AN INVESTIGATION INTO THE INTESTINAL PARASITIC LOAD AND ITS EFFECTS ON THE BODY CONDITION SCORE OF THE WHITE RHINOCEROS (*Ceratotherium simum simum*) IN AN ISLAND-BOUND AREA.

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BY

Cveta Pudar

201405604

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Supervisor: Dr Mark Jago (MA, Vet.M.B.)

Co-supervisor: Dr Diethardt Rodenwoldt (B.V.Sc., B.Sc)

Abstract

A commensal relationship of low clinical significance usually occurs between wildlife and parasites. It is important to properly manage the well-being and care of rhinoceroses once placed in an island-bound area, and this includes from a parasitological perspective. The aim of this study is to determine whether the intestinal parasitic load, in terms of faecal egg count, has an effect on the body condition score of the white rhinoceroses. Analysis of faecal egg counts was carried out with the McMaster method on samples collected from various rhinoceroses in an undisclosed location, and their body condition scores were also determined. This was conducted in the wet and the dry season. The results show that there was no significant difference in faecal egg counts between the two seasons and that their body conditions scores remained the same for both seasons. Considering the limitations and challenges encountered, the results tentatively indicate that there is no correlation between the faecal egg count and body condition score of the white rhinoceros.

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1. Introduction

1.1 Background

The Cambridge Dictionary (2008) defines a parasite as "an animal or plant that lives on or in another animal or plant of a different type and feeds from it". Although extensive studies involving parasites and wildlife are limited, it is known that a commensal relationship between wildlife and parasites can occur, and is usually of a low clinical significance (Knapp et al, 1997). A commensal relationship refers to one of the organisms benefiting (usually the parasite) while the other is unaffected (the host in this case).

Rhinos are valuable and endangered – once placed in an island-bound area, it is our responsibility to ensure proper management of their health and wellbeing.

The purpose of this study is to determine the relationship between the faecal helminth egg count and the clinical health status of the white rhinoceros (*Ceratotherium simum simum*) as measured by the body condition score.

1.2.Statement

This study focuses on the commensal relationship between intestinal parasites and the white rhino (*Ceratotherium simum simum*) to demonstrate whether there is a correlation between the intestinal parasitic load and the body condition score (BCS) of the rhinos. This also aims to provide documentation to assist with decision-making regarding the well-being of the rhinos. The aim was to conduct the study in the wet and dry seasons.

1.3. Objective

The objective of this study is to quantify the intestinal helminth load of *Ceratotherium simum simum* and determine whether the load has any observable effects on the health of the rhinos as measured by their body condition score. Identification of the parasites encountered is only done to genus level.

1.4. Hypothesis

The intestinal parasitic load does not have an effect on the clinical health status of the white rhinoceros as measured by the body condition scores in this island-bound area.

H0: There is no correlation between the faecal egg count and the body condition score of the white rhinoceros.

H1: There is a correlation between the faecal egg count and the body condition score of the white rhinoceros.

1.5. Significance

The correlation between the intestinal parasitic load and the clinical health of the rhinos may assist with decision-making regarding their well-being, and whether intervention is required. Data was collected during the wet and dry seasons, to determine whether the change in seasons plays a role in the intestinal and body condition score. Although the target population is small, one could view this as a pilot study for future research.

1.6. Limitation

The area in question had a limited group of rhinoceroses to study. This may be considered too small a target population for any study and thus conclusions drawn from this study cannot be generalized to the rest of the region or country. The location of the rhinoceroses and the total number cannot be disclosed due to ethical considerations. The body condition scoring chart used was designed for black rhinoceroses; there is no chart available for white rhinoceroses. Another limiting factor is that body condition score evaluations were done visually from a distance and these may be considered too subjective

1.7. Delimitation

This study is only concerned with the target population in the area in question, in order to assist with decision-making regarding their well-being. Results from this study, however, may be used as a pilot for more extensive study. One could repeat this study on other populations and compare the results, as a means to solve the limitation. It is important to note that this type of study has not been done in Namibia before and thus any new information brought to light would be valuable information forming a basis for further studies.

2. Literature review

Wild animals and parasites can have a commensal relationship which is usually of low clinical significance (Penzhorn et al., 1997). Faecal egg counts by means of faecal flotation are used as a non-invasive tool to evaluate intestinal parasitic load. Studies on black rhino (*Diceros bicornis*) have shown that it is best to collect a sample from the middle of the faecal ball within 6 hours after defecation, as this does not affect faecal egg counts (Stringer et al., 2014). This is aimed at minimizing sampling errors and may be useful for studies in the white rhino.

Body condition scoring (BCS) aims to give one an idea of nutritional status and fitness and is a crucial component of animal management (Reuter & Adcock, 1998). A standardized body condition scoring chart has been developed for black rhinoceroses with a 5-point scale ranging from very poor (BCS of 1) and excellent (BCS of 5). Acceptable visual scoring of free-ranging rhinos can only be done with the use of binoculars and at a distance of not more than 100 meters (Reuter & Adcock, 1998).

3. Materials and methods

The methodology involves quantitative and qualitative components. Quantitatively, the McMaster method was used to determine a numerical count of faecal eggs; and body condition scoring of the rhinoceroses was done using a scale of 1 to 5. Qualitatively, identification of eggs was only done up to genus level, based on photographs obtained from microscopically evaluated specimens. This was done by means of passive direct faecal flotation. Convenient sampling was used.

Materials:

Table 1. Materials

Sample collection	Laboratory analysis
Sample bags Gloves Markers Cool box Ice packs	Microscope Microscope slides and cover slips McMaster slide OvaTector® (Kyron) Faecal flotation fluid: sodium nitrate, SG 1.22 Plastic containers Tongue depressor sticks Tea strainer Electronic scale 20ml syringes Plastic pipettes Laptop Stopwatch

Method:

Fresh faecal samples were collected early morning, from the rhinoceroses, every day for 4 days. Each sample was identified as being from specific individuals. These individuals were observed from a distance and a body condition score out of 5 was given, according to the guidelines of Reuter and Adcock (1998). This included assessing the neck, shoulders, ribs, spine, gluteal region, tail base and abdominal region.

Table 2. Body condition scoring chart (Reuter & Adcock, 1998).

Body Condition Score	Description
1	Very poor
2	Poor
3	Fair
4	Good
5	Excellent

The samples were collected from the centre of the faecal balls. Each sample was placed in a separate bag, which was sealed and labelled. All samples were placed in a cool box with ice until they were able to be analysed. Analysis was done within 7 hours of sample collection.

The method used to determine faecal egg count was the modified McMaster faecal egg counting procedure. This method is a flotation test that allows the eggs to float to the surface.

<u>Modified McMaster protocol</u> (USDA guidelines):

1. Two grams of faeces were measured in a container.
2. 28ml of flotation solution was added to the faeces and mixed.
3. The solution was left to stand for 5 minutes.
4. The sample was mixed again and then poured through a tea strainer into a second container, using a tongue depressor to press the fluid through.
5. Using a pipette, the solution was immediately transferred to both chambers of the McMaster slide.
6. The McMaster slide was left to stand for 5 minutes.
7. The McMaster slide was placed under the microscope and examined at 4x and 10x objective.
8. All the eggs within the grid areas, in both chambers, were counted under the 10x objective.

9. The faecal egg count per gram (FEC/gram) was calculated as follows:

[chamber 1 and chamber 2] *50 = eggs per gram

This calculation is specific to the ratio of faeces to flotation fluid. Each egg represents 50 eggs per gram. The FEC/gram was calculated for each sample of the individual rhinoceroses.

To identify the eggs up to genus level, passive faecal flotation was performed on each sample, the specimens observed under 100x magnification and photos were taken of the eggs observed.

<u>Direct faecal flotation protocol</u>:

1. 2 grams of faeces was weighed out and placed into the receptacle of the OvaTector®.
2. The cylinder was placed over the receptacle.
3. The cylinder was filled halfway with flotation fluid and the solution was mixed with an applicator stick.
4. The OvaTector® strainer was inserted into the cylinder and more floatation fluid was added until it formed a convex meniscus at the top.
5. A cover slip was placed on the meniscus and left to stand for 15 minutes.
6. After 15 minutes, the cover slip was lifted off, placed on a microscope slide and viewed at 100x magnification under the microscope.

4. Results

The FEC/gram for each rhinoceros sample was as follows:

Table 3. The FEC/gram per rhino for each day's sampling.

Date	Rhino ID	FEC/gram
4.12.2017	D	50
4.12.2017	E	50
5.12.2017	D	50
5.12.2017	A	300
5.12.2017	B	100
6.12.2017	E	50
6.12.2017	B	100
6.12.2017	A	200
6.12.2017	B	100
7.12.2017	C	150
7.12.2017	F	100

Table. 4. The FEC/gram per rhino for each day's sampling.

Date	Rhino ID	FEC/gram
2.07.2018	C	100
2.07.2018	F	50
2.07.2018	E	250
3.07.2018	C	250
3.07.2018	D	100
3.07.2018	F	250
4.07.2018	B	400
4.07.2018	F	200
4.07.2018	E	400
5.07.2018	A	450
5.07.2018	B	250
5.07.2018	F	200

The mean FEC/gram for each rhinoceros was calculated for each season that the samples were collected. All rhinoceroses had the same body condition score (BCS) for both seasons, that being 3.5/5.

Table 5. Mean FEC/gram per rhino for December 2018.

Rhino ID	December 2017 Parasitic Load (FEC/gram)	December 2017 BCS
A	250	3.5
B	100	3.5
C	150	3.5
D	50	3.5
E	50	3.5
F	100	3.5

Table 6. Mean FEC/gram per rhino for July 2018.

Rhino ID	July 2018 Parasitic Load (FEC/gram)	July 2018 BCS
A	450	3.5
B	325	3.5
C	175	3.5
D	100	3.5
E	325	3.5
F	175	3.5

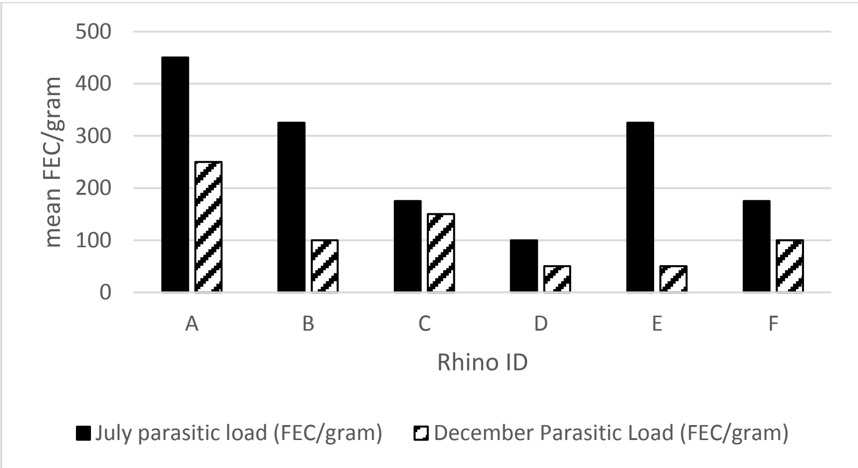

Figure 1. The mean FEC/gram per rhino for December 2017 and July 2018.

The Two-sample Assuming Equal Variances T-Test (Excel) was used to determine the significance of the parasitic load in the rhinoceroses, and it was found that there is no significant difference between the parasitic loads in the rhinoceroses between December 2017 and July 2018.

Table 7. T-Test: Two-sample Assuming Equal Variances

	July parasitic load (FEC/gram)	December Parasitic Load (FEC/gram)
Mean	258.3333333	116.6666667
Variance	16916.66667	5666.666667
Observations	6	6
Pooled Variance	11291.66667	
Hypothesized Mean Difference	0	
df	10	
t Stat	2.309134756	
P(T<=t) one-tail	0.02178577	
t Critical one-tail	1.812461123	
P(T<=t) two-tail	0.043571539	
t Critical two-tail	2.228138852	
[P(T<=t) two-tail] < [t Critical two-tail] =TRUE		

It was shown that the body condition scores of the rhinoceroses remained the same for both of the months that sampling was done, despite the different FEC/gram results.

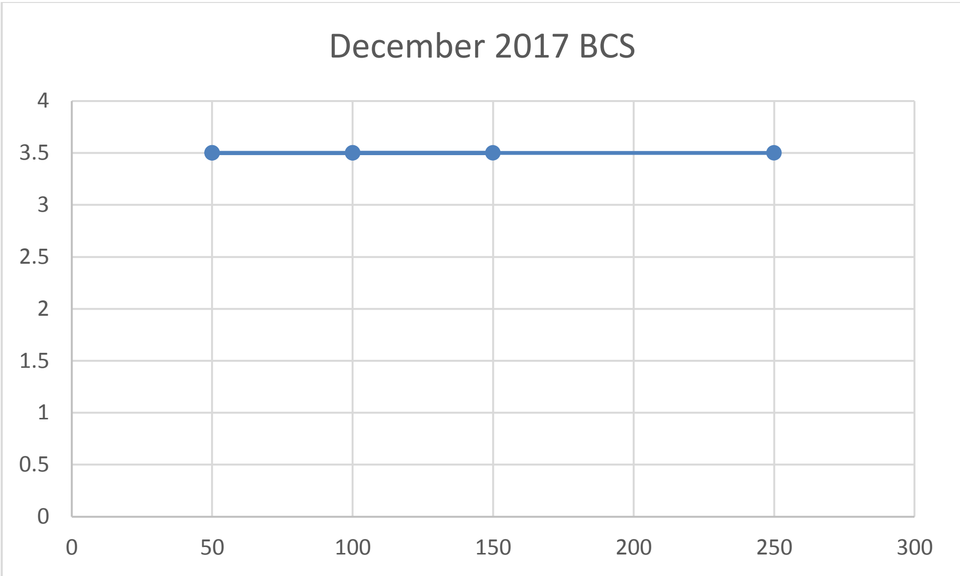

Figure 2. Body condition scores for December 2017 versus the FEC/gram.

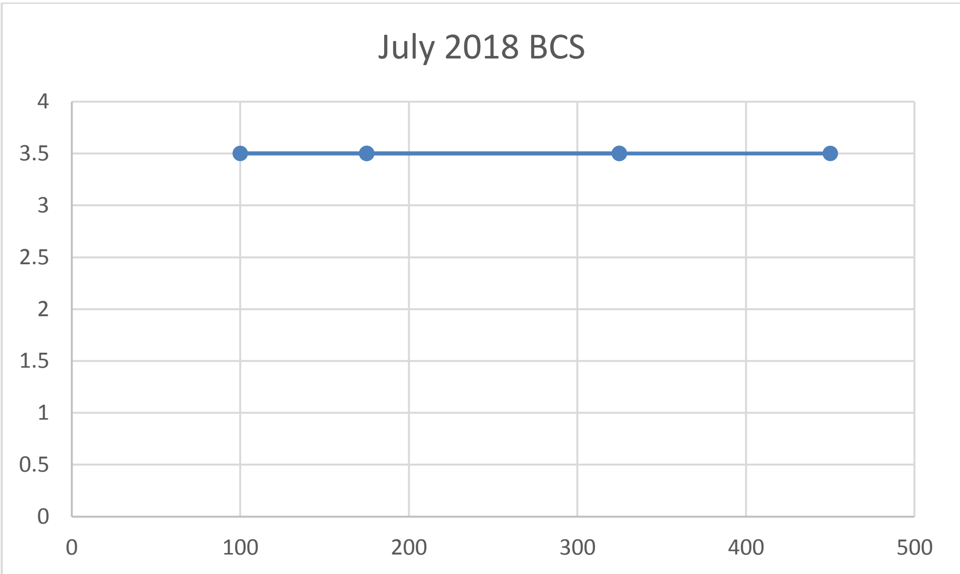

Figure 3. Body condition scores for July 2018 versus the FEC/gram.

Two egg types were observed and identified up to genus level, namely *Trichostrongylus* and *Nematodirus*.

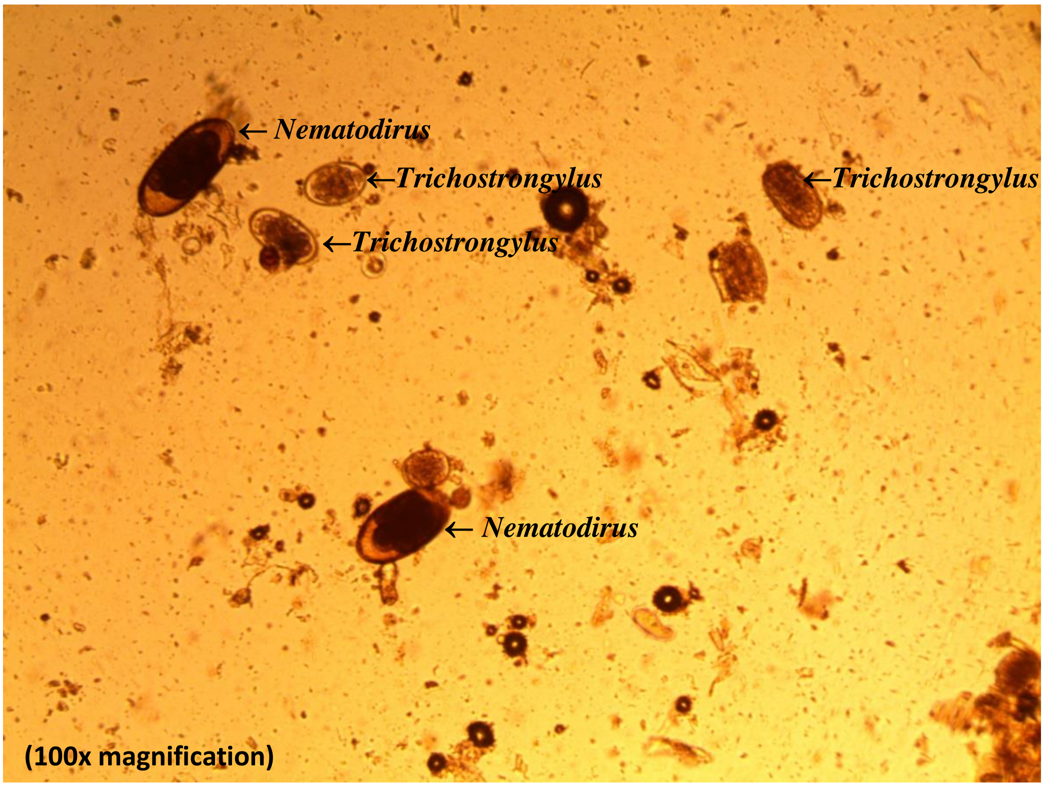

Figure 4. ***Trichostrongylus*** **and** ***Nematodirus*** **eggs.**

5. Discussion

The mean faecal egg count per gram per rhinoceros, for December 2017 and July 2018 showed no statistical difference. This may have been due to the fact that sampling was done in two consecutive "dry" seasons, as opposed to one wet and one dry season. This was due to the rains starting late in the 2017 – 2018 wet season. It had been expected that by December some rain would have fallen with a resultant increase in the faecal egg count in December 2017, as the wet conditions would have been ideal for parasites to proliferate.

The body condition score for each of the rhinoceroses remained the same for both December 2017 and July 2018. This could tie in with the fact that there was no statistical difference in the faecal egg counts for the two months.

Identification of the eggs was done up to genus level only, as limited time and resources would not allow hatching for more identification up to species level. This author is also confident with the genus identification. *Trichostrongylus* was a more prominent finding, making up approximately 90% of the eggs observed. *Nematodirus* eggs made up 10% of the observed eggs. It is not known what significance these helminths have on rhinoceroses.

6. Conclusion

It was observed that there was no significant difference in the faecal egg counts for each of the months that sampling was done. The body condition score for the two months remained the same for all the study subjects. This study therefore demonstrates that the parasitic load in terms of faecal egg count did not have any effect on the body condition score of the white rhinoceros. This falls in place with the null hypothesis: there is no correlation between the faecal egg count and the body condition score of the white rhinoceros.

7. Recommendations

Regarding the laboratory analysis of the faeces, it is of this author's opinion that there are methods of faecal flotation superior to that of the passive flotation technique used. One might consider the use of centrifugal flotation techniques to perhaps obtain a higher sensitivity with regards to results.

A consistent, pre-calculated number of faecal samples per rhinoceros would provide a more accurate representation of the faecal egg count per individual. In this study, convenient sampling was used, and an inconsistent number of faecal samples per rhinoceros were collected. A longer period of sample collection, timed to the actual rainfall, instead of the 4 days (based on availability) used for each season would more accurately detect a seasonal difference in faecal egg count, if indeed one does occur.

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8. Appendices

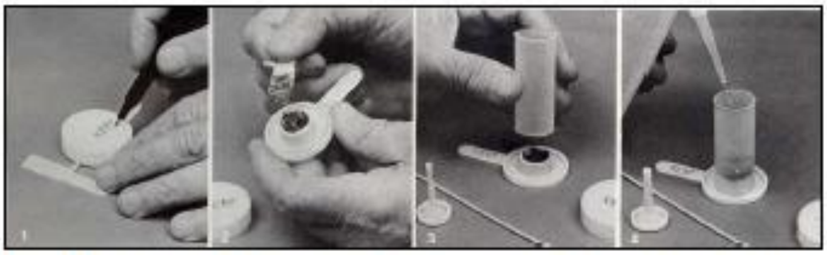
Appendix 1: OvaTector® (Kyron) faecal flotation method.

KYRON

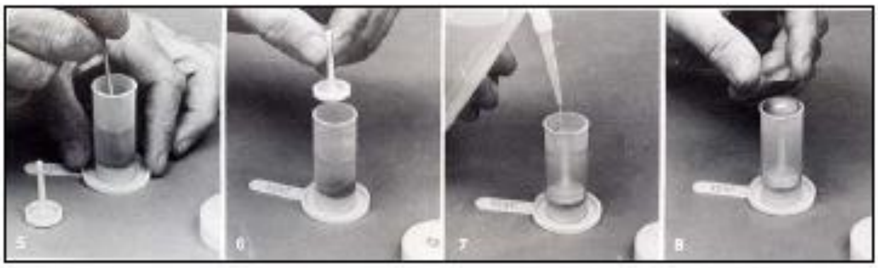
OVATECTOR® disposable fecal diagnostic system

The original standard of excellence.

Follow this simple procedure to set up the OvaTector® system in less than 45 seconds



). Using a marking pen, identify the faecal collection container and dispense to client. **(2).** Client separates atula from container and fills centre receptacle with faecal matter (holds a 2g sample). **(3).** When faecal ntainer is returned, the cylinder with lip-end up, is placed over the centre receptacle and snapped into sition forming the floatation system. **(4).** Fill the cylinder halfway with *Kyron Egg Flotation Fluid.*



(5). Mix the faecal specimen and solution thoroughly with applicator stick provided. **(6).** Push strainer gently down into cylinder until handle is below top lip. **(7).** Add more *Kyron Egg Flotation Fluid* until convex meniscus is formed at the top of the cylinder. **(8).** Float a 22mm cover slip on the meniscus. Allow to stand at least 15 min. for ova to float through the strainer and adhere to the cover slip. Lift cover slip with a smooth motion and place on microscope slide. Examine under low power and 100x for ova.

Appendix 2: Body condition scoring chart by Reuter & Adcock (1998).

	CONDITION Assessment site	Numerical scale Descriptive scale	5 excellent (heavy)	4 good (ideal)	3 fair (average)	2 poor (thin)	1 very poor (emaciated)
A	Neck	General appearance	thick, well muscled, rounded	well muscled, rounded	rounded	flat, narrow neck; nuchal ligament visible	narrow, angular (bony) neck; nuchal ligament prominent
		Prescapular groove		-	slightly visible	obvious	deep groove very obvious
B	Shoulder	General appearance	well-muscled, rounded	rounded	flat	flat, slightly angular (bony)	angular, bony
		Scapula	covered	covered	spine visible	obvious	very obvious
C	Ribs		well covered (skin folds)	covered (skin folds)	visible	obvious	very obvious
D	Spine	General appearance	rounded	slightly angular	back groove back visible	groove deep obvious	back groove very obvious
		Spinous processes	covered	slightly visible	visible	prominent	very prominent
E	Rump	General appearance	well rounded	flattened	slightly concave	concave	obvious depression
		Bony protuberances	covered	slightly visible	visible	prominent	very prominent
F	Abdomen	General appearance	distended, taught	filled	slightly tucked in	tucked in	tucked in
		Flank-fold	none	sometimes slightly visible	slightly visible	visible	obvious
G	Tail base		rounded (bulging)	rounded	narrow	slightly bony	very thin and bony

Appendix 3: Regions assessed for body condition scoring (Reuter & Adcock, 1998)