other co-variates were added as above;
 - v. Residuals were also checked, and Kruskal-Wallis analyses were undertaken if no appropriate normally distributed residuals could be obtained
 - d. This was all then repeated 10,000 times. This generated a distribution of statistical significance which was then be checked with the analytical results obtained in (iii) above. The frequently with which the bootstrap results differed from the entire data set in terms of statistical/non-statistical significance ($P < 0.05$) was considered.

All of these analyses presented above are repeated for the various dependent variables. At all times the residuals of any model were checked prior to reporting. If there were any parameters for which the residuals were not normally distributed, transformation of this parameter was attempted. Any dependent variables for which no transformation was possible were merely reported as a simple non-parametric comparison (Kruskal-Wallis). Statistical significance was taken as 0.05 through out, and degrees of freedom are quoted as subscripts.

Results

Appendix A contains tables showing summary statistics of the raw data for each of the variables recorded.

Haemolysis

Haemoglobin (see *Table A1* in the Appendix for a numerical summary of the raw data)

There were only seven deer in which the plasma haemoglobin concentration was greater than 0.6 g/l of these six were from the helicopter assisted culling group and one from the collaborative culled group. There was no detectable haemoglobin in any of the other samples.

Total bilirubin (*Table A2*)

There were 18 total bilirubin values that were positive, but less than the minimum detection threshold (0.2 $\mu\text{mol/l}$). Therefore, the 18 very low total bilirubin values were reallocated using a random value generated from a uniform distribution with a minimum of 0.01 and a maximum of 0.1. There was a univariate statistically significant difference in the square root transformed plasma total bilirubin concentrations between the five culling methods ($F_{4,128}=7.0$, $P < 0.001$, Fig. 3). Post-hoc analysis revealed that these differences were between deer culled with the assistance of a helicopter and collaborative ($P=0.02$), single rifle ($P < 0.01$), night ($P < 0.01$) and field ($P < 0.03$) methods. The highest total bilirubin concentrations were in the group killed at the slaughterhouse. This significant result did not remain when the analyses were repeated using a multivariate analysis that included the following nine covariates: sex, age, site of entry, and six time variables: time between first shot and death, start of cull and death, death to bleeding, death to blood sampling, blood sampling to plasma separation and storage and site of entry wound; and the two random effects: location with event nested within ($P=0.202$). Excluding the 24 deer that were from locations

where only one culling event occurred, or if the event was only one deer, did not affect this change in statistical significance ($P=0.508$).

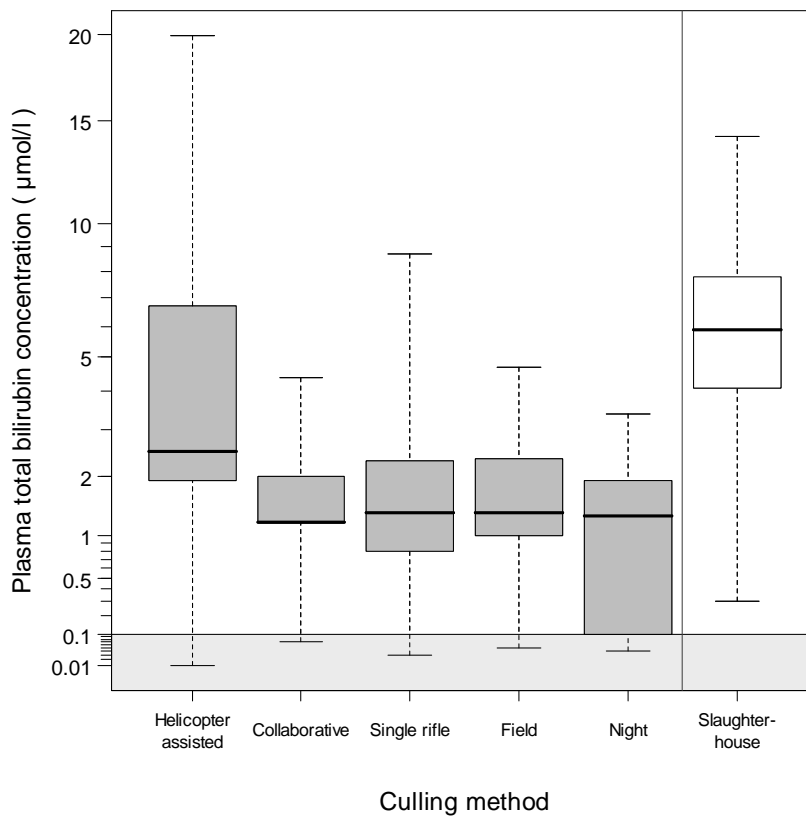


Figure 3. Boxplot of square root transformed plasma total bilirubin concentration ($\mu\text{mol/l}$). Horizontal line within the box shows median Q_2 , the box shows the interquartile range Q_1 to Q_3 and the dashed line shows the range of values. The light grey box indicates values that are positive, but are less than the minimum detection threshold (0.2), and have therefore been reallocated using a random value generated from a uniform distribution with a minimum of 0.01 and a maximum of 0.1.

Time to blood sampling (Table A3)

There was a univariate statistically significant difference between the five culling methods in the square root transformed time from death to blood sampling ($F_{4,114}=17.7$, $P<0.001$, Fig. 4). Post-hoc analysis revealed that these differences were between deer culled in a field and the four other culling methods: via helicopter assisted, collaborative, night and single rifle (all four $P<0.01$).

When the analyses were repeated with the multivariate analyses and the five covariates: sex, age, site of entry wound and time between first shot and death, start of cull and death; and the two random effects: location with event nested within, these differences remained statistically significant ($P=0.004$), with the statistical differences again between field and the other four culling methods. Excluding the 24 deer that were from either locations where only one culling event occurred, or if the event was only one deer, made no qualitative difference ($P=0.011$).

All of the statistical analyses for each of the plasma variables were repeated with the three blood samples obtained $>0.5\text{h}$ after death removed. For univariate and multivariate analyses, no statistically significant results at ($P<0.05$) changed to a non-significant result ($P>0.05$) and no results that were not significant changed to statistically significant by the exclusion of these three samples.

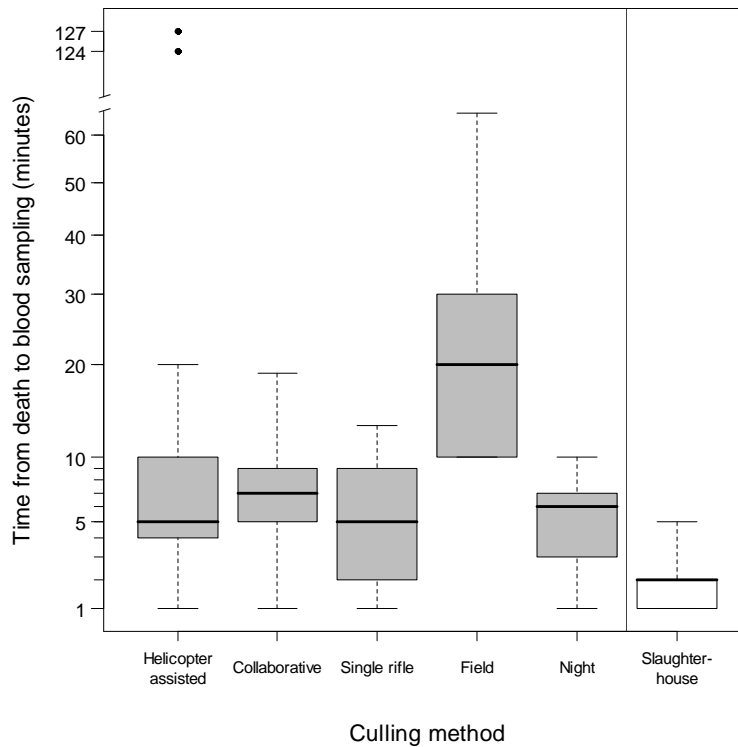


Figure 4. Boxplot of square root transformed time (minutes) between death and blood sampling.

Storage temperature

All of the statistical analyses for each of the plasma variables were repeated (a) with the 26 blood samples not stored at $<10^{\circ}\text{C}$ at the time of collection removed, (b) with the 18 blood samples not stored $<10^{\circ}\text{C}$ after 0.5h of storage removed and (c) with the 10 blood samples stored at $<0^{\circ}\text{C}$ removed. For univariate and multivariate analyses, no statistically significant results at ($P<0.05$) changed to a non-significant result ($P>0.05$) and no results that were not significant changed to statistically significant by the exclusion of any of the above samples.

Shooting

Time from start of cull to first shot (Table A4)

For 70 deer (58%), the time between the start of the cull and the first shot was 0s. An analysis on whether this time was zero or not was performed. GLM with binomial errors showed that there was a significant difference between the methods ($P<0.001$), with a greater number of deer shot $>0\text{s}$ after the start of the cull for helicopter assisted culling than for night, collaborative and single rifle methods. Table A5 shows statistics for the time between the start of the cull and death.

Time from first shot to death (Table A6)

For eighty three of the deer, the time between the first shot and death was recorded as 0s (Table 7).

Table 7 No. of deer that died immediately after the first shot (0s) and those in which there was an interval between the first shot and death.

	Time from first shot to death	
	0 s	>0 s
Helicopter assisted	28	17
Collaborative	12	5
Single rifle	21	10
Field	5	2
Night	17	4

To compare whether there was a difference between culling methods in the number of deer that died immediately after the first shot, generalised linear models with binomial errors of whether the time from the first shot to death was >0 s were used. For those deer where this time was >0 s, analyses of positive times were performed using the methods described above. There were no statistically significant differences between culling methods in whether the time from the first shot to death was greater than zero in the univariate ($\chi^2_4=2.52$, $P=0.641$, Table 7), or the multivariate mixed-effect model ($P=0.480$). As there were, only two deer culled in a field for which the time from first shot to death was greater than zero these were excluded from the analysis of positive times. There was no statistically significant differences between culling methods in the \log_{10} transformed time from first shot to death in the remaining deer in the univariate ($F_{3,13}=0.65$, $P=0.600$, Fig. 5), or the multivariate mixed-effect model ($P=0.827$).

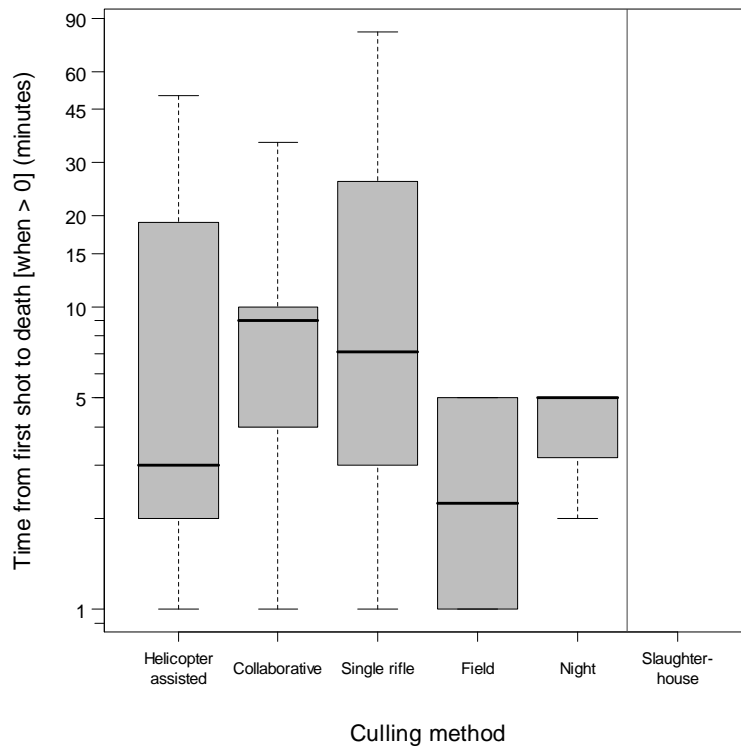


Figure 5. Boxplot of \log_{10} transformed time (minutes) from first shot to death (excluding deer that died immediately)

Time from first to second shot (Table A7)

The only comparisons possible were between deer culled with a single rifle and those culled with the assistance of a helicopter. 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. There was no statistically significant difference in the square root transformed time between deer culled with the assistance of a helicopter and those culled with a single rifle in the univariate ($F_{1,15}=0.64$, $P=0.437$), or the multivariate mixed-effect model ($P=0.960$, Fig. 6).

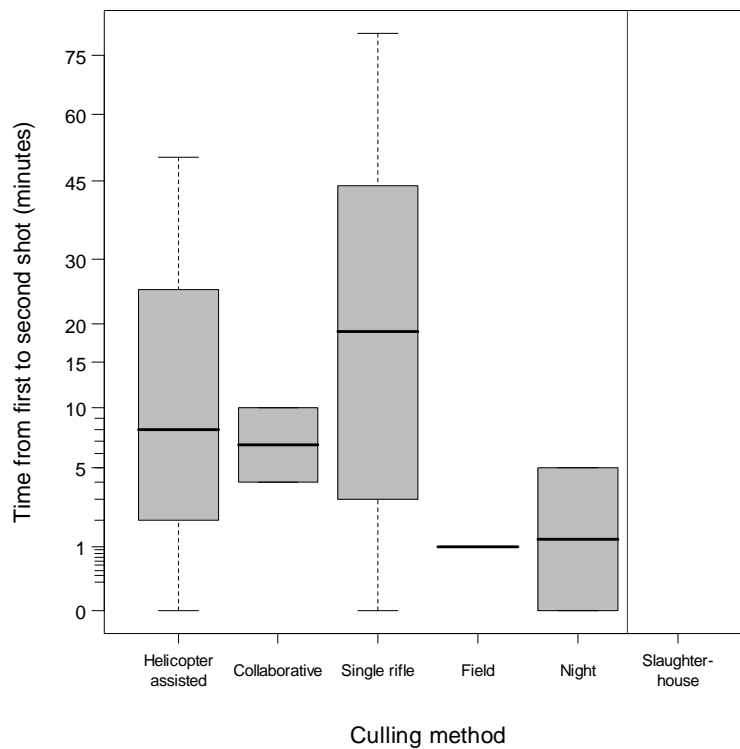


Figure 6. Boxplot of square root transformed time (minutes) from first to second shot.

Site of wound (Tables A8 and A9)

Field shooting was 100% accurate in achieving a shot in the head (Table 8) and this culling method has been excluded from subsequent analyses of wound sites.

Table 8. Percentages of deer with a wound in the head, neck or lower chest/heart and those with at least one entry wound not in the head, neck or chest

	Accuracy of shooting	
	% of deer with at least one entry or exit wound in either the head, neck or lower chest/heart	% of deer with at least one entry wound not in either the head, neck or chest
Helicopter assisted	74	16
Collaborative	67	13
Single rifle	89	7
Field	100	0
Night	50	11

There was a univariate 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 ($\chi^2_3=9.9$, $P=0.019$). Post-hoc analysis revealed that these differences were between deer culled at night and single rifle stalking ($P=0.022$). When the analyses were repeated with the multivariate analyses with the two covariates: sex and age, and the two random effects: location with event nested within, this difference remained ($P=0.007$). There was no univariate 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 ($\chi^2_3=1.4$, $P=0.702$). When the analyses were repeated with the multivariate analyses with the two covariates: sex and age, and the two random effects: location with event nested within, the lack of differences remained.

Behaviour

Behaviour of individual deer before first shot (Table A10)

For the 108 deer in which it was reported, the vast majority (87, 81%) of the deer were standing but alert, before they were shot (Table 9).

Table 9. Number and percentage of deer standing alert before the first shot

	Behaviour of individual deer before first shot		
	No. of deer not standing alert	No. of deer standing alert	% standing alert
Helicopter assisted	11	30	73
Collaborative	4	10	71
Single rifle	6	25	81
Field	0	1	-
Night	0	21	100

There was only one deer culled in the field for which this information was observed and so this data was excluded. Fisher's exact test was used to examine whether the number of deer standing alert differed between culling methods. There was a statistically significant difference between the four culling methods in whether the behaviour before the first shot was standing alert ($P=0.030$, Table 9), with the difference apparently due to the night culled deer all standing alert, whereas only 70-80% of the deer in the other culling methods were standing alert. If field culling is excluded from the analysis (only one observation was recorded), and activity categorised as either: walking, running or trotting and inactivity as either: lying down or standing, Fisher's exact test of whether the deer were active or inactive before the first shot showed that there was a significant difference ($P<0.001$) between culling methods. Helicopter assisted culling resulted in the greatest activity (one third 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.

Behaviour of individual deer after first shot (Table A11)

For the 113 deer in which it was reported, the majority (62, 55%) of deer after being shot, collapsed immediately (Table 10). A further 40 deer (35%) ran some distance (from <5 to 50 m) before collapsing, the remaining 11 deer either ran or walked away.

Table 10. Number (%) of deer that collapsed, ran then collapsed or ran / walked away after the first shot

	Behaviour of individual deer after it was shot		
	Collapsed (%)	Ran then collapsed (%)	Ran / walked away without collapsing (%)
Helicopter assisted	19 (43)	4 (9)	21 (48)
Collaborative	10 (67)	0	5 (33)
Single rifle	22 (71)	6 (19)	3 (10)
Field	0	0	2
Night	11 (52)	1 (5)	9 (43)

Therefore, for the analyses these three groups were considered. However, there were only two deer shot in the field for which this information was observed and so this data was excluded. Fisher's exact test of whether the behaviour after the first shot differed between culling methods revealed a statistically significant difference between the four culling methods in the behaviour after the first shot ($P=0.008$). The highest percentages of deer that after the first shot, ran or walked away without collapsing were in the helicopter assisted and the night culled groups.

Behaviour of group after first shot (Table A12)

For the 103 deer in which it was reported, the vast majority (84, 82% of deer in groups ran away after the 1st shot (Table 11).

Table 11. Number (%) of times that a group ran away after the first shot

	Behaviour of group of deer after the first shot	
	Did not run away (%)	Ran away (%)
Helicopter assisted	6 (16)	31 (84)
Collaborative	2 (12)	14 (88)
Single rifle	6 (20)	24 (80)
Field	1	1
Night	4 (22)	14 (78)

There was only information on the behaviour of the group after the first shot for two deer shot in the field and so this data was excluded. Fisher's exact test of whether the behaviour of the group after the first shot differed between culling methods was carried out. 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 11).

Injury

Creatine kinase activity (Table A13)

Plasma creatine kinase activity data required box-cox transformation to normalise the residuals ($\lambda=-0.058$) [*i.e.* close to log]. There were no statistically significant differences between the five culling methods and the box-cox transformed plasma creatine kinase activities in the univariate ($F_{4,128}=1.0$, $P=0.408$, Fig. 7), or multivariate ($P=0.364$) analyses.

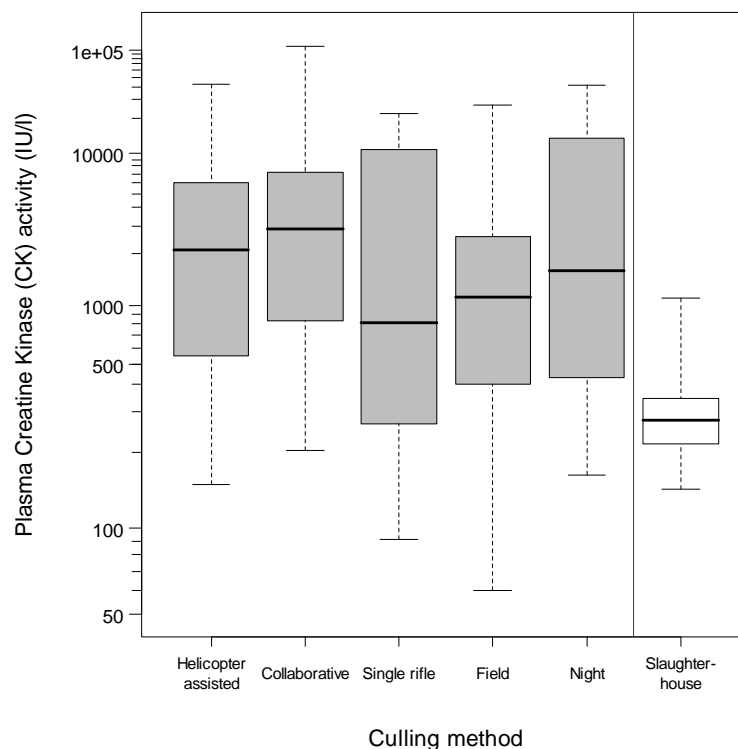


Figure 7. Boxplot of box-cox transformed plasma creatine kinase activity (IU/l)

Aspartate aminotransferase activity (Table A14)

There were no statistically significant overall differences between the five culling methods in the \log_{10} transformed aspartate aminotransferase activities in the simple univariate ($F_{4,128}=0.7$, $P=0.594$, Fig. 8), or multivariate ($P=0.701$) analyses.

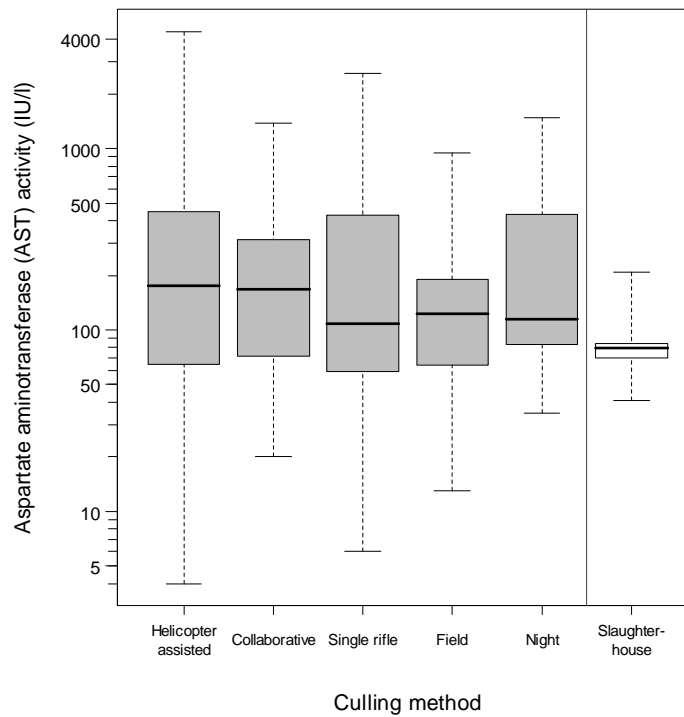


Figure 8. Boxplot of \log_{10} transformed aspartate aminotransferase activity (IU/l)

Muscle exercise

Time from death to muscle freezing (Table A15)

There was a univariate statistically significant difference between the five culling methods in the \log_{10} transformed time from death to muscle freezing ($F_{4,124}=36.7$, $P<0.001$, Fig. 9).

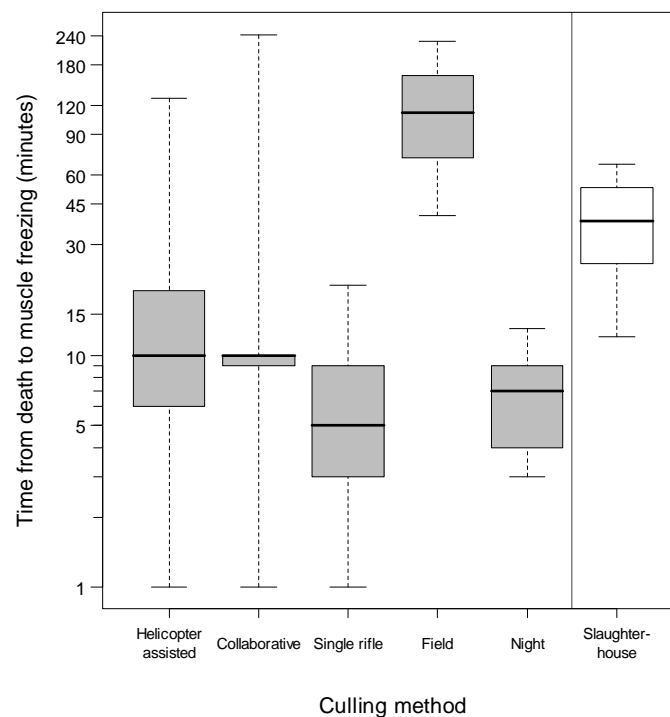


Figure 9. Boxplot of \log_{10} time (minutes) between death and freezing of the muscle sample

Post-hoc analysis revealed that these differences were between deer shot in a field and the four other culling methods: via helicopter assisted, collaborative, night and single rifle (all four $P < 0.01$). There were also apparent differences between deer culled by the single rifle method and those culled with the assistance of a helicopter ($P < 0.01$) or collaborative culling ($P = 0.041$).

When the analyses were repeated with the multivariate analyses with the five covariates: sex, age, site of entry wound and time between first shot and death, start of cull and death; and the two random effects: location with event nested within, these differences remained statistically significant ($P < 0.001$), with the statistical differences again between field and the other four culling methods, and between single rifle and collaborative methods. Excluding the 24 deer that were from either locations where only one culling event occurred, or if the event was only one deer, made no qualitative difference ($P = 0.002$).

Muscle glycogen (Table A16)

There was a univariate statistically significant difference between the five culling methods in the \log_{10} transformed muscle glycogen concentration ($F_{4,127} = 7.1$, $P < 0.001$, Fig. 10).

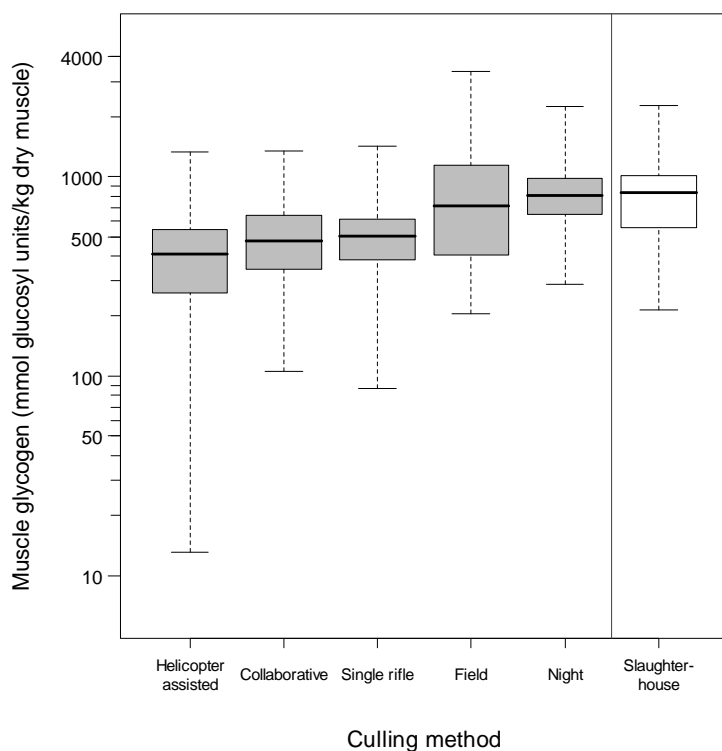


Figure 10. Boxplot of \log_{10} transformed muscle glycogen concentration (mmol glucosyl units/dry kg muscle)

Post-hoc analysis revealed that these differences were between deer culled at night and those culled via helicopter assisted ($P < 0.01$), collaborative ($P = 0.049$), and single rifle ($P = 0.020$); and between those culled with the assistance of a helicopter and those shot in the field ($P < 0.01$). When the analyses were repeated with the multivariate analyses with the six covariates : sex, age, site of entry wound and three time variables : time between first shot and death, start of cull and death, death to muscle sampling, death to muscle freezing; and the two random effects : location with event nested within, this difference remained statistically significant ($P = 0.043$). Muscle glycogen concentrations from helicopter assisted and single rifle culling were lower than that of night culled deer. Excluding the 24 deer that were from either locations where only one culling event occurred, or if the event was only one deer, resulted in a loss of statistical significance ($P = 0.093$). The results from the

10,000 bootstrap analyses revealed that statistically significant differences between culling methods were observed for 85% of the univariate, and 40% of the overall multivariate comparisons. This dropped to 33% if only deer that were culled from locations with multi-deer events were considered.

The statistical analyses for muscle glycogen concentration were repeated (a) with the 18 muscle samples frozen >1h after death removed, (b) with the 10 muscle samples frozen >2h after death removed, (c) with the 4 muscle samples frozen >3h after death removed, and (d) with the one muscle samples frozen >4h after death removed. For univariate and multivariate analyses, the statistically significant results at ($P < 0.05$) did not change to a non-significant result ($P > 0.05$) and no results that were not significant changed to statistically significant by the exclusion of any of the above samples.

Muscle pH

Time from death to muscle pH reading

There was a univariate statistically significant difference between the five culling methods in the reciprocally transformed time from death to the muscle pH reading ($F_{4,104}=2.8$, $P < 0.031$). Post-hoc analysis revealed that these differences were between deer shot in a field and three other culling methods: via helicopter assisted ($P=0.014$), collaborative ($P=0.023$) and single rifle ($P=0.037$) (Fig. 11).

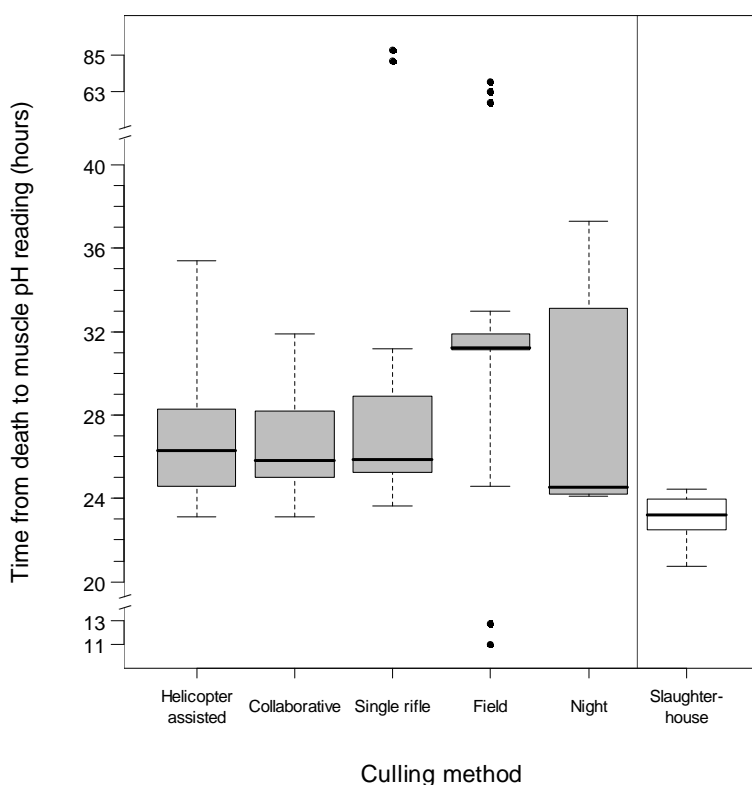


Figure 11. Boxplot of time from death to muscle pH reading ()

When the analyses were repeated with the multivariate analyses with the five covariates: sex, age, site of entry wound and time between first shot and death, start of cull and death; and the two random effects: location with event nested within, these differences disappeared ($P=0.719$). Excluding the 24 deer that were from either locations where only one culling event occurred, or if the event was only one deer did not change this result ($P=0.708$).

Muscle pH – neck/shoulder (M. Triceps brachii) (Table A17)

The residuals for neck/shoulder muscle pH indicated that parametric statistical approaches were problematic and that transformation of the data was not helpful (the pH readings were clustered around a mean of 5.71, but with some values at either extreme (Fig. 12)). There were no statistically significant overall differences between culling methods for the simple univariate ($F_{4,116}=2.1$, $P=0.096$), multivariate ($P=0.353$), or Kruskal-Wallis tests ($P=0.205$).

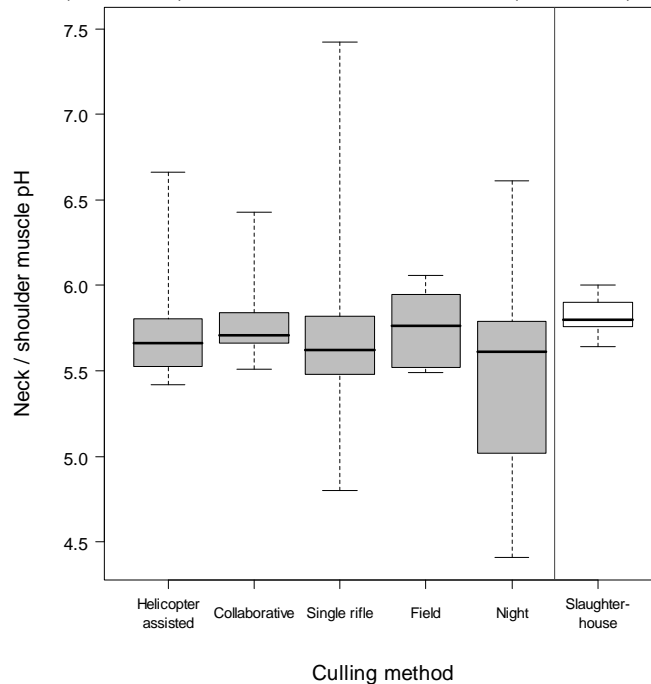


Figure 12. Boxplot of neck/shoulder muscle pH

Muscle pH – back (M. Longissimus dorsi) (Table A18)

The residuals for back muscle pH indicated that parametric statistical approaches were problematic and that transformation of the data was not helpful (Fig. 13). There were no statistically significant overall differences between culling methods in the simple univariate ($F_{4,116}=1.5$, $P=0.202$), multivariate ($P=0.806$), or Kruskal-Wallis univariate tests ($P=0.131$).

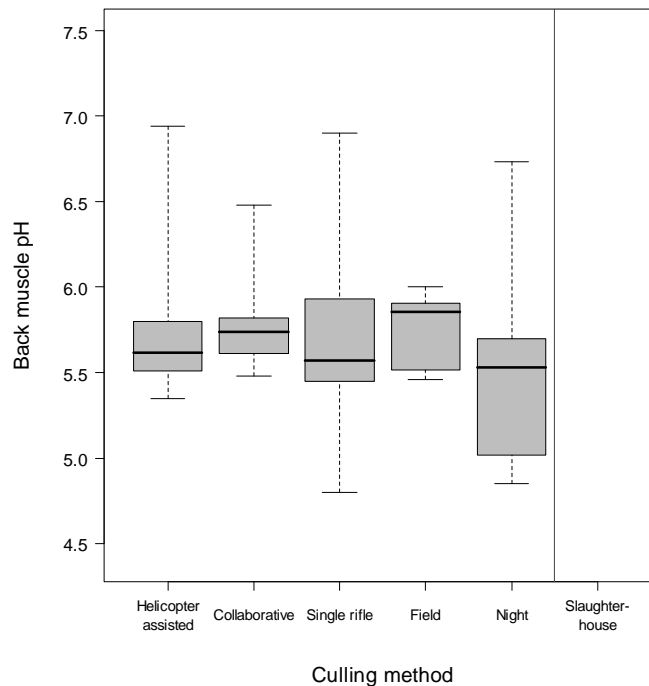


Figure 13. Boxplot of back muscle pH

Muscle pH – back leg (M. Biceps femoris) (Table A19)

The residuals of the back leg muscle pH were better than those for neck/shoulder and back, but were still problematic (Fig. 14). There was a univariate statistically significant difference between the five culling methods in the back leg muscle pH ($F_{4,116}=6.3$, $P<0.001$). Post-hoc analysis revealed this difference to be between deer culled at night and those culled with the assistance of a helicopter ($P<0.01$), collaborative ($P<0.01$), and single rifle ($P<0.01$).

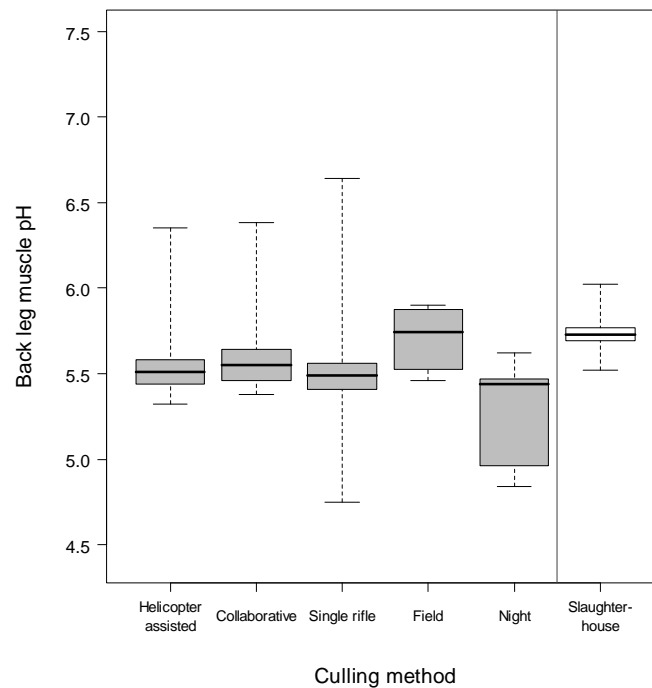


Figure 14. Boxplot of back leg muscle pH

However, when the analyses were repeated with the multivariate analyses with the five covariates : sex, age, site of entry wound and two time variables : time between first shot and death, start of cull and death, death to muscle pH sampling; and the two random effects : location with event nested within, this difference was lost ($P=0.060$). The main apparent reason for this was the statistically significant impact of time between death and muscle pH sampling on the back leg muscle pH ($P=0.041$). Excluding this variable resulted in the multivariate analyses indicating a statistically significant difference between the culling methods ($P=0.033$), with back leg muscle pH values from helicopter assisted and single rifle culling greater than that of night culled deer. Excluding the 24 deer that were from locations where only one culling event occurred did not change this result ($P=0.034$). The results from the 10,000 bootstrap analyses revealed that statistically significant differences between culling methods were observed for only 48% of the univariate, and 38% of the overall multivariate comparisons. This dropped to 30% if only deer that were culled from locations with multi-deer events were considered.

Blood lactate concentration (Table A20)

There was a univariate statistically significant difference between the five culling methods in the \log_{10} transformed blood lactate concentration ($F_{4,120}=7.7$, $P<0.001$, Fig. 15). Post-hoc analysis revealed that these differences were between deer culled in a field and those culled at night ($P<0.01$), collaborative ($P=0.046$), and single rifle ($P<0.01$) and between those culled with the assistance of a helicopter and single rifle culled deer ($P<0.01$).

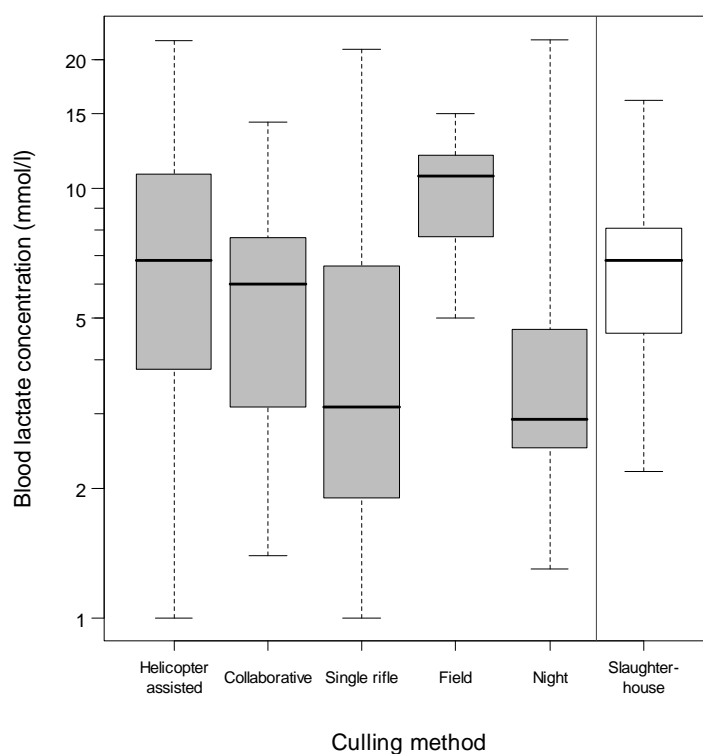


Figure 15. Boxplot of \log_{10} transformed blood lactate concentration (mmol/l)

However, when the analyses were repeated with the multivariate analyses with the nine covariates: sex, age, site of entry, and six time variables: time between first shot and death, start of cull and death, death to blood sampling, death to bleeding, blood sampling to plasma separation and storage and site of wound entry; and the two random effects: location with event nested within, this difference was lost ($P=0.056$). Excluding the 24 deer that were from locations where only one culling event occurred did not change this result ($P=0.292$).

Stress

Cortisol (Table A21)

Of the 162 recorded plasma cortisol concentrations, 36 (22%) were apparently positive, but below the minimum detection level of 13.5 nmol/l. These values were not distributed randomly between culling methods, and so they cannot just be ignored. The minimum sensitivity for the analyser to give a positive result is 5.5. The 36 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. There was a univariate statistically significant difference between the five culling methods in the \log_{10} transformed plasma cortisol concentrations ($F_{4,128}=11.3$, $P<0.001$, Fig. 16). Post-hoc analysis revealed that these differences were between deer culled with the assistance of a helicopter and those culled at night ($P<0.01$), field ($P<0.01$), and single rifle ($P<0.01$); and between deer culled via collaborative methods and those culled at night ($P<0.01$). This overall result remained with the multivariate analyses with ten covariates: sex, age, site of entry, number of deer previously culled and six time variables: time between first shot and death, start of cull and death, death to blood sampling, death to bleeding, blood sampling to plasma separation and storage and site of entry wound; and the two random effects: location with event nested within ($P=0.002$). Deer culled at night and single rifle culled had lower plasma cortisol concentration than those culled with the assistance of a helicopter.

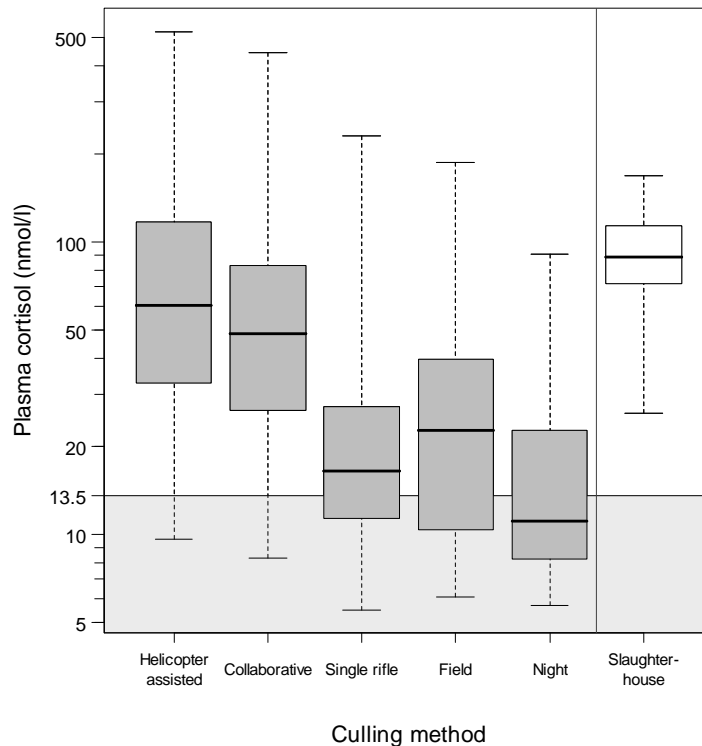


Figure 16. Boxplot of \log_{10} transformed plasma cortisol concentration (nmol/l). The light grey box indicates values that are positive, but are less than the minimum detection threshold (13.5), and have therefore been reallocated using a random value generated from a uniform distribution with a minimum of 5.5 and a maximum of 13.5.

Excluding the 24 deer that were from locations where only one culling event occurred did not affect the statistical difference ($P=0.028$). The bootstrap analyses revealed that statistically significant differences between culling methods were observed for 99.8% of the univariate, but only 3% of the overall multivariate comparisons.

Red deer that had more than one entry wound in the carcass had a greater plasma cortisol concentration than those with one entry wound. A two-way ANOVA of number of entry wounds ($F_{1,114}=24.3$, $P<0.001$) and culling method ($F_{4,114}=7.75$, $P<0.001$) was performed. Due to the absence of a second shot in field shot deer, the analysis was repeated with field shot deer excluded. The main effects were still significant and there was no interaction between culling method and number of entry wounds.

Free fatty acid concentration (Table A22)

There was a univariate statistically significant difference between the five culling methods in the \log_{10} transformed plasma free fatty acid concentration ($F_{4,128}=15.7$, $P<0.001$, Fig. 17). Post-hoc analysis revealed differences between deer culled with the assistance of a helicopter and those culled via collaborative methods ($P<0.01$), at night ($P<0.01$), field ($P<0.01$), and single rifle methods ($P<0.01$). This result remained with a multivariate analyses with eleven covariates: sex, age, site of wound entry, body condition score, percentage of kidneys covered by fat and six time variables: time between first shot and death, start of cull and death, death to blood sampling, death to bleeding, blood sampling to plasma separation and storage; and two random effects: location with event nested within ($P<0.001$). Deer culled with the assistance of a helicopter had a higher plasma free fatty acid concentration than those culled at night, via single rifle or collaborative methods. Excluding the 24 deer that were from one culling event did not affect the statistical significance ($P=0.003$), nor did only including results from the two main observers ($P=0.003$). 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. This decreased to 30% if only deer that were culled from locations with multi-deer events were considered.

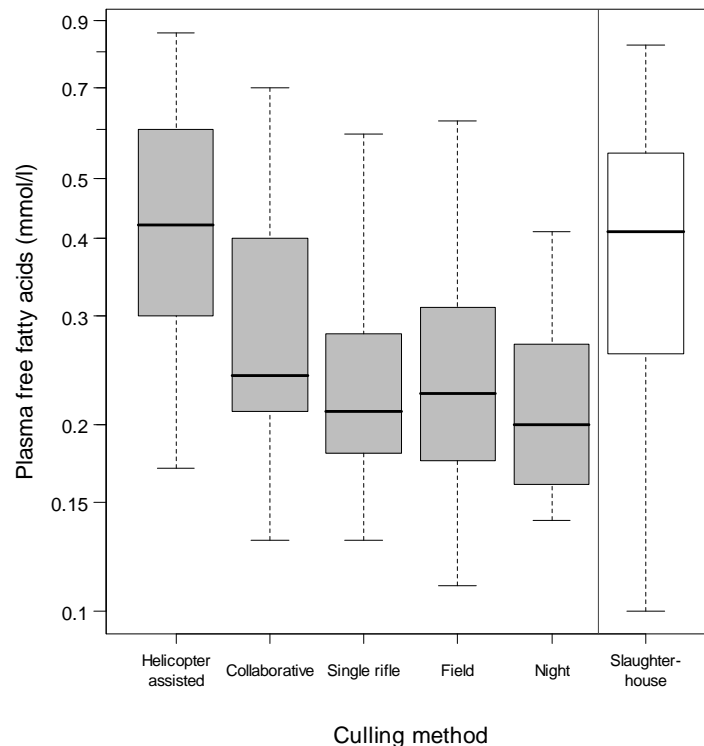


Figure 17. Boxplot of plasma free fatty acid concentration (mmol/l)

Nutrition

Body condition classification (Tables A23 and A24)

There was no univariate statistically significant difference between the culling methods in the percentage of deer that were thin/normal ($\chi^2_4=7.7$, $P=0.104$). However, when the analyses were repeated with the multivariate analyses with the two covariates: sex and age; and the two random effects: location with event nested within, there was a statistically significant difference ($F_{4,112}=2.9$, $P=0.032$), with a lower percentage of thin/normal in field shot deer compared to helicopter assisted and collaborative culling. There was a univariate statistically significant difference between the culling methods in the percentage of deer that were thin or thin/normal ($\chi^2_4=13.5$, $P=0.009$). However, this was due to no field shot deer being thin or thin/normal, if field shot deer are excluded there is no difference ($\chi^2_3=4.0$, $P=0.265$). In addition, when the analyses were repeated with the multivariate analyses with the two covariates: sex and age; and the two random effects: location with event nested within, there was no statistically significant difference ($F_{3,95}=1.4$, $P=0.252$).

Percentage of kidneys covered with fat (Tables A25 and A26)

There was no univariate statistically significant difference between the four culling methods in the percentage of deer with <25% of the kidneys covered with fat ($\chi^2_3=2.8$, $P=0.591$). When the analyses were repeated with the multivariate analyses with the two covariates: sex and age, and the two random effects: location with event nested within, the lack of differences remained ($P=0.645$).

β-hydroxybutyrate (Table A27)

There was a univariate statistically significant difference between the five culling methods in the \log_{10} transformed plasma β -hydroxybutyrate concentration ($F_{4,128}=13.5$, $P<0.001$, Fig. 18). Post-hoc analysis showed that there were differences between deer shot in the field and those culled via a helicopter assisted ($P<0.01$), via collaborative methods ($P<0.01$), at night ($P<0.01$), and single rifle ($P<0.01$).

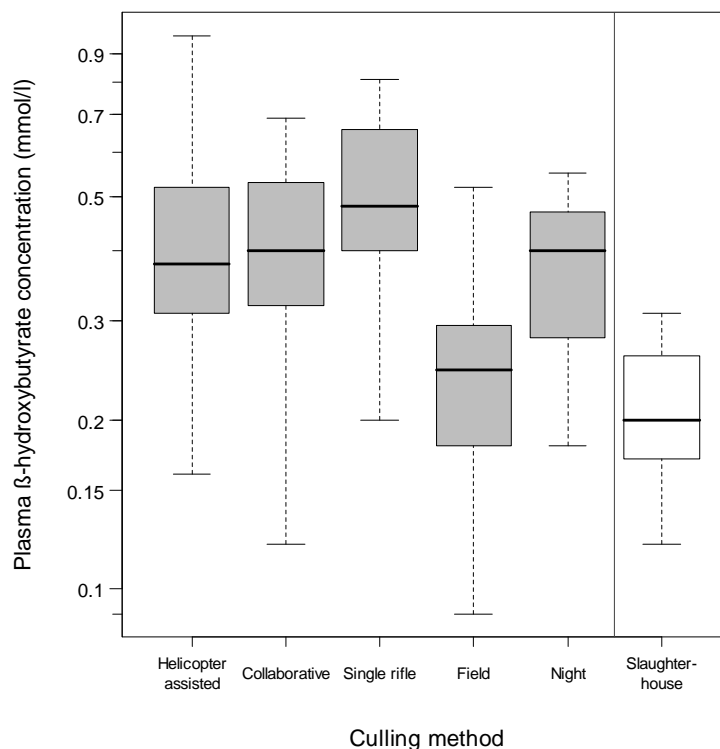


Figure 18. Boxplot of \log_{10} transformed plasma β -hydroxybutyrate concentration (mmol/l)

This result remained with the multivariate analyses with eleven covariates: sex, age, site of wound entry, body condition score, percentage of kidneys covered by fat and six time variables: time between first shot and death, start of cull and death, death to blood sampling, death to bleeding, blood sampling to plasma separation and storage; and two random effects: location with event nested within ($P=0.023$). Deer culled via single rifle methods had higher plasma β -hydroxybutyrate concentrations than those shot in a field. However, excluding the 24 deer that were from one culling event did change this result ($P=0.160$), as did only including results from the two main observers ($P=0.064$). The bootstrap results revealed that statistically significant differences between culling methods were observed for 85% of the univariate, but only 27% of the overall multivariate comparisons. This decreased to 9% if only deer that were culled from locations with multi-deer events were considered.

Urea (Table A28)

There was a univariate statistically significant difference between the five culling methods in the plasma urea concentration ($F_{4,128}=19.7$, $P<0.001$, Fig. 19). Post-hoc analysis showed that these differences were between deer (i) shot in a field and those culled with the assistance of a helicopter ($P<0.01$), via collaborative methods ($P<0.01$) and single rifle methods ($P<0.01$); (ii) via single rifle methods and those culled with the assistance of a helicopter ($P=0.024$) and night ($P<0.01$); (iii) helicopter assisted and at night ($P=0.015$).

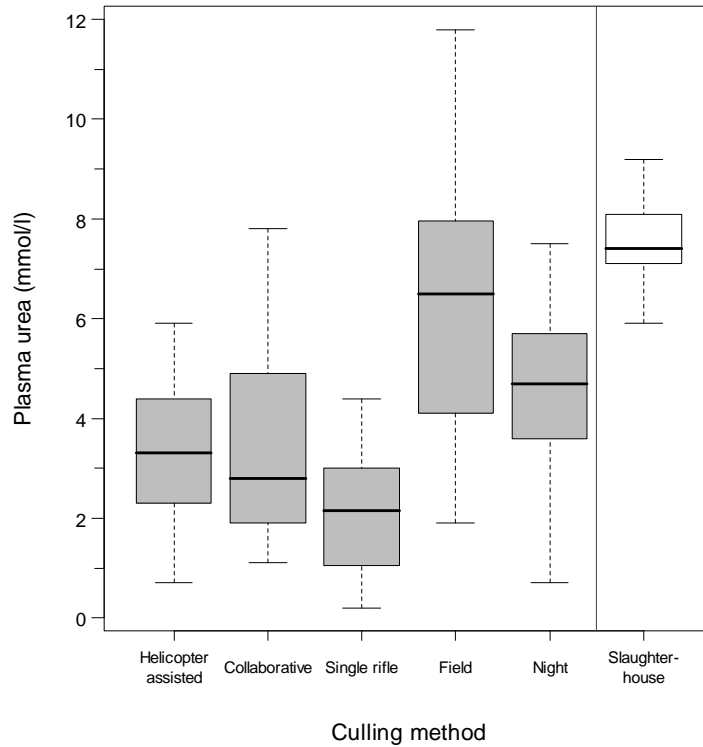


Figure 19. Boxplot of plasma urea concentration (mmol/l)

This result remained with the multivariate analyses with eleven covariates: sex, age, site of entry, body condition score, percentage of kidneys covered by fat and six time variables: time between first shot and death, start of cull and death, death to blood sampling, death to bleeding, blood sampling to plasma separation and storage; and two random effects: location with event nested within ($P=0.002$). Deer culled at night had higher plasma urea concentrations than those culled with the assistance of a helicopter, single rifle or collaborative methods. Excluding the 24 deer that were from locations where only one culling event occurred did not change this statistical significance ($P=0.012$), nor did only including results from the two main observers ($P=0.017$). The bootstrap results revealed that statistically significant differences between culling methods were observed for 99.8% of the univariate, and 52% of the overall multivariate comparisons. This decreased to 31% if only deer that were culled from locations with multi-deer events were considered.

Glucose (Table A29)

There was a univariate statistically significant difference between the five culling methods in the square root transformed plasma glucose concentration ($F_{4,127}=3.5$, $P=0.008$, Fig. 20). Post-hoc analysis revealed that these differences were between deer shot in a field and those culled with the assistance of a helicopter ($P<0.01$) and at night ($P=0.015$). This result remained when the data was analysed with the multivariate analyses with eleven covariates: sex, age, site of wound entry, body condition score, percentage of kidneys covered by fat and six time variables: time between first shot and death, start of cull and death, death to blood sampling, death to bleeding, blood sampling to plasma separation and storage; and the two random effects: location with event nested within ($P=0.764$).

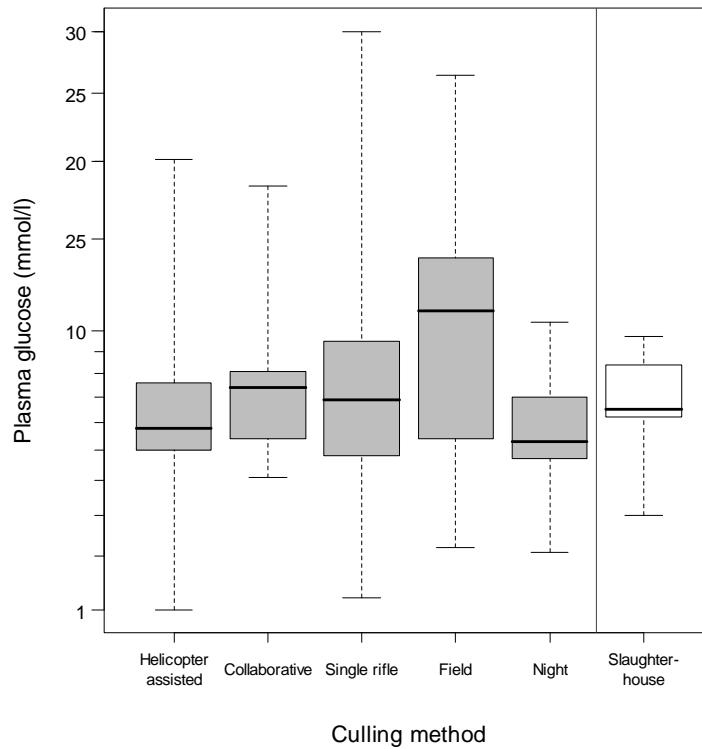


Figure 20. Boxplot of square root transformed plasma glucose concentration (mmol/l)

Discussion

The difficulties in obtaining sufficient numbers of separate culling events for each culling method meant that the statistical analyses was problematic and the results might have been influenced by factors other than differences between culling methods. Therefore, the confidence in which conclusions can be drawn regarding the welfare implications of the various culling methods is less than we would have liked. However, it has been possible to come to some tentative conclusions regarding the various culling methods.

Blood Sample collection

Problems associated with the method of sample collection and storage could potentially have affected the measurements made using blood and muscle samples. The rate of post-mortem change in a deer carcass is affected by the weather and as such was part of the sample variation in the study. The rate of loss of heat from a deer carcass is affected by air movement and air temperature (Moen and Jacobsen, 1974). Johnston (1984) reported that deer carcasses that had been 'gralloched', transported and placed outside at a temperature of between -1 and 5.5°C for 12h, had a muscle temperature of between 25.6 and 36.7°C. This indicates the variability between carcasses, but suggests that carcasses sampled within a time period of no greater than 4h would all still have been warm.

Most blood samples were stored at an ideal temperature (the median temperature was 3.5°C) before centrifugation. The time between death and blood sampling was short, and on average less than 9 minutes. The longer time (4h) between blood sampling and plasma separation should not have caused any great difficulty, as the storage temperature of the blood was low. Bradshaw and Bateson (2000) in their paper on culling of red deer reported a time from death to blood sample collection of between 1 and 10 minutes, mean 5.5 minutes. The average time to collection was similar in the current study. However, three samples were collected several hours after the culling, with the remainder all collected within 30 minutes of culling. The median time from death of the deer to centrifugation of blood samples in the study by Bradshaw and Bateson (2000) was 4.06h, Q_1 2.88 and Q_3 5.69. This again is very similar to the times taken in the current study. They stored their blood samples at ambient temperature until the first samples could be centrifuged (after 24 or 54 minutes) then half of the samples were stored at 4°C and half at room temperature until centrifugation. The plasma concentration of free fatty acid was affected by the time from collection and centrifugation and/or storage temperature as the value increased with both time and temperature. Bateson and Bradshaw (1997) used a correction factor of 970 μ mol/l/h to deal with this issue. The plasma concentrations of cortisol, urea, haemoglobin and total bilirubin and the plasma activities of creatine kinase and aspartate aminotransferase were not affected. The plasma concentration of lactate was affected, but their methodology is not comparable with the 'on-site' measurement of blood lactate performed in the current study. If the blood lactate readings were made shortly after death, reliable readings would have been obtained. A mean time of 9 minutes between death and sampling should have resulted in reasonably reliable blood lactate measurements. There was variation between methods of culling in the time between death and sampling. Except for field shot deer, where the median time to blood sampling was 20 minutes and the minimum time was 10 minutes, the median time between death and blood sampling was between 2 and 7 minutes. The readings for blood lactate for field shot deer might therefore have been unreliable. The delay in sampling field shot deer was due to the owners request for sampling to take place after the carcasses had been bought inside for preparation. Bradshaw and Bateson (2000) findings are consistent with other reports in the literature, *e.g.* Ferrante and Kronfeld (1994) that reported increases in lactate in equine blood stored at 22°C for 30 minutes. In equine blood or plasma stored at 4°C or 22°C for 18-22h, the plasma cortisol concentration was not affected by storage temperature or haemolysis (Reimers *et al.*, 1991). In bovine blood stored at 20°C for 6h, the

plasma aspartate aminotransferase activity was not significantly affected (Blincoe and Marble, 1985). In heparinised sheep and cattle plasma, the activity of aspartate aminotransferase is stable at either 4°C or 20°C for at least 4 days and that of creatine kinase is stable at 4°C for at least 2 days and at 20°C for at least one day (Jones 1985a and b).

A major concern is the possibility of haemolysis of the red blood cells after death and during the handling of the blood, *e.g.* shaking, raised temperature, freezing etc. As some of the plasma samples had red discoloration this gives cause for concern as this would normally indicate that haemolysis artefacts are present and these could affect the biochemical analysis and in particular the measurement of plasma creatine kinase activity. As visual discoloration of plasma samples is subjective and tends to over estimate the degree of haemolysis that has actually taken place, the actual plasma haemoglobin concentration was measured. Janatpour *et al.* (2004) reported that as little as 0.4g/l of haemoglobin can cause obvious red discoloration of human plasma. It is of interest that in the current study only seven out of the 162 (4%) plasma samples analysed had detectable concentrations of haemoglobin. These ranged from 0.7 to 1.7g/l of haemoglobin. In culling methods where any haemoglobin was detected (helicopter assisted and collaborative culling) the mean haemoglobin concentration was between 0.08 and 0.13g/l. Other than the culling method, (six samples were from the helicopter assisted culling group and one was from the collaborative culled group), there was no other apparent common factor between the samples in which haemoglobin was detected in the plasma, *i.e.* between the plasma cortisol concentration, the plasma bilirubin concentration; the times between the start of the cull and death, between the first shot and death or death to blood sampling; the number of entry wounds, and the behaviour of the deer before or after the first shot. Knox *et al.* (1988) reported a median plasma haemoglobin concentration in farmed red deer sampled by jugular venepuncture of 0.63g/l (range 0.44 to 0.92) and values lower than 0.43 and greater than 0.82 were considered to exceed tolerance limits. In red deer shot during single rifle stalking, Bateson and Bradshaw (1997) reported a median plasma haemoglobin concentration of 0.05g/l (Q₁ 0.03, Q₃ 0.08) and Bradshaw and Bateson (2000) reported a median plasma haemoglobin concentration of 0.07g/l (Q₁ 0.05, Q₃ 0.14).

Bateson and Bradshaw (2000) reported that the plasma of deer shot in the chest was more deeply coloured than those shot in the head or neck. The difference in colour of the plasma between the sites of the entry wound might have indicated that haemolysis of the red blood cells had taken place and this might have been responsible for the increased measurements of plasma creatine kinase and aspartate aminotransferase activities in deer shot in the chest compared with those shot in the head/neck. It is possible that (a) the trauma to the chest at the time of death could cause damage to the heart and major blood vessels that is sufficient to cause haemolysis and discoloration of the plasma and (b) deer shot in the chest take longer to die and have greater activity after they are shot compared with deer shot in the head or neck and this could cause haemolysis before death.

This problem in differentiating between pre- and post-mortem haemolysis was faced by Bateson and Bradshaw (1997) in their study of hunted red deer. They suggested that in their study the haemolysis occurred before death as a consequence of hunting. If before death, haemoglobin is released into the circulation as a result of haemolysis of red blood cells, it is metabolised to form bilirubin. Bateson and Bradshaw (1997) found that the plasma concentration of haemoglobin was positively correlated with that of bilirubin in both hunted and non-hunted animals and that deer which were hunted for longer had a greater plasma bilirubin concentration. Hunted deer had a median total bilirubin concentration of 14µmol/l. In the current study, the highest total bilirubin concentrations were in the group killed at the slaughterhouse (6µmol/l). Those culled with the assistance of a helicopter had a greater total bilirubin concentration (4µmol/l) than those culled by the other methods (1-2µmol/l). In red deer shot during single rifle stalking, Bateson and Bradshaw (1997) reported a median plasma bilirubin concentration of 5.0µmol/l (Q₁ 4.4, Q₃ 5.9) and Bradshaw and Bateson (2000) reported a median plasma bilirubin concentration of 6.1µmol/l (Q₁

5.4, Q₃ 7.4). Kent *et al.* (1980) reported that the serum bilirubin concentration in park red deer shot in the head without any chasing was <10.5µmol/l. In wild red deer captured using chemical restraint, the serum bilirubin concentration was 3.23µmol/l, after physical restraint it was 3.93µmol/l (Marco and Lavin, 1999), after using a net from a helicopter it was 3.42µmol/l and in those herded by two helicopters for about 2h into a fenced enclosure it was 11.97µmol/l (Millsbaugh *et al.*, 2000). In comparison with other studies where the deer had experienced extensive exercise before blood sampling, the plasma total bilirubin concentration in the current study was lower and was not indicative of major pre-mortem haemolysis. There was some evidence that there might have been some pre-mortem haemolysis in the deer culled with the assistance of a helicopter and in those killed at the slaughterhouse. However, other potential causes of raised plasma total bilirubin concentration are liver disorders and feed restriction. There is no reason to suggest any affect of culling method on liver function and in any case, there was no difference in the plasma activity of aspartate aminotransferase between culling methods that would have been evident if liver function had been impaired (Tennant, 1997). As there is, evidence that in fasted horses, the bilirubin concentration can increase (Gronwall and Mia, 1972) there is a possibility that the higher total bilirubin concentration in the deer slaughtered at the slaughterhouse could have been associated with a period of feed restriction. The plasma free fatty acid concentration in the slaughterhouse killed deer in comparison with other culling methods is not inconsistent with this explanation; however, the plasma β -hydroxybutyrate concentration was not raised in comparison with the other culling methods.

Shooting

The information collected provides an indication of the relative efficiency of the various culling methods and whether the timing of events could potentially have been associated with any welfare issues. In the current study, no stags shot by guests were included. The likelihood of wounding will be expected to be lower in deer culled by professional stalkers compared with those shot by guests. The location of the entry wounds provide a measure of the accuracy of the shooting performed during each of the culling methods. Baker (2003) noted that there were three recommended shot areas in deer:

1. Thoracic, the largest area and therefore with more margin for error, but with the disadvantage that the animal will often run some distance before dropping
2. Posterior cervical area, which is smaller in lethal area, but will provide an instant knock down, and
3. Shoulder area, which if the spinal cord is severed, will produce an instant effect.

Headshots are not recommended because of the small target area and the high risk of a badly injured deer that is likely to escape. The BASC Deer Stalking Code of Practice (British Association for Shooting and Conservation, 2008) recommends a perpendicular shot through the heart or lungs. Shots aimed to kill red deer by severing the cervical spinal cord are used, but the risk of missing the spinal cord and causing a non-fatal wound or a delayed death is high (Urquhart and McKendrick, 2003a). In a study of wild red deer carcasses, Urquhart and McKendrick (2006) found head wounds in 7% of the carcasses. It was estimated that in 4% of the carcasses, the marksman was considered to have aimed for the head, and the remaining head wounds resulted from close range shots to kill a wounded deer. Of the carcasses with a single permanent wound tract in the carcass trunk, 12% cent had damage limited to cervical structures, indicating the use of an elective 'neck shot'. In the current study, the most efficient means of stunning/killing in terms of accuracy of hitting the target location was captive bolt stunning at the slaughterhouse and field shooting as both methods were 100% successful in causing an entry wound in the head. If an entry wound in either the head, neck or lower chest is considered to be an acceptable location in which the deer are likely to be killed relatively quickly, 45% of wounds from helicopter assisted culls using a single rifle, 71% of wounds from helicopter assisted culls using multiple rifles, 71% of wounds from collaborative culls, 80% of wounds from single rifle culls and 45% of wounds from night culls were in an

acceptable location. If a wound in either the abdomen or the leg is considered to be ineffective in achieving a quick kill, 27% of wounds from helicopter assisted culls using a single rifle, 22% of wounds from helicopter assisted culls using multiple rifles, 12% of wounds from collaborative culls, 9% of wounds from single rifle culls and 15% of wounds from night culls were in an inappropriate location. There was a significant difference between the culling methods in the number of deer with at least one entry or exit wound in the head, neck or lower chest/heart. Field shooting was 100% accurate, but only 50% of deer shot at night had a wound in the head, neck or lower chest/heart. The highest percentage of carcasses with a wound in an acceptable location and the lowest percentage of carcasses with a wound in the abdomen or leg were in the single rifle stalking group and this degree of accuracy was similar to a previous report by Urquhart and McKendrick (2003a). There was no significant difference between the culling methods in the number of deer with at least one entry wound not in the head, neck or chest. However, the higher percentages of wounding in the abdomen or leg in the culling methods for helicopter assisted or collaborative suggest that it was more difficult to shoot the deer accurately. In night culled deer, the low percentage of carcasses that were shot in an 'acceptable location' was due to the high percentage that were shot in the upper chest. Whether this was significant in terms of the efficiency of killing the deer is not clear.

Urquhart and McKendrick (2003a) examined carcasses (after removal of the head and lower legs) of red deer culled in Scotland to identify sites of entry wounds. Thirty-six per cent of the wounds were in the lower chest, 32% in the upper chest, 21% in the neck, 7% in the abdomen and 4% in the leg. Fourteen per cent of carcasses had more than one entry wound. Thirty-three per cent of the plucks (heart and lungs) examined had a wound tract involving the heart and 48% had a wound tract through only the lungs. Urquhart and McKendrick (2006) examined carcasses (that included the head and lower legs) of red deer culled in Scotland to identify sites of entry wounds. Thirty-three per cent of the wounds were in the lower chest, 32% in the upper chest, 18% in the neck, 10% in the abdomen, 3% in the leg (no wounds were found in the lower legs) and 3% in the head. Fourteen per cent of carcasses had more than one entry wound. Thomas and Allen (2003) interpreted the implications of the sites of entry wounds in the deer carcasses reported in the study by Urquhart and McKendrick (2003a) as follows, deer shot:

- (a) in the heart would have died almost instantaneously
- (b) in the thorax might have died almost instantaneously from massive haemorrhage, but this would not be certain
- (c) in the anterior or posterior cervical regions could have died almost instantaneously if the shot had hit the spinal cord or had caused a massive haemorrhage by severing cervical blood vessels
- (d) with one permanent wound tract not involving the thoracic cavity or cervical region would probably not have died instantaneously
- (e) with more than one tract may not have died instantaneously from the first shot
- (f) with no permanent wound tract could have died instantaneously from a shot to the head or through the thoracic inlet.
- (g) In the abdomen, fore-leg, hind-leg or rump are likely to have remained alive for a considerable time after they were first shot.

Thomas and Allen (2003) concluded that at least 65% of the deer would not have died almost instantaneously from the first shot. Urquhart and McKendrick (2003b) considered that the conclusions drawn by Thomas and Allen (2003) went beyond those appropriate for the data and that up to 89% of the deer probably died instantaneously. Urquhart and McKendrick (2003b) rejected an approach based on the anatomical distribution of individual entry/exit wounds to assess, retrospectively the welfare consequences for the culled deer. The outcome of the complex pathological processes that occur when a deer is struck by an expending high-velocity bullet was considered to be too difficult to predict. Without considering whether a vital structure was injured

e.g. 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 its likely trajectory could underestimate the effect that the bullet may have caused to the surrounding tissues, *i.e.* even if the bullet does not directly penetrate a vital structure, the extensive peripheral damage might have been sufficient to kill the deer effectively. Pavletic (1986) and Coupland (2003) noted that the wounds that occur in deer shot with bullets that expand or flatten easily (thereby depositing more kinetic energy early in the tract) are likely to cause more damage than that in humans shot with bullets that do not expand or flatten. Pavletic (1996) described the potential effects of a high velocity rifle bullet on an animal. Tissue in the immediate path of a bullet is lacerated and crushed and those in the vicinity are also damaged by shock waves released by the high velocity 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. The vacuum formed by the cavity draws in contaminants and surface debris. Bones can be fractured, vessels torn, bowels ruptured and massive contusions caused without direct contact with the bullet. A shot to the neck may cause serious injuries to the larynx, trachea, spinal cord, cranial nerves, carotid and jugular vessels, pharynx and oesophagus. Gun shot wounds to the abdomen can cause peritonitis secondary to bowel perforation and those to the thorax can cause haemorrhage. Traumatized tissue, vascular compromise, foreign debris and the inoculation of bacteria into the wound increases the risk of infection. Thomas *et al.* (2001) considered that the small number of deer that escape after they are shot either will die through starvation and sepsis or will adapt to their wounds.

In situations where deer are only wounded, the time between the first and second 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. The accuracy of a second shot is also likely to be more difficult than the first shot. 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 required on 22 occasions and the median time between the first and second shot was 6.5 minutes (Q_1 1.8 and Q_3 29 minutes). The time between the first shot and the apparent death of the deer is another key indicator of the efficiency of the culling method. For 69% of the deer, the time between the first shot and death was recorded as 0s. For field shot deer, there were only two deer for which the time from the 1st shot to death was greater than 0s. There was no statistically significant difference between culling methods in the time from 1st shot to death. There was no statistically significant difference between the culling methods in the time between the first and second shot (comparisons were only possible between deer culled for helicopter assisted and those culled with a single rifle). However, if the data is correct and the first shot did wound the deer, the time between the first shot and death may have been significant for some deer. The upper quartile of times for each method ranged from about 1 minute for field shot deer to about 3 minutes for single rifle stalking. The maximum time between the first and second shot was 1.35 h in the single rifle group.

Baker and Harris (2005) considered that the welfare issues arising from shooting are (a) the rate at which animals are wounded and (b) the level of suffering caused by these wounds. Bradshaw and Bateson (2001) stated, "How much deer suffer when fatally shot in the heart, spine, liver or lungs is hard to ascertain. Loss of consciousness will not be instantaneous as with head and upper-neck shot deer." Bateson and Bradshaw (2000) reported that nine stags and one hind killed with more than one shot had a greater plasma cortisol concentration (median 41 nmol/l, Q_1 4, Q_3 88) than 21 stags and 30 hinds killed with one shot (median <2.7 nmol/l, Q_1 3, Q_3 14). A similar difference was obtained in the current study with deer shot more than once having a greater plasma cortisol concentration than those shot only once. In humans shot by a rifle, pain within the first minutes is

not reported as a major feature of the injury, even when there is a fracture or abdominal wound (Coupland, 2003).

If the deer are not killed by a shot that causes damage to the central nervous system, many will die as a result of haemorrhage from major blood vessels in the neck or chest. 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 hypoxemia. Based on experiments in rats, Gregory estimated that a deer would need to lose at least 2l of blood to ensure rapid death. In sheep, severance of both carotid arteries plus jugular veins takes 14s to induce a loss of brain responsiveness, whereas severing only one carotid artery plus one jugular vein takes 70s. Cortical responsiveness lasts for about 5 minutes when only one of the jugular veins are cut (Gregory and Wotton, 1984).

The behaviour of the deer after it was shot provides useful information on the effectiveness of the culling methods in killing the deer as soon as possible, *i.e.* an effective kill would be one where the deer collapsed immediately after it was shot. Fifty-five per cent of the deer that were observed collapsed immediately after being shot. A further 35% ran some distance (from <5 to 50m) before collapsing, the remaining 10% deer either ran or walked away. With the exclusion of the field shot deer (where only two deer did not collapse immediately), there was a statistically significant difference between the other four culling methods in the behaviour after the first shot. The highest percentage of deer that after the first shot, ran or walked away without collapsing were in the helicopter assisted (48%) and the night culled (43%) groups. Thomas and Allen (2003) noted that experienced stalkers considered that even deer shot through the heart could run 30 to 40m before collapsing. Bradshaw and Bateson (2000) reported a retrospective analysis of the recollections by eight stalkers (three accompanied amateurs and five professionals) of 372 deer shot during the previous season. A mean (\pm S.D.) of $7.5 \pm 1.7\%$ survived for more than 2 minutes after the first shot, $12.0 \pm 3.0\%$ were killed using 2 or more shots and $3.5 \pm 1.5\%$ escaped wounded. They also report a retrospective analysis of the records made by a further seven experienced stalkers who shot 171 deer during the 1995-1996 season. A mean of $4.4 \pm 2.3\%$ survived for more than 2 minutes after the first shot. $9.8 \pm 3.0\%$ were killed using two or more shots and no deer escaped wounded. The mean distance run by deer after they were shot in the chest was $31.9 \pm 0.6\text{m}$ ($n=44$) and by deer after they were shot in the head or neck was $3.2 \pm 0.5\text{m}$ ($n=45$). An 'expert opinion' based on carcase diagrams returned from game dealers suggested that $14.6 \pm 3.3\%$ of the deer had survived for more than 2 minutes after the first shot and $10 \pm 2.8\%$ required two or more shots to be killed. Bateson and Bradshaw (2000) analysed records from two stalkers in 1995-1996 who shot 30 adult hinds. Twenty-four were shot in the head/neck and six were shot in the chest. Only one hind required more than one shot. Deer that had been shot in the chest were reported to have ran a short distance, median 20m (Q_1 10 and Q_3 35), whereas those shot in the head/neck did not run after they were shot. The estimated median time between the first shot and the deer falling to the ground was 0s (Q_1 0, Q_3 15) for those shot only once, but it was 60s (Q_1 40, Q_3 250) for those that were shot more than once. Culling of wild deer cannot compare with the efficiency of slaughtering farmed deer under relatively controlled conditions in a slaughterhouse. Blackmore *et al* (1993) described the response of four red deer to captive bolt stunning at a slaughterhouse. After 20s the pupils were dilated, the jaw relaxed and the mouth was open. There was no muscular movement after 45s in three of the animals and no movement after 70s in the fourth deer.

Behaviour

The time between the start of the cull and the first shot was longer during the helicopter assisted cull with multiple rifles (about 40 minutes) than for other culling methods (between 1 and 20 minutes). The overall times between the start of the cull and the apparent death of the deer were longest for

the helicopter assisted cull with multiple rifles and the slaughterhouse slaughter. 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. Farmed red deer on moorland spend most of their daylight hours grazing (46%), lying down (46%) and only about 4% moving (Hester *et al.*, 1999). Most deer (70-80%) were standing alert before they were shot. There was a significant difference between culling methods as all night culled deer were observed standing alert before they were shot. The deer were active during the helicopter assisted cull and the collaborative cull. Helicopter assisted culling resulted in the greatest activity (one third 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. Although the intention of using a helicopter is limited to the deployment of personnel and the removal of carcasses, deer can be disturbed by the presence of a helicopter and when chased will walk with occasional bursts of running (Bleich, 1983; Cattet *et al.*, 2004; DeYoung, 1985). In the USA, helicopters are used to conduct population surveys of red deer (Noyes *et al.*, 2000; McCorquodale, 2001). 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 minutes 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 injury. Only one occurrence of injury as a result of trauma was recorded. This one injury was recorded to the hind-leg muscle of one deer culled with the assistance a helicopter and multiple rifles. No fractures, bruising or other injuries were recorded. Grigor *et al.* (1997) transported farmed hinds for 3h to a slaughterhouse and found no evidence of skin damage. Bruising was described as insignificant on most carcasses. This compares with a survey of hind red deer carcasses in a New Zealand slaughterhouse by Jago *et al.* (1996) where they reported that 5.6% of carcasses were downgraded because of bruising or recent injury, 0.3% had severe bruising and 0.3% had recently broken bones. There were also no significant differences between culling methods in the plasma activities of creatine kinase and aspartate aminotransferase. Plasma creatine kinase activity is a useful indicator of muscle damage that can occur following bruising or exercise. Plasma aspartate aminotransferase activity is not organ specific, but is a useful indicator of soft tissue damage (Kramer and Hoffman, 1997). Haemolysis can interfere with both enzyme assays and the time lag between an injury and a significant rise in plasma creatine kinase activity may have been too long for significant increases to be detected in blood sampled at the time of death. In sheep, the half life of creatine kinase is about 1h and that of aspartate aminotransferase is longer (Boyd, 1976). As creatine kinase activity generally peaks 12-24h after muscle damage, the lack of a significant difference between culling methods may have been due to the short time between the start of the cull and blood sampling.

Other than, for the deer slaughtered at the slaughterhouse (274 IU/l), the median plasma creatine kinase activity in each of the culled groups was high. The plasma creatine kinase activity of farmed deer after transport for 2h can increase from 257 to 465 IU/l and remain at 577 IU/l 2.75h after transport (Grigor *et al.*, 1998) and at the time of slaughter after 3h of transport, it can rise to 997 IU/l (Grigor *et al.*, 1997). Jago *et al.* (1997) reported that the plasma creatine kinase activity in farmed hinds transported for 80km rose from 160 to 3211 IU/l. The plasma creatine kinase activity in deer slaughtered at the slaughterhouse was similar to those reported in farmed deer under 'control' conditions. The serum creatine kinase activity in farmed red deer can be 221 IU/l (79 to 409 IU/l) (Padilla *et al.*, 2000) and in park red deer shot in the head without any chasing, it can be as low as 23 IU/l (Kent *et al.*, 1980). Wilson and Pauli (1983) reported a serum creatine kinase

activity in farmed red deer of 198 IU/l (range 23-1054 IU/l) and from the confidence limits suggested that values greater than 600 IU/l could be regarded as elevated. Knox *et al.* (1988) reported a median plasma creatine kinase activity in farmed red deer sampled by jugular venepuncture of 179 IU/l (range 83 to 383) and values lower than 57 and greater than 315 were considered to exceed tolerance limits).

Even though the median plasma creatine kinase activity of 816 IU/l in deer shot by single rifle culling was the lowest numerical value amongst the culling methods, it was greater than that reported in other studies. Bateson and Bradshaw (1997) reported a median plasma creatine kinase activity of 270 IU/l (Q₁ 203, Q₃ 320) in 40 deer shot during single rifle stalking. Bradshaw and Bateson (2000) reported a median plasma creatine kinase activity of 266 IU/l (Q₁ 221, Q₃ 387) in 8 deer shot by stalkers. Bateson and Bradshaw (2000) reported a median plasma creatine kinase activity of 262 IU/l (Q₁ 208, Q₃ 306) in 31 deer shot in the head/neck by stalkers. Pollard *et al.*, (2002) reported a plasma creatine kinase activity of 260 IU/l in farmed stags shot in the paddock, whereas in those collected, transported for 3h and held overnight at a slaughterhouse lairage before stunning with a captive bolt it was 2441 IU/l. Exertion associated with handling can raise the plasma creatine kinase activity in red deer hinds. In wild red deer captured using chemical immobilisation, Marco and Lavin (1999) reported a value of 198 IU/l, but after physical restraint it was 1109 IU/l. Other than, for the samples collected at the slaughterhouse, the plasma creatine kinase activity in each of the culling groups should be considered to be 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.

As for plasma creatine kinase activity, the lowest median plasma activity of plasma aspartate aminotransferase was in the deer slaughtered at the slaughterhouse (80 IU/l); the median plasma aspartate aminotransferase activity in each of the culled groups was higher than that reported in normal deer. Padilla *et al.* (2000) reported a serum aspartate aminotransferase activity in farmed red deer of 54 IU/l (36 to 123 IU/l). Kent *et al.* (1980) reported a serum aspartate aminotransferase activity in park red deer shot in the head without any chasing of 28 IU/l. Knox *et al.* (1988) reported a median plasma aspartate aminotransferase activity in farmed red deer sampled by jugular venepuncture of 80 IU/l (range 59 to 150) and values lower than 51 and greater than 130 were considered to exceed tolerance limits. The median value obtained for the field shot group was greater, but in those slaughtered in the slaughterhouse it was lower than reported by Pollard *et al.* (2002). Pollard *et al.* (2002) shot farmed stags in a paddock and found a plasma aspartate aminotransferase activity of 67 IU/l, whereas in those collected, transported for 3h and held overnight at a slaughterhouse lairage before stunning with a captive bolt it was 183 IU/l. Bateson and Bradshaw (1997) reported a median plasma aspartate aminotransferase activity of 62 IU/l (Q₁ 493, Q₃ 83) in 40 deer shot during single rifle stalking. Bateson and Bradshaw (2000) reported a median plasma aspartate aminotransferase activity of 64 IU/l (Q₁ 55, Q₃ 79) in 31 deer shot in the head/neck by stalkers. These values are slightly lower than in those in the single rifle culled group (median 110, Q₁58, Q₃430 IU/l). In wild red deer, the serum aspartate aminotransferase activity can be raised after physical restraint (111 IU/l) compared with capture using chemical restraint (63 IU/l) (Marco and Lavin, 1999).

Muscle glycogen

The time between death and muscle sampling (25 minutes) was too long to ensure that relevant results were obtained from the muscle glycogen analysis. In an ideal situation, the muscle sample should be snap frozen immediately after death. The glycogen in the muscle will start to be metabolised from death and therefore the concentration of glycogen in a muscle sample frozen 25 minutes after the death of the animal will be less than would have been present at death. The measurement of muscle pH 24 hours after death (muscle pH₂₄ or muscle pH_u) would be expected to

provide an additional way of assessing the muscle glycogen content at the time of death. The acidity of the muscle at 24 hours after death is related to the degree of lactic acid formed in the tissue as a result of glycogen metabolism after death. By 24h almost all muscle glycogen would have been metabolised. If the muscle glycogen content was normal at the time of death the muscle pH₂₄ would be low, but if lower than normal muscle glycogen was present *e.g.* due to prolonged exercise, the muscle pH₂₄ would be higher than normal.

Hoppeler and Billeter (1991) described the utilisation of energy substrates in exercising muscles. Muscle glycogen is utilised for muscle activity, but sustained submaximal exercise depends on the delivery of exogenous energy to muscles. Increases in plasma glucose derived from liver glycolysis or gluconeogenesis, increases in plasma free fatty acids from adipose tissue triglycerides and increases in plasma lactate resulting from muscle glycolysis can provide energy to the muscles. Red deer can walk at speeds of up to 1.5m/s on a treadmill and gallop for short periods at a speed of 2m/s. However, increased exercise is associated with increased energy expenditure. The increase in heat production of red deer with increased speed is similar on a body weight basis to that of cattle and sheep (Brockway and Gessaman, 1977). The energy expenditure of red deer walking at speeds of up to 1.7m/s is about 7 J/kg/s, trotting at 1.7 to 3m/s is about 14 J/kg/s and galloping at 3 to 5m/s is about 21 J/kg/s (Parker *et al.*, 1984).

The lower muscle glycogen concentration in deer culled with the assistance of a helicopter compared to those culled at night or shot in the field suggests that glycogen was utilised as an energy source in the muscle during prolonged exercise. The raised muscle pH_u was consistent with the utilisation of muscle glycogen before death and decreased lactic acid formation during post-mortem glycogen metabolism. The muscle glycogen concentration and the muscle pH_u suggest that deer shot in the field experienced the least prolonged exercise before death. The method used to analyse the muscle glycogen quantifies the concentration in a manner comparable with human exercise studies, but is not comparable with previous reports for farmed deer. The muscle (*M. longissimus*) glycogen concentration of farmed deer has been reported as 139 mmol/g dry tissue (Wiklund *et al.*, 2001). The muscle (*M. longissimus*) glycogen concentration of farmed deer at pasture has been reported as 205 mmol/g dry tissue and in those offered pellets it was 218 mmol/g dry tissue (Wiklund *et al.*, 2003). Pollard *et al.* (2002) reported that farmed stags shot in the paddock had a neck muscle glycogen concentration of 9.81 mg/g wet wt whereas in those collected, transported for 3h and held overnight at a slaughterhouse lairage before stunning with a captive bolt it had decreased to 5.72mg/g wet wt.

Muscle pH

In red deer, the muscle pH (*M. longissimus*) falls after slaughter from about 6.9 to reach a minimum value of about 5.7, 20 hours post-mortem (Wiklund *et al.*, 2001). There was a statistically significant difference between the five culling methods in the back leg (*M. Biceps femoris*) muscle pH_u. Deer culled at night had a lower back leg muscle pH_u than those culled via helicopter assisted, collaborative and single rifle methods. This result is consistent with the lower muscle glycogen concentration found in deer culled with the assistance of a helicopter compared to those culled at night. There was no effect of culling method on the neck/shoulder muscle pH_u (*M. Triceps brachii*) or back (*M. Longissimus dorsi*). Although there were some maximum muscle pH readings greater than 6 for all culling methods, all median muscle pH_u and upper-quartile (Q₃) values were less than 6. A muscle pH \geq 6.0 is considered to be high and likely to show dark cutting (MacDougall *et al.*, 1979).

Although the muscle pH_u tends to be mainly influenced by muscular activity depleting muscle glycogen, a stress response involving catecholamine induced muscle glycogenolysis could possibly affect the muscle pH_u. The method of slaughter might have a small effect on the muscle pH_u. Smith

and Dobson (1990) compared neck muscle pH_{24} in farmed red deer stags shot in the neck in a field and then stunned with a captive bolt, 5.74, with those (a) handled and housed before stunning in a crush on the farm with a captive bolt (pH_u 5.80) and (b) transported for 25 minutes, lairaged for 1h before stunning at a slaughterhouse farm with a captive bolt (pH_u 5.78). Whereas, Pollard *et al.* (2002) reported that farmed stags shot in the paddock had a muscle pH_u of about 5.8 and in those collected, transported for 3h and held overnight at a slaughterhouse lairage before stunning with a captive bolt it was about 5.7. Kay *et al.* (1981) measured the muscle pH_{24} (Neck: triceps brachialis, Back: longissimus dorsi and Rump: biceps femoris) in red deer stags and hinds slaughtered:

- (a) after holding overnight in an unfamiliar paddock : Neck 6.00, Back 6.08 and Rump 5.88 or in a small pen Neck 6.10, Back 6.23 and Rump 5.97,
- (b) by shooting in the field Neck 5.83, Back 5.65 and Rump 5.63, after gathering Neck 5.97, Back 5.68 and Rump 5.72, after holding overnight in darken pens Neck 6.0, Back 5.8 and Rump 5.8 or after holding overnight in small pens Neck 6.26, Back 6.02 and Rump 6.15.,
- (c) by shooting in the neck after holding overnight in individual covered pens: Neck 5.65, Back 5.80 and Rump 5.80, by captive bolt stunning after holding overnight in a group pen : Neck 5.70, Back 5.69 and Rump 5.75, or by captive bolt stunning after holding overnight in a group pen after social mixing: Neck 5.76, Back 5.80 and Rump 5.83
- (d) In shot wild hinds, the pH_u was Neck 5.75, Back 5.56 and Rump 5.55.

There was no effect of sequence of slaughter on muscle pH_u . MacDougall *et al.* (1979) measured the muscle pH_u in *M. semimembranosus* in farmed hinds brought in from moorland and penned overnight in either (a) individual adjacent pens and shot in the neck 5.3-5.4, (b) a circular unroofed pen and then shot using a captive bolt 5.4 or (c) kept outside in a pen, mixed and then shot using a captive bolt 5.3-5.4.

Lactate

Raised blood lactate concentrations can occur following breakdown of muscle glycogen after extreme muscular exertion and from catecholamine induced glycogenolysis (Shaw and Tume, 1992). It can rise in farmed hinds from 3.85 to 3.96 mmol/l after transportation for 80-380km (Jago *et al.*, 1997). Farmed stags shot in a paddock were reported to have a plasma lactate concentration of 3.54 mmol/l whereas in those collected, transported for 3h and held overnight at a slaughterhouse lairage before stunning with a captive bolt it was 8.81 mmol/l (Pollard *et al.*, 2002). 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. In cattle, the plasma lactate concentration is increased by handling and is further increased after transport and slaughter using captive bolt stunning (Mitchell *et al.* (1988).

Stress

Measurement of the 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 and Boissy, 2007). Although raised plasma cortisol concentration is thought to be associated with psychological stress, it is not possible to differentiate between raised plasma cortisol following suffering and that following excitement. Therefore, it is not appropriate to infer that a raised plasma cortisol concentration necessarily indicates poor welfare (Webster, 1998). That said, The Welfare of Animals (Slaughter or Killing) Regulations, 1995 (Great Britain Parliament, 1995) governing the slaughter of farmed animals in a slaughterhouse, contain 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. Although a cortisol response can follow exercise (Marc *et al.*, 2000) and deer culled by some culling methods appeared to have experienced more exercise than those culled by other methods, it is not appropriate to interpret a greater cortisol response in deer culled by some methods as necessarily indicating only a physiological response to exercise associated with the mobilisation of body energy reserves (Hyypä, 2005). It is likely 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 and 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.

The overall times between the start of the cull and the apparent death of the deer were longest for the helicopter assisted cull with multiple rifles and the slaughterhouse slaughter. 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 that can occur following psychological stress 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. There was a significant difference between the five culling methods in plasma cortisol concentrations. Deer culled using the assistance of a helicopter had a greater plasma cortisol concentration (91 nmol/l) than those culled at night (21 nmol/l), by single rifle methods (47 nmol/l) or shot in a field (36 nmol/l), and those culled via collaborative methods (82 nmol/l) had a greater plasma cortisol concentration than those culled at night. Slaughterhouse slaughtered deer had a mean plasma cortisol concentration of 92 nmol/l.

Although some high maximum plasma cortisol concentrations were found, the median and upper-quartile (Q₃) plasma cortisol concentrations were less than the maximum plasma cortisol concentration of 180 nmol/l reported by Ferre *et al.* (1998) in red deer hinds 45 minutes after the adrenals had been stimulated with ACTH. As part of the hypothalamic-pituitary-adrenal axis response to stressors, ACTH (adrenocorticotropic hormone) stimulates the cortex of the adrenal gland to produce cortisol. If a sufficient dose of synthetic ACTH is administered, a maximal adrenal response is obtained and the plasma cortisol concentration reflects the maximum capacity of the adrenals to produce cortisol at that time. Therefore, a plasma cortisol concentration of 180 nmol/l produced in response to ACTH stimulation provides an indication of the magnitude of a maximum cortisol response in red deer. Shooting farmed deer in the field or wild deer on open moorland using single rifle stalking methods is less stressful than the handling, transport and novelty of slaughter at a slaughterhouse. After farmed hinds had been transported for 3h, Grigor *et al.* (1997) reported a plasma cortisol concentration at the time of slaughter of 154 nmol/l. Smith and Dobson (1990) compared the plasma cortisol concentration in farmed red deer stags shot in the neck in a field and then stunned with a captive bolt (16 nmol/l), with those (a) handled and housed before stunning in a crush on the farm with a captive bolt (58 nmol/l), and (b) those transported for 25 minutes, lairaged for 1h before stunning at a slaughterhouse farm with a captive bolt (80 nmol/l). Also, Pollard *et al.* (2002) shot farmed stags in a paddock and reported a plasma cortisol of 15 nmol/l whereas in those collected, transported for 3h and held overnight at a slaughterhouse lairage before stunning with a captive bolt it was 59 nmol/l. Farmed deer respond to confinement in a pen, drafting while in a pen and placement in a crush with an increase in plasma cortisol concentration from 15 nmol/l to 83 nmol/l (Carragher *et al.*, 1997). In cattle, the plasma cortisol concentration is increased by handling and is further increased after transport, but is not increased again by slaughter using captive bolt

stunning (Mitchell *et al.* (1988). It is also greater after slaughter at a commercial slaughterhouse compared with a quiet research slaughterhouse (Tume and Shaw, 1992). Bateson and Bradshaw (1997; 2000) and Bradshaw and Bateson (2000) reported relatively low median plasma cortisol concentrations (<2.7 to 7.5 nmol/l) in deer shot during single rifle stalking. Although these values were lower than those in the current study they were comparable (median 16 nmol/l, Q₁ 14, Q₃ 31). After wild red deer had been herded by two helicopters for about 2h into a fenced enclosure, a serum cortisol concentration of 105 nmol/l was reported by Millsbaugh *et al.* (2000). This is greater than the median plasma cortisol concentration of 61 nmol/l (Q₁ 31, Q₃ 122) in the deer culled with the assistance of a helicopter where the intention was not to affect the behaviour of the deer. Deer are susceptible to a variety of stressors. The approach by an unfamiliar person or the sound of a loud noise can increase the heart rate of farmed deer by 20 beats/minutes over resting values (Price *et al.*, 1993). Farmed red deer hinds show increased heart rate from about 75 beats/minute while standing foraging to about 85 beats/minute while either standing with the neck raised or walking, to about 108 beats/minute while walking with the neck raised and to about 120 beats/minute while trotting (Price *et al.*, 1993).

Free fatty acids

The deer culled with the assistance of a helicopter had a greater plasma free fatty acid concentration than those culled via collaborative methods, at night, by single rifle methods or shot in the field. The plasma free fatty acid concentration in the slaughterhouse deer was similar to that in the helicopter assisted culled group. The values obtained at the slaughterhouse (median 0.41 mmol/l) were similar to the 0.43 mmol/l at the time of slaughter found by Grigor *et al.* (1997) after farmed hinds had been transported for 3h. Bradshaw and Bateson (2000) reported a median plasma free fatty acid concentration (adjusted for sample handling) of 0.30 mmol/l (Q₁ 0.12, Q₃ 0.50) in 8 deer shot by stalkers. This is similar to the values found in the current study for single rifle culled deer (median 0.21 mmol/l, Q₁ 0.18, Q₃ 0.28). However, Bateson and Bradshaw (2000) reported lower values, a median plasma free fatty acid concentration (adjusted for sample handling) of 0.059 mmol/l (Q₁ 0.034, Q₃ 0.102) in 28 hinds shot in the winter. Knox *et al.* (1988) reported a median plasma free fatty acid concentration in farmed red deer sampled by jugular venepuncture of 0.314 mmol/l (range 0.027 to 0.947) and values lower than 0.034 and greater than 0.777 were considered to exceed tolerance limits. Wolkers *et al.* (1994) reported that the plasma free fatty acid concentration in farmed red deer hinds was not affected by a 20% food restriction for 13-23 weeks. However, it is likely that the plasma free fatty acid concentration was influenced by both the nutritional state of the deer and the stress response, *e.g.* catecholamine response, to each of the culling methods. In cattle, the plasma catecholamine concentration is not increased by handling, but the plasma adrenaline concentration is increased after transport and does not increase further after slaughter using captive bolt stunning, however the plasma noradrenaline concentration is not markedly increased by transport, but it is increased after slaughter (Mitchell *et al.* (1988). In a review of the effects of slaughter on the composition of blood collected at exsanguination, Shaw and Tume (1992) found reports of increases in plasma adrenaline concentration in cattle and pigs. In sheep, the plasma free fatty acid concentration is affected by both handling procedures and fasting, whereas the plasma concentration of β -hydroxybutyrate reflects the degree of fasting and is not influenced by handling (Warriss *et al.*, 1989).

Body condition

Farmed deer shot in the field appeared to have been in a better nutritional state than the wild deer. Farmed deer were not thin, and had greater plasma concentrations of urea and glucose and a lower plasma concentration of β -hydroxybutyrate than wild deer. Wild red deer hinds lose condition in the winter and during lactation (Audige *et al.*, 1998). During the winter months, wild yield (non-lactating) red deer hinds have better body condition (as assessed by carcase weight, amount of

kidney and rump fat) than lactating hinds (Mitchell *et al.*, 1976). Body condition score (Audige *et al.*, 1998) and the amount of fat surrounding the kidneys are established methods for assessing the condition of wild red deer. The level of fat reserves gives an indication of body condition. Fat is first deposited in the bone marrow then around the kidneys and finally subcutaneously. Although kidney fat is considered to provide an index of condition (Albon *et al.*, 1986), starved deer may still have some kidney fat (Suttie, 1983). Bateson and Bradshaw (2000) reported a mean kidney fat percentage coverage of 48 SD 21.5 in 28 hinds shot in the winter.

β -hydroxybutyrate

The plasma β -hydroxybutyrate concentration in farmed deer in the field was lower than in wild deer culled using the assistance of a helicopter, via collaborative methods, at night and by single rifle methods. The low plasma β -hydroxybutyrate concentration in the farmed deer at the slaughterhouse was similar to those shot in the field. The median plasma β -hydroxybutyrate concentration in farmed red deer sampled by jugular venepuncture has been reported as 0.17 mmol/l (range 0.038 to 0.372) and values lower than 0.032 and greater than 0.318 were considered to exceed tolerance limits (Knox *et al.* 1988). Although in the current study, the plasma β -hydroxybutyrate appears to have been affected by the relative nutritional state of farmed versus wild deer, Wolkers *et al.* (1994) did not find that the concentration of plasma β -hydroxybutyrate in farmed red deer hinds was affected by food restriction of 20% for 13-23 weeks.

Urea

The plasma urea concentration was greater in farmed deer shot in the field than in wild deer culled using the assistance of a helicopter, via collaborative methods and single rifle methods. Deer culled at night had higher plasma urea concentrations than those culled using the assistance of a helicopter, single rifle or collaborative methods. Some of the deer that were shot at night could have been shot in locations that had vegetation that provided for a higher plane of nutrition than those culled mainly on moorland, via a helicopter assisted, single rifle or collaborative methods. The farmed deer at the slaughterhouse had similar high plasma urea concentrations to those shot in the field. The plasma urea concentration can be affected by a number of factors that influence urea metabolism and cycling. Increased plasma urea concentration can be associated with an excess of dietary protein, with deficiency of energy in the ration, with catabolism of body proteins or with renal disease (Padilla *et al.*, 2000). In farmed red deer, the plasma urea concentration is greater when offered a high-nitrogen diet (8.4-10/9 mmol/l) than when offered a low-nitrogen diet (0.7-0.9 mmol/l) (Maloiy and Scott, 1969). In farmed red deer hinds, Wolkers *et al.* (1994) reported a plasma urea concentration of 7.9 mmol/l, but in those that had been food restricted it was 10.8 mmol/l. The values obtained in the current study for farmed deer are consistent with previous reports for farmed deer, Knox *et al.* (1988) reported a mean plasma urea concentration of 11.3 mmol/l (range 4.7 to 18.0), Wilson and Pauli (1983) a serum urea concentration of 8.56 mmol/l and Padilla *et al.* (2000), a serum urea concentration of 6.32 mmol/l (4.85 to 8.11 mmol/l). Bateson and Bradshaw (2000) reported a mean plasma urea concentration (adjusted for sample handling) of 3 mmol/l SD 1.8 in 28 hinds shot in the winter. This is similar to the plasma urea concentration found in single rifle shot deer (median 2.15 mmol/l, Q₁ 1.04, Q₃ 3.00).

Glucose

The plasma glucose concentration in deer shot in a field was greater than in those culled using the assistance of a helicopter and at night. Plasma glucose concentration can be affected by stress, but also by the nutritional state of the animal. However, Pollard *et al.* (2002) found that farmed stags shot in a paddock had a plasma glucose concentration of 7.04 mmol/l whereas in those collected, transported for 3h and held overnight at a slaughterhouse lairage before stunning with a captive bolt

it was 7.22 mmol/l. Millspaugh *et al.* (2000) reported a serum glucose concentration in wild red deer captured using a net from a helicopter of 8.59 mmol/l and in those herded by two helicopters for about 2h into a fenced enclosure it was 7.81 mmol/l. Wolkers *et al.* (1994) reported a plasma glucose concentration in farmed red deer hinds of between 11 and 15 mmol/l, but in those food restricted it was only 5 to 6 mmol/l. A median plasma glucose concentration in farmed red deer sampled by jugular venepuncture of 1.56 mmol/l (range 0.49 to 4.15) was found by Knox *et al.* (1988), values lower than 0.18, and greater than 3.32 were considered to exceed tolerance limits. Bateson and Bradshaw (1997) reported a mean plasma glucose concentration of 6 mmol/l SD 3.0 in 7 deer shot during single rifle stalking. This is comparable to the values found in the current study (median 6.90 mmol/l, Q₁ 4.80, Q₃ 10.0). After farmed hinds had been transported for 80-380 km, Jago *et al.* (1997) found that the plasma glucose concentration had risen from 6.17 to 9.02 mmol/l. In the current study, the values in the slaughterhouse deer were median 6.50 mmol/l (Q₁ 6.05, Q₃ 8.43). In cattle, the plasma glucose concentration is increased by handling and is further increased after transport and slaughter using captive bolt stunning (Mitchell *et al.* (1988). In a review of the effects of slaughter on the composition of blood collected at exsanguination, Shaw and Tume (1992) found reports of slight increases in plasma glucose concentration in sheep and cattle.

Conclusions

The difficulties in obtaining sufficient numbers of separate culling events for each culling method and potential issues with sample handling meant that the statistical analyses was problematic and the results might have been influenced by factors other than differences between culling methods. Although the reliability with which we can report the results is less than we would have liked, it has been possible to come to some tentative conclusions regarding the various culling methods.

1. Culling wild red deer with the assistance of a helicopter was more stressful than single rifle, field and night culling, but was not more stressful than collaborative culling
2. 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.
3. Single rifle culling and field shooting were the most accurate and night shooting was the least accurate in achieving at least one wound likely to have quickly killed the deer. However, there was no significant difference between the culling methods in the number of deer that appeared to have died immediately after the first shot. The highest percentages of deer that after the first shot, ran or walked away without collapsing were in the helicopter assisted and the night culled groups. There was no significant difference between culling methods in the percentage of deer that were wounded in the abdomen or leg.

Not all of the above conclusions are supported by the most conservative statistical analyses. Some of the apparent differences might have been due to factors other than those related to a particular culling method. 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. The use of night culling appeared to associated with little disturbance to the deer, but the effectiveness of this method is dependent on whether the high proportion of deer shot in the upper rather than in the lower chest is a significant factor in achieving a quick and humane death. The effects of location of wounds when shooting wild deer on the effectiveness of the kill merits further research.

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Appendix A

Tables showing summary statistics of the raw data by culling method for each of the variables recorded

Blood sampling

Table A1 Plasma haemoglobin concentration (g/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	0.11	0.04	8	0	0	0	0	0.9
Helicopter assisted / multiple rifles	14	0.13	0.01	37	0	0	0	0	1.7
Field	5	0.00	0.00	20	0	0	0	0	0.0
Collaborative	9	0.08	0.02	17	0	0	0	0	1.3
Single rifle	16	0.00	0.00	30	0	0	0	0	0.0
Night	14	0.00	0.00	21	0	0	0	0	0.0
Slaughterhouse	1	0.00	0.00	29	0	0	0	0	0.0

Table A2 Plasma total bilirubin concentration ($\mu\text{mol/l}$)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	4.92	0.79	8	1.10	2.0	2.3	3.8	19.9
Helicopter assisted / multiple rifles	14	4.24	0.11	37	0.03	1.9	2.6	6.7	17.7
Field	5	1.82	0.06	20	0.02	1.1	1.4	2.3	4.7
Collaborative	9	1.66	0.07	17	0.03	1.2	1.2	2.0	4.4
Single rifle	16	1.74	0.06	30	0.03	0.8	1.4	2.2	8.7
Night	14	1.29	0.05	21	0.03	0.1	1.3	1.9	3.4
Slaughterhouse	1	6.05	0.11	29	0.30	4.1	5.9	7.8	14.2

Table A3 Time between death and blood sampling (minutes)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	12.00	0.86	8	4	5.5	13	17.8	20
Helicopter assisted / multiple rifles	13	13.24	0.85	34	1	4.0	5	8.8	127
Field	3	22.07	1.00	15	10	10.0	20	30.0	65
Collaborative	9	7.12	0.24	17	1	5.0	7	9.0	19
Single rifle	16	5.42	0.14	26	1	2.3	5	8.8	13
Night	14	5.33	0.11	21	1	3.0	6	7.0	10
Slaughterhouse	1	1.85	0.04	27	1	1.0	2	2.0	5

Shooting

Table A4 Time between start of cull and first shot (minutes)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	10.38	1.57	8	0	1.5	5.5	15.0	30
Helicopter assisted / multiple rifles	14	41.11	1.63	37	0	0.0	16.0	65.0	233
Field	2	6.29	1.06	7	0	0.0	5.0	9.5	20
Collaborative	9	19.24	3.41	17	0	0.0	0.0	13.0	241
Single rifle	16	0.26	0.02	31	0	0.0	0.0	0.0	2
Night	14	1.10	0.19	21	0	0.0	0.0	0.0	18
Slaughterhouse	0	-	-	0	-	-	-	-	-

Table A5 Time between start of cull and death (minutes)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	16.38	2.18	8	0	4.3	8	30.0	48
Helicopter assisted / multiple rifles	14	45.35	1.63	37	0	4.0	20	65.0	233
Field	3	3.33	0.39	15	0	0.0	0	5.0	20
Collaborative	9	22.71	3.38	17	0	0.0	2	20.0	241
Single rifle	16	6.42	0.54	31	0	0.0	0	3.5	81
Night	14	1.90	0.25	21	0	0.0	0	0.0	23
Slaughterhouse	1	101.73	2.92	30	0	15.5	70	132.0	248

Table A6 Time between first shot and death (minutes)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	6	2.12	8	0	0	0	0.0	48
Helicopter assisted / multiple rifles	14	4.24	0.28	37	0	0	0	2.0	50
Field	2	0.86	0.27	7	0	0	0	0.5	5
Collaborative	9	3.47	0.51	17	0	0	0	1.0	35
Single rifle	16	6.16	0.54	31	0	0	0	3.0	81
Night	14	0.81	0.09	21	0	0	0	0.0	5
Slaughterhouse	0	-	-	0	-	-	-	-	-

Table A7 Time between first and second shot (minutes)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	2	27.5	13.79	2	8	17.8	27.5	37.3	47
Helicopter assisted / multiple rifles	6	13.3	1.94	9	0	2.0	2.0	19.0	50
Field	1	1.0	-	1	1	1.0	1.0	1.0	1
Collaborative	2	7.0	2.12	2	4	5.5	7.0	8.5	10
Single rifle	5	27.8	5.11	6	0	5.5	19.5	39.5	81
Night	2	2.5	1.77	2	0	1.3	2.5	3.8	5
Slaughterhouse	0	-	-	0	-	-	-	-	-

Table A8 Sites of entry wound (no. of wounds)

Culling method	No. of culling events	No. of carcasses examined	Head	Neck	Lower chest	Upper chest	Abdomen	Leg
Helicopter assisted / single rifle	3	8	0	1	4	3	2	1
Helicopter assisted / multiple rifles	14	35	4	5	20	3	9	0
Field	5	20	20	0	0	0	0	0
Collaborative	8	15	0	1	11	3	1	1
Single rifle	15	28	4	6	15	3	1	2
Night	14	18	0	0	9	8	3	0
Slaughterhouse	1	30	30	0	0	0	0	0

Please note for above – there are more entry sites than deer

Table A9 Sites of entry wound (%. of wounds)

Culling method	No. of culling events	No. of carcasses examined	Head	Neck	Lower chest	Upper chest	Abdomen	Leg
Helicopter assisted / single rifle	3	8	0	9	36	27	18	9
Helicopter assisted / multiple rifles	14	35	10	12	49	7	22	0
Field	5	20	100	0	0	0	0	0
Collaborative	8	15	0	6	65	18	6	6
Single rifle	15	28	13	19	48	10	3	6
Night	14	18	0	0	45	40	15	0
Slaughterhouse	1	30	100	0	0	0	0	0

Behaviour

Table A10 Behaviour of individual deer before it was shot (no. of deer)

Culling method	No. of culling events	No. of deer observed	Lying down	Standing alert	Standing idling	Walk-ing	Runn-ing	Trott-ing
Helicopter assisted / single rifle	3	8	0	8	0	0	0	0
Helicopter assisted / multiple rifles	12	33	0	22	0	2	1	8
Field	1	1	0	1	0	0	0	0
Collaborative	8	14	0	10	1	1	2	0
Single rifle	16	31	2	25	4	0	0	0
Night	14	21	0	21	0	0	0	0
Slaughterhouse	0	-	-	-	-	-	-	-

Table A11 Behaviour of individual deer after it was shot (no. of deer)

Culling method	No. of culling events	No. of deer observed	Collapsed	Ran <5m	Ran 5-20m	Ran 20-50m	Walked away	Ran away
Helicopter assisted / single rifle	1	8	0	0	6	0	0	2
Helicopter assisted / multiple rifles	5	36	19	2	7	6	0	2
Field	1	2	0	1	1	0	0	0
Collaborative	2	15	10	0	4	1	0	0
Single rifle	2	31	22	0	0	3	1	5
Night	1	21	11	3	3	3	1	0
Slaughterhouse	1	0	0	0	0	0	0	0

Table A12 Behaviour of group of deer after the first deer was shot (no. of occurrences)

Culling method	No. of deer observed	No response	Increased activity	Walked away	Ran away
Helicopter assisted / single rifle	8	2	0	0	6
Helicopter assisted / multiple rifles	29	0	4	0	25
Field	2	0	1	0	1
Collaborative	16	0	2	0	14
Single rifle	30	2	2	2	24
Night	18	0	4	0	14
Slaughterhouse	-	-	-	-	-

Injury

Table A13 Summary statistics for plasma creatine kinase activity (IU/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	10090.0	1741.5	8	612	2914.0	4557	10903.3	42702
Helicopter assisted / multiple rifles	14	5666.7	242.1	37	148	398.0	1782	5479.0	38197
Field	5	3355.2	317.8	20	60	415.3	1116	2537.3	26475
Collaborative	9	10665.5	1546.8	17	204	825.0	2847	7130.0	111105
Single rifle	16	5038.1	230.4	30	91	290.3	816	9801.8	22198
Night	14	8705.7	599.0	21	161	432.0	1573	13368.0	41243
Slaughterhouse	1	339.1	7.3	29	141	218.0	274	344.0	1103

Table A14 Summary statistics for plasma aspartate aminotransferase activity (IU/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	1136.1	209.63	8	76	231.8	308.0	1105.5	4416
Helicopter assisted / multiple rifles	14	402.1	14.87	37	4	59.0	168.0	447.0	2469
Field	5	179.3	10.25	20	13	67.3	123.5	184.8	946
Collaborative	9	294.4	22.17	17	20	72.0	168.0	313.0	1375
Single rifle	16	298.9	16.37	30	6	61.0	109.5	424.5	2590
Night	14	372.7	20.79	21	35	83.0	115.0	435.0	1483
Slaughterhouse	1	81.6	1.03	28	41	70.8	79.5	83.8	209

Muscle glycogen and pH

Table A15 Time between death and muscle freezing (minutes)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	27.62	1.74	8	8	20.5	29.5	35.0	50
Helicopter assisted / multiple rifles	14	15.54	0.75	37	1	5.0	8.0	12.0	129
Field	3	119.80	4.02	15	40	72.0	112.0	162.0	227
Collaborative	9	23.76	3.34	17	1	9.0	10.0	10.0	243
Single rifle	16	6.29	0.15	31	1	3.0	5.0	9.0	20
Night	14	6.95	0.15	21	3	4.0	7.0	9.0	13
Slaughterhouse	1	37.93	0.55	30	12	25.0	38.0	52.3	67

Table A16 Muscle glycogen concentration (mmol glucosyl units/kg dry muscle)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	376.54	14.84	7	255.4	301.9	386.34	413.03	564.2
Helicopter assisted / multiple rifles	14	444.35	7.49	36	13.1	256.3	412.97	547.27	1329.9
Field	5	960.91	40.83	20	205.1	410.5	714.02	1125.61	3367.0
Collaborative	9	528.01	18.79	17	105.1	344.7	474.78	638.27	1349.2
Single rifle	16	516.73	8.49	31	86.8	381.8	502.42	613.45	1419.7
Night	14	914.75	23.74	21	288.7	644.9	802.03	974.52	2236.5
Slaughterhouse	1	901.01	15.97	30	214.8	577.6	833.58	990.42	2279.8

Table A17 Neck/shoulder muscle pH (*M. Triceps brachii*)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	2	5.79	0.03	7	5.58	5.69	5.74	5.88	6.08
Helicopter assisted / multiple rifles	14	5.70	0.01	37	5.42	5.50	5.63	5.80	6.66
Field	4	5.75	0.02	12	5.49	5.53	5.77	5.94	6.06
Collaborative	9	5.81	0.02	17	5.51	5.66	5.71	5.84	6.43
Single rifle	16	5.69	0.02	29	4.80	5.48	5.62	5.82	7.42
Night	13	5.48	0.03	19	4.41	5.02	5.61	5.79	6.61
Slaughterhouse	1	5.82	0.00	29	5.64	5.76	5.80	5.90	6.00

Table A18 Back muscle pH (*M. Longissimus dorsi*)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	2	5.65	0.03	7	5.37	5.50	5.66	5.81	5.88
Helicopter assisted / multiple rifles	14	5.69	0.01	37	5.35	5.52	5.60	5.76	6.94
Field	4	5.74	0.02	12	5.46	5.52	5.86	5.90	6.00
Collaborative	9	5.79	0.02	17	5.48	5.61	5.74	5.82	6.48
Single rifle	16	5.66	0.02	29	4.80	5.45	5.57	5.93	6.90
Night	13	5.5	0.03	19	4.85	5.02	5.53	5.7	6.73
Slaughterhouse	0	-	-	0	-	-	-	-	-

Table A19 Back-leg muscle pH (*M. Biceps femoris*)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	2	5.52	0.02	7	5.32	5.47	5.51	5.54	5.79
Helicopter assisted / multiple rifles	14	5.56	0.01	37	5.32	5.45	5.51	5.58	6.35
Field	4	5.70	0.01	12	5.46	5.53	5.74	5.87	5.9
Collaborative	9	5.63	0.02	17	5.38	5.46	5.55	5.64	6.38
Single rifle	16	5.46	0.01	29	4.75	5.41	5.49	5.56	6.64
Night	13	5.29	0.02	19	4.84	4.96	5.44	5.47	5.62
Slaughterhouse	1	5.74	0.0	29	5.52	5.69	5.73	5.77	6.02

Table A20 Blood lactate concentration (mmol/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	7.39	0.95	7	3.2	3.7	5.1	6.95	22.1
Helicopter assisted / multiple rifles	13	7.69	0.13	34	1.0	3.9	7.2	10.95	21.1
Field	5	9.94	0.15	19	5.0	7.8	10.7	11.95	15.0
Collaborative	9	6.14	0.22	17	1.4	3.1	6.0	7.70	14.3
Single rifle	16	5.10	0.18	29	1.0	1.9	3.1	6.60	21.2
Night	13	5.32	0.32	19	1.3	2.5	2.9	4.70	22.3
Slaughterhouse	1	6.77	0.10	30	2.2	4.6	6.8	8.05	16.1

Stress

Table A21 Plasma cortisol concentration (nmol/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	103.0	21.79	8	7.0	19.5	36.6	75.1	523
Helicopter assisted / multiple rifles	14	88.3	1.77	37	9.9	36.7	83.0	117.0	267
Field	5	33.5	2.02	20	5.5	9.8	22.6	38.4	186
Collaborative	9	81.0	6.23	17	10.1	26.5	48.5	82.8	444
Single rifle	16	45.5	2.14	30	5.9	10.5	16.4	26.7	229
Night	14	19.2	0.87	21	8.0	9.4	13.3	22.6	91
Slaughterhouse	1	91.9	1.08	29	25.9	71.7	88.6	113.0	168

Table A22 Plasma free fatty acid concentration (mmol/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	0.37	0.02	8	0.17	0.30	0.32	0.42	0.61
Helicopter assisted / multiple rifles	14	0.46	0.01	37	0.19	0.30	0.42	0.61	0.86
Field	5	0.26	0.01	20	0.11	0.18	0.23	0.30	0.62
Collaborative	9	0.30	0.01	17	0.13	0.21	0.24	0.40	0.70
Single rifle	16	0.24	0.00	30	0.13	0.18	0.21	0.27	0.59
Night	14	0.22	0.00	21	0.14	0.16	0.20	0.27	0.41
Slaughterhouse	1	0.40	0.01	29	0.10	0.26	0.41	0.55	0.82

Nutrition

Table A23 Body condition classification (no. of deer)

Culling method	No. of culling events	No. of deer observed	Thin	Thin / normal	Normal	Normal / fat	Fat
Helicopter assisted / single rifle	3	8	0	1	3	3	1
Helicopter assisted / multiple rifles	14	36	10	1	21	0	4
Field	5	20	0	0	12	0	8
Collaborative	9	17	4	2	8	0	3
Single rifle	16	31	4	0	17	1	9
Night	14	21	4	0	11	1	5
Slaughterhouse	1	30	5	0	25	0	0

Table A24 Body condition classification (percentage of deer)

Culling method	No. of culling events	No. of deer observed	Thin	Thin/normal	Normal	Normal/fat	Fat
Helicopter assisted / single rifle	3	8	0	12.5	37.5	37.5	12.5
Helicopter assisted / multiple rifles	14	36	27.8	2.8	58.3	0	11.1
Field	5	20	0	0	60.0	0	40.0
Collaborative	9	17	23.5	11.8	47.1	0	17.6
Single rifle	16	31	12.9	0	54.8	3.2	29.0
Night	14	21	19.0	0	52.4	4.8	23.8
Slaughterhouse	1	30	16.7	0	83.3	0	0

Table A25 Percentage of kidneys covered with fat (no. of deer)

Culling method	No. of culling events	No. of deer observed	% of kidneys covered with fat			
			<25	25-50	51-75	>75
Helicopter assisted / single rifle	2	8	1	1	3	2
Helicopter assisted / multiple rifles	14	35	9	17	9	0
Field	4	19	4	4	8	3
Collaborative	9	17	7	3	7	0
Single rifle	15	30	6	12	11	1
Night	13	18	5	9	4	0
Slaughterhouse	1	30	30	0	0	0

Table A26 Percentage of kidneys covered with fat (percentage of deer)

Culling method	No. of culling events	No. of deer observed	% of kidneys covered with fat			
			<25	25-50	51-75	>75
Helicopter assisted / single rifle	2	7	14.3	14.3	42.9	28.6
Helicopter assisted / multiple rifles	14	35	25.7	48.6	25.7	0
Field	4	19	21.1	21.1	42.1	15.8
Collaborative	9	17	41.2	17.6	41.2	0
Single rifle	15	30	20.0	40.0	36.7	3.3
Night	13	18	27.8	50.0	22.2	0
Slaughterhouse	1	30	100	0	0	0

Table A27 Plasma β -hydroxybutyrate concentration (mmol/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	0.40	0.02	8	0.21	0.24	0.36	0.53	0.75
Helicopter assisted / multiple rifles	14	0.42	0.00	37	0.16	0.32	0.38	0.47	0.97
Field	5	0.24	0.00	20	0.09	0.18	0.24	0.29	0.52
Collaborative	9	0.42	0.01	17	0.12	0.32	0.40	0.53	0.69
Single rifle	16	0.51	0.01	30	0.20	0.40	0.48	0.66	0.81
Night	14	0.38	0.00	21	0.18	0.28	0.40	0.47	0.55
Slaughterhouse	1	0.21	0.00	29	0.12	0.17	0.20	0.26	0.31

Table A28 Plasma urea concentration (mmol/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	2.25	0.15	8	0.7	1.5	2.1	2.9	4.4
Helicopter assisted / multiple rifles	14	3.52	0.04	37	1.2	2.5	3.5	4.4	5.9
Field	5	6.20	0.14	20	1.9	4.2	6.5	7.8	11.8
Collaborative	9	3.43	0.11	17	1.1	1.9	2.8	4.9	7.8
Single rifle	16	2.06	0.04	30	0.2	1.1	2.2	2.9	4.4
Night	14	4.75	0.09	21	0.7	3.6	4.7	5.7	7.5
Slaughterhouse	1	7.49	0.03	29	5.9	7.1	7.4	8.1	9.2

Table A29 Plasma glucose concentration (mmol/l)

Culling method	No. of culling events	Mean	SE	N	Min	Q1	Median	Q3	Max
Helicopter assisted / single rifle	3	6.17	0.39	8	1.1	4.9	6.5	8.5	10.2
Helicopter assisted / multiple rifles	14	6.45	0.09	37	1.0	5.0	5.8	7.4	20.1
Field	5	10.68	0.33	19	2.2	5.4	11.0	13.9	26.4
Collaborative	9	7.91	0.23	17	4.1	5.4	7.4	8.1	18.3
Single rifle	16	7.97	0.20	30	1.2	4.8	6.9	9.5	30.0
Night	14	5.82	0.09	21	2.1	4.7	5.3	7.0	10.4
Slaughterhouse	1	6.93	0.06	29	3.0	6.2	6.5	8.4	9.7