NOTTINGHAM TRENT UNIVERSITY

PROXIMATE AND MINERAL COMPOSITION OF SELECTED WHOLE INVERTEBRATES AND NUTRITIONAL EFFECTS OF DIFFERENT DIETS ON THE FIELD CRICKET, *GRYLLUS BIMACULATUS*.

BY

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ABSTRACT

Over the past five years the Durrell Wildlife Conservation Trust based in Jersey has been developing its amphibian husbandry practices and several health issues have been encountered. These health issues encountered at Durrell may well be attributed to nutritionally inappropriate and/or imbalanced diets. Imbalanced diets and poor feeding management in amphibians commonly lead to developmental and reproductive problems. In this study, the nutritional compositions of the invertebrate species that are cultured on site at Durrell in Jersey were analysed and the effect of different diets on *G. bimaculatus* was compared. Crude protein was the major component of all of the species it was analysed for in this study (>40% DMB). The dry matter component of each sample varied from 14.76% (earthworm) to 50.80% (bean weevil). The lipid content of the invertebrates sampled was variable with the lowest value of 3.25% (giant African land snail, with shell) and highest value of 34.88% (Argentinean cockroach DMB). Ash values ranged from 1.88% DMB (bean weevil) to 52.62% DMB (giant African land snail, with shell). The DM content was higher in the groups fed diet A and B. The crickets fed diet A had a lower lipid and crude protein content and higher ash content than the control group. The crickets fed on diet B had a higher lipid content and lower crude protein and ash content than the control group.

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TABLE OF CONTENTS

LIST OF TABLES & FIGURES

1. INTRODUCTION

Amphibians are currently more threatened than either mammals or birds and are also declining more rapidly (Stuart et al., 2004). A recent report from the International Union for Conservation of Nature's (IUCN) Global Amphibian Assessment indicates that of the 5743 described species of amphibian, 1856 are globally threatened (listed in IUCN's Red List categories as vulnerable, endangered or critically endangered) and as many as 2468 amphibian species are in some form of population decline (Stuart et al., 2004).

Over the past five years the Durrell Wildlife Conservation Trust based in Jersey has been developing its amphibian husbandry practices and due to the large numbers of amphibians maintained, several health issues have been encountered (Tapley, pers. com).

Appropriate and ideal husbandry requirements are unknown or at best incompletely studied for the vast majority of amphibians. The three lineages of extant amphibians, caecilians (Gymnophiona, Apoda, or Caecilia), salamanders (Urodela or Caudata) and frogs and toads (Anura) are a massively diverse class (Pough, 2007). As such providing an adequate and suitable diet is perhaps no more than an educated guess based on general characteristics of amphibian biology, inferences drawn from available natural history information and field observations of feeding behaviours (Pough, 2007). Although behavioural aspects of feeding should not be ignored it is important to consider the nutritional composition of prey items when formulating and evaluating diets (Barker *et al.,* 1998).

The varying health issues encountered at Durrell may well be attributed to nutritionally inappropriate and/or imbalanced diets. Imbalanced diets and poor feeding management in amphibians commonly lead to developmental and reproductive problems such as metabolic bone disease, tetany, failure to thrive and death (Donoghue, 1998; Pramuk & Galiardo, 2008).

This study, in collaboration with Durrell seeks to analyse the nutritional composition of the invertebrate species used as food for the captive amphibian populations at Durrell in Jersey and compare the effects of two different diets on the nutritional composition of the field cricket, *Gryllus bimaculatus.*

The particular nutritional information required by Durrell are the proximate compositions of dry matter, protein, fat, fibre and ash and a mineral suite analysis for copper, zinc, magnesium, calcium, potassium, phosphorus, sodium and iron.

Although the results of this study will not provide the information required to formulate ideal diets for captive amphibians, it is hoped this study will yield nutritional value information required to ensure amphibian diets are formulated in a much more informed way.

2. LITERATURE REVIEW

Invertebrates are an important food source for both animals and humans and as a result the nutritional values of many species of invertebrate have been published (Ademolu *et al.*, 2004; Banjo, 2006; Barker *et al.*, 1998; Bernard & Allen, 1997; Finke, 2002; Finke, 2007 and Omotoso & Adedire, 2007; Oonincx & van der Poel, 2009; Raksakantong *et al.*, 2009 and Ramos-Elorduy *et al.*, 1997). This literature review seeks to establish what is known about the nutritional composition of invertebrates in context with the nutritional requirements of amphibians. It also seeks to ascertain the most appropriate and commonly used analytical methods used to determine proximate compositions of dry matter, ash, fat, protein and mineral concentrations of invertebrates.

2.1. Nutritional Composition of Invertebrates

Invertebrates commonly fed to captive amphibians include fruit flies (*Drosophila hydei* and *Drosophila melanogaster*), blackflies (*Musca* spp), ants (various genera), crickets (*Gryllus* spp, *Acheta* spp), locusts (*Melanoptus* spp), springtails (*Collembola* spp) and earthworms (*Lumbricus* spp) (McWilliams, 2008). Although generally, insects are a good source of many nutrients, they are deficient in several others; therefore, providing adequate nutrition for insectivores can be challenging (Finke & Winn, 2004). Proximate compositions and mineral concentrations of some species commonly fed to amphibians are shown in tables 2.1 and 2.2. The species to be analysed in this study are given in table 2.3. Of these species only the vestigial winged fruit fly (*Drosophila melanogaster)* has been previously studied for both

proximate and mineral composition (Barker *et al.*, 1998; Bernard & Allen, 1997). The brown cricket (*Gryllus assimilis)* has been the subject of a study investigating the effect of dietary calcium supplementation on calcium content (Eidhoff *et al.,* 2006). No other data are available for any of the other species to be analysed in this study. The majority of the literature regarding the mineral content of invertebrates is concerned with the calcium content and the calcium: phosphorus ratios (Allen & Oftedal, 1989; Anderson, S., J., 2000; Barker *et al.*, 1998 and Eidhoff *et al.,* 2006).

Table 2.1 Proximate composition of selected whole invertebrates

Species	Water (%)	Crude Fat (%DMB)	Crude Protein (%DMB)	Fibre (%DMB)	Ash (%DMB)
Mealworms *Tenebrio molitor*[1]	62.9	31.1	51.9	14.5	4.3
Cricket (Adult) *Acheta domesticus*[1]	73.2	22.8	64.4	19.1	5.1
Fruit fly *Drosophila melanogaster*[1]	67.1	17.9	56.3	16.2	5.2
Earthworm *Lumbricus terrestrius*[1]	75.8	10.6	50.6	20.9	24.9
Palm weevil (Adult) *Rhynchophorus phoenicis*[2]	4.8	52.4	8.4	21.8	1.4
American cockroach *Periplaneta americana*[3]	61.3	28.4	53.9	9.4	3.3
African giant land snail (without shell) Archachatina marginata*	72.8	1.4	59.6	0.1	1.3

[1] Data from Barker *et al.* (1998) fibre measured as NDF.
[2] Data from Omotoso & Adedire (2007) fibre measured as crude fibre.
[3] Data from Bernard & Allen (1997) fibre measured as ADF.
* Data from Ademolu *et al.* (2004) fibre measured as crude fibre.

Data available on some species could be comparable with similar species used in this study, such as the giant African land snail (*Archachatina marginata*) (Ademolu *et al.* 2004), the palm weevil (*Rhynchophorus phoenicis*)

(Omotoso & Adedire, 2007), the American cockroach (*Periplaneta americana*), the house cricket *(Acheta domesticus*) and earthworms *(Lumbricus terrestrius*) (Barker *et al.*, 1998; Bernard & Allen, 1997).

Wild amphibians may often eat hundreds to thousands of prey daily and the invertebrate prey provided to amphibians in captivity rarely resemble their natural diets (McWilliams, 2008). The nutritional requirements of most amphibian species, throughout all life stages are poorly understood (Hadfield *et al.*, 2006; McWilliams, 2008; Pough, 1992).

Table 2.2 Macrominerals and trace minerals in selected whole invertebrates

Species	Ca (%)	P (%)	Mg (%)	Na (%)	K (%)	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
American cockroach *Periplaneta americana*[1]	0.20	0.50	0.08	0.27	0.87	14	90	57
Earthworm *Lumbricus terrestrius*[1]	1.52	0.96	0.16	0.44	0.87	9	1945	1119
Cricket (Adult) *Acheta domesticus*[1]	0.14	0.99	0.13	0.49	1.29	28	58	188
Mealworms *Tenebrio molitor*[1]	0.11	0.77	0.22	0.14	0.91	19	43	100
Fruit fly *Drosophila melanogaster*[1]	0.10	1.05	0.08	0.42	1.06	18	138	171

[1]Data from Bernard & Allen (1997)

The proximate nutritional composition of some invertebrates, commonly fed to amphibians, are given in table 2.1. The proximate compositions are variable with wide ranges (on dry matter basis) for dry matter (24.2-5.2%), crude fat (1.4-52.4%), crude protein (8.4-64.4%), crude fibre (0.1-21.8%) and ash (1.3-24.9). With the exception of earthworms *(Lumbricus terrestrius*), all the invertebrates given in table 2.2 have inverse Ca:P ratios.

General natural diets of insectivorous amphibians are given by McWilliams (2008) as consisting of 30% to 60% protein and 40% to 70% fat with negligible amounts of (all types) of carbohydrates. Hadfield *et al.* (2006) suggests a diet consisting of 50% protein, 45% fat, 5% carbohydrate and fibre with a positive Ca:P ratio for the purposes of short term nutritional support. The recommended dietary Ca:P ratio for vertebrate insectivores is 1.5:1 (Eidhoff *et al.*, 2006).

Table 2.3 Invertebrate species for analysis in this study

Scientific name	Common name
Gryllus bimaculatus	field cricket
Gryllus assimilis	brown cricket
Thermobia domestica	Firebrat
Blaptica dubia	Argentinean cockroach
Pachnoda marginata	fruit beetle (adult & larvae)
Callosobruchus maculates	bean weevil
Achatina fulica	giant African land snail (with & without shell)
Dendrobaena sp	compost worm
Drosophila melanogaster	vestigial winged fruit fly
Drosophila hydei	giant fruit fly
Trichorhina tomentosa	tropical woodlouse
Dermestes sp.	dermestial beetle larvae

Insectivores in the wild consume a variety of species of invertebrates, which, when consumed in the right proportions, provides a complete and balanced diet. In contrast, insectivores fed in captivity are likely fed only two or three species of insects (typically, crickets, mealworms, and wax worms) and so, when using a diet of cultured insects; nutrient deficiencies commonly occur (Finke & Winn, 2004). In order to prevent these deficiencies, Invertebrates are commonly supplemented; both internally and externally (Allen & Oftedal, 1989; Barker *et al.*, 1998; Browne, 2009; Eidhoff *et al.*, 2006; Wright & Whitaker, 2001). The 'dusting' of invertebrates with powdered vitamin and mineral supplements is widely advised (Browne, 2009; Donoghue, 1998; Pough, 1992) but generalisations about the quantities required are difficult to

formulate and may result in the occurrence of nutrient toxicities if overdone or deficiencies if underdone (Donoghue, 1998; McWilliams, 2008). An alternative method to dusting is to raise and maintain prey on a diet that provides them with complete nutrition, known as gut-loading, which increases the animals' nutrient content due to the food retained in their gastrointestinal tract (Allen & Oftedal, 1989; McWilliams, 2008). The diet of invertebrates has shown it can significantly impact upon their nutritional quality (Ademolu *et al.* 2004; Hatt *et al.*, 2003; Oonincx & van der Poel, 2009 and Wright & Whitaker, 2001) and as such this study will also investigate the effect of two different types of diet on the nutrient composition of the field cricket (*Gryllus bimaculatus*).

2.2 Proximate Analysis

Proximate analysis determines the total moisture, ash, crude fat, protein and fibre content of a food sample, given as the percentage composition of the sample (Self, 2005).

As most foods are relatively heterogeneous in their nature, it is important to ensure that, prior to compositional analysis, samples of food required for analysis are truly representative of the product to be analysed (James, 1999). The most commonly employed technique to prepare homogeneous samples given in the literature is the grinding of samples to approximate particle size of <1mm after the removal of moisture (Barker *et al.*, 1998; Hatt *et al.*, 2003).

2.2.1 Moisture Content

The moisture content of a sample is measured as the difference in mass after dehydration (Self, 2005). The mass after dehydration is the dry matter (DM) or total solids value. The two most commonly used drying techniques for invertebrate analyses are oven-drying (Banjo, 2006; Ramos-Elorduy *et al.*, 1997) and freeze-drying (Barker *et al.*, 1998; Bernard & Allen, 1997; Hatt *et al.*, 2003). It is well known that processing may affect the physical, chemical and/or biological characteristics of foodstuffs. Structural alterations and degradation of nutritional substances may occur (Ratti, 2003). Chan *et al.* (1997) compared the effects of sun-drying, oven-drying, and freeze-drying methods on the nutritional composition of the seaweed *Sargassum hemiphyllum.* Chan *et al.* (1997) found that although the fast drying rate that oven-drying allows preserved the ash and mineral contents, the high temperatures (60°C for 15hrs) used during the method caused the greatest nutrient losses among the three drying methods with freeze-drying providing the best nutritional quantity.

Freeze-drying or lyophilisation is based on the dehydration by sublimation of a frozen sample (Ratti, 2003). Due to the low temperatures required for freeze drying and the absence of liquid water, the majority of deterioration and microbiological reactions are stopped (Ratti, 2003). Vacuum freeze-drying is the best method of water removal with final products of highest quality when compared to other methods of food drying (Ratti, 2003). Freeze drying will be used to determine the moisture/dry matter content for this study.

2.2.2 Ash Content

The ash content is the mineral or inorganic residue left after high temperature combustion (>500°C) (James, 1999; Self, 2005) and is used as a crude measure of mineral content. The use of a muffle furnace to incinerate samples is an Association of Analytical Communities (AOAC, 1995) approved method and used commonly for proximate analysis of invertebrates (Ademolu *et al.*, 2004; Banjo, 2006; Barker *et al.*, 1998; Bernard & Allen, 1997; Finke, 2007 Omotoso & Adedire, 2007; Oonincx & van der Poel, 2009 and Ramos-Elorduy *et al.,* 1997). Samples will be incinerated in a muffle furnace to determine the ash content for this study.

2.2.3 Lipid Content

The determination of the fat content of a food almost invariably involves the estimation of the lipid fraction of the sample, not the true fat content (which makes up approximately 99% of the lipid fraction) (James, 1999).The most common method of lipid determination is by solvent extraction (Self, 2005). The Soxhlet method is universally applied and exhibits good accuracy and reproducibility (James, 1999). The use of petroleum ether as a solvent in the Soxhlet method is an approved AOAC (1995) method and used for proximate analysis of invertebrates by Banjo (2006), Barker *et al.* (1998), Omotoso & Adedire (2007) and Ramos-Elorduy *et al.* (1997). The lipid content of each species will be determined with Soxhlet extraction using petroleum ether.

2.2.4 Crude Protein Content

Crude protein content of samples is an estimate of protein based on the nitrogen content of a sample, crude protein is calculated by multiplying the nitrogen content by a conversion factor (Self, 2005). The most commonly used method for nitrogen determination among the literature for invertebrate proximate analysis is the Kjeldahl method (Banjo, 2006; Barker *et al.*, 1998; Bernard & Allen, 1997; Hatt *et al.,* 2003; Finke, 2007; Ramos-Elorduy *et al.,* 1997) and is AOAC approved (1995). The universality, high precision and good reproducibility of the Kjeldahl method make it the most widely used method for nitrogen determination (James, 1999). For invertebrate studies the conversion factor is usually 6.25 (Crude protein=nitrogen x 6.25) (Finke, 2002; Finke, 2007; Raksakantong *et al.*, 2009). Some authors however do not calculate the crude protein but instead give the value for total nitrogen (Barker *et al.*, 1998; Bernard & Allen, 1997; Hatt *et al.,* 2003). This is because it is widely thought that large amounts of non-protein nitrogen may be contributed by chitin (amino-cellulose). As such, estimating protein by nitrogen x 6.25 may result in an overestimate of the true protein content. Finke (2007) analysed a number of invertebrate species to estimate the amount of chitin they contained. It was found that the quantity of chitin nitrogen (as a percentage of total nitrogen) is actually small and that a conversion factor of nitrogen x 6.25 does provide a reasonable estimate of total protein in invertebrates. Nitrogen content will be determined using the Kjeldahl method and crude protein calculated using a conversion factor of 6.25.

2.2.5 Fibre Content

The crude fibre content is a measure of the structural carbohydrates in a sample (Banjo, 2006; Ramos-Elorduy *et al.,* 1997). The amounts of fibre in insects are measured using acid detergent fibre (ADF) and neutral detergent fibre (NDF) (Finke, 2007). ADF is composed typically of cellulose and lignin and NDF is composed of cellulose, lignin and hemicellulose, as such NDF is considered to represent the entire dietary fibre fraction of the feed (Van Soest, 1991). NDF was used as a measure of dietary fibre of invertebrates by Barker *et al.* (1996).

2.2.6 Mineral Analysis

Various methods have been used in the literature to analyse the mineral compositions of invertebrates such as atomic absorption spectrometry (AAS) (Ademolu *et al.*, 2004; Barker *et al.*, 1998; Bernard & Allen, 1997), atomic emission spectrometry (AES) (Bernard & Allen, 1997), flame photometry (FP) (Omotoso & Adedire, 2007) and inductively coupled plasma optical emission spectrometry (ICP-OES) (Oonincx & van der Poel, 2009). Oonincx & van der Poel (2009) used ICP-OES to simultaneously determine the elements Ca, K, Mg, Na, P, Cu, Fe, and Zn. These are the same elements required by Durrell. ICP-OES is a powerful tool for fast, sensitive, multi-element analysis that is relatively free of chemical interferences and can analyse over ranges of widely varying concentrations (Duxbury, 2003). Aqua regia digestion (digestion of samples in nitric acid) was used by Mitchell (2007) to prepare bone, soil and plant samples for ICP-OES and also by Duxbury (2003) for the preparation of apple samples for ICP-OES. This method of sample

preparation does not require the use of ashed samples. This method will be used in this study to prepare the samples for ICP-OES analysis.

3. MATERIALS AND METHODS

Proximate analysis and preparation of samples for ICP-OES analysis was carried out at Brackenhurst Campus, NTU. The ICP-OES was carried out at Clifton Campus, NTU.

3.1 Species for Analysis

All invertebrates for this study were provided by Durrell Wildlife Conservation Trust in Jersey, UK. The diets and other available husbandry information for the sampled invertebrates are found in Table 3.1.

Table 3.1 Husbandry information of species for analysis (from Tapley, pers. com)

Species	Husbandry information
field cricket (control)	Crickets frozen as soon as they arrive from supplier. Fed just oats for at least 24 hrs.
field cricket (diet A)	Fed on standard feeding regime (carrot, potato, orange and cat pellet).
field cricket (diet B)	Fed on tadpole food.
brown cricket	Fed on slices of potato, orange, carrot and herbivore pellet.
firebrat	Housed on egg cartons. Fed ready brek and fish flake, at 40°C.
Argentinean cockroach	Fed on slices of potato, orange, carrot and herbivore pellet.
fruit beetle adult & larvae	Housed on substrate of organic peat free compost. Sweet chestnut leaves and decaying wood are mixed in with the substrate. Fed banana, papaya, potato, orange apple and carrot. At temp 25-30°C.
bean weevil	Housed in jars. Fed a diet of black eyed peas. Temp 25° C.
giant African land snail	Housed on organic peat free compost. Fed a mix (apple, pear, papaya, cucumber, carrot, potato, tomato, oranges, lettuce and Chicory). They are also provided with cuttlefish as additional source of calcium. Temp approx 25°C.
compost worm	Housed in bins filled with organic peat free compost. Fed leaf litter, mango and a worm mix (potato, apple, carrot, powdered herbivore pellet and the dietary supplement nutrobal). Temp range from 19 - 23°C.
vestigial winged fruit fly	Fed our fruit mix (banana, apple, pear, oats, carrots, vinegar, yeast and the vitamin supplement nutrobal) and commercial powder mix. Temps range from 24 - 26 °C.
giant fruit fly	As above
tropical woodlouse	Housed on a substrate of neopeat. Fed ready brek and our tadpole diet Temp 25° C.
dermestial beetle larvae	Taken from bag of crickets delivered to Durrell, feeding on crickets (dead or alive).

The control cricket group were frozen as soon as they arrived at Durrell from their supplier and were fed on just oats for at least 24 hrs prior to arrival. Diet A is the standard cricket diet used at Durrell and diet B is the standard tadpole

diet used at Durrell. The cricket diet consists of carrot, potato, orange and cat pellet and the tadpole diet consists of calcium powder, fish flake, ground river shrimp, ground tubifex, and spirulina algae (Tapley, pers. com). Samples were live-frozen at Jersey (ca -20°C) and sent by courier to Brackenhurst.

3.2 Proximate Analysis

Due to time constraints of the project and to ensure as many species are analysed for as many proximate components as possible, a single analysis of each proximate component for each species was performed. A limited supply of dermestial beetle larvae, tropical woodlouse and both fruit fly species meant that a smaller amount of each of these four species was used for each analysis and that protein analysis could not be carried out carried out. The protein analysis for the giant African land snail and compost worm and fibre analysis for all species was incomplete at the time of writing this report. Details of each sample are found in Table 3.2.

Table 3.2 Sample details and ID

Sample ID	Sample details
A	field cricket – control
B	field cricket – diet A
C	field cricket – diet B
D	brown cricket
E	firebrat
F	Argentinean cockroach
G	fruit beetle - adult
H	fruit beetle - larvae
I	bean weevil
J	giant African land snail – with shell
K	giant African land snail - without shell
L	compost worm
M	vestigial winged fruit fly
N	giant fruit fly
O	tropical woodlouse
P	dermestial beetle larvae

3.2.1 Moisture content

Method

Each of the 16 samples was partially thawed at room temperature and subsamples were weighed into also weighed 60ml sample pots. The total weight was recorded. Each pot was covered with parafilm, which was then pierced to allow any water to escape. The samples were placed in a vacuum freeze dryer (Lyotrap) and freeze-dried to constant weight (approx. 7 days). The total dry weight was recorded and proximate moisture content and DM calculated using the following equations:

Where, pot weight is A, sample weight is B, total weight is C, total dry weight is D and dry sample weight is E (E = D-A).

%DM = E/Bx100
%Moisture = 100-%DM

After freeze-drying samples were ground using a coffee grinder and passed through a 1mm screen. In between analyses all samples were kept in sealed sample pots in a dessicator until required.

3.2.2 Ash Content

Method

Individual ceramic crucibles were marked and their weight recorded. A subsample (between 0.5g-1.2g for both fruit fly species, tropical woodlouse and dermestial beetle larvae and 2.4g-5.2g for the rest) of each sample was accurately weighed into a crucible and the total weight recorded. The samples were then incinerated at 650°C in a muffle furnace. The total weight of each

crucible after ashing was recorded and proximate ash content calculated using the following equation:

Where, crucible weight is A, sample weight is B, total weight is C, total ashed weight is D and ashed sample weight is E (E = D-A).

%Ash = E/Bx100

3.2.3 Lipid Content

Chemicals

Petroleum ether 40%/60% (see Appendix for Risk assessment and COSHH)

Method

A subsample of each sample (0.3g-1.4g for L, M, N, O and P and 2.4g-5g for the rest) was accurately weighed and recorded and placed into an extraction thimble. A few anti-bumping granules were placed into a 250ml round bottom, ground neck flask and the weight was recorded. 150ml of petroleum ether was added to the flask in the fume cupboard. The flask was then assembled into the Soxhlet apparatus and extracted for 4½ hours. This was repeated for each sample. Each flask was then placed into a fume cupboard and any remaining solvent left to evaporate. The total weight of flask, granules and extracted fat was recorded and the proximate lipid content calculated from the following equation:

Where flask and granules weight is A, sample weight is B and flask, granules and extracted fat weight is C.

$$\% \text{ Lipid} = \frac{(C-A)}{B} \times 100$$

3.2.4 Crude Protein Content

Chemicals

Nitrogen-free Sulphuric acid 90-98% (see Appendix for Risk assessment and COSHH)

Hydrochloric acid 1M (see Appendix for Risk assessment and COSHH)

4% Boric Acid Solution with Indicator (see Appendix for Risk assessment and COSHH)

Kjeltab catalysts

Method

A subsample (0.5) of each sample was weighed and placed in a digestion tube. For each set of 6 digestion tubes one was prepared as a blank, with 0.1g of starch. One each of the two different (copper and selenium) Kjeltab catalyst tablets was placed in each tube. 12.5mls of Nitrogen-free Sulphuric acid was then added to each tube. The tubes were placed in the preheated digester (450°C) and the heat shields and exhaust caps fitted. After 45 minutes digesting the tubes were removed from the heater and left to cool. 75ml of distilled water was carefully added to each tube. Each tube and a corresponding conical flask, with 25ml of 4% boric acid solution with indicator in was placed into the Foss 2100 Kjeltec Distillation Unit and distillation cycle initiated. After distillation, 1M hydrochloric acid was titrated against the boric acid in the conical flask. The amount of acid used was recorded and the nitrogen and crude protein content was calculated using the following equations:

Where weight of sample is A, volume of acid used in sample titration (ml) is B, volume of acid used in blank titration (ml) is C and molarity of titration acid (M) is D.

$$\%\text{Nitrogen} = \frac{1.4x(B-C)xM}{A}$$

% Crude Protein = %Nitrogen x 6.25

3.3 Mineral Analysis

3.3.1 Aqua Regia Digestion

Chemicals

Concentrated Hydrochloric Acid 32% (see Appendix for Risk assessment and COSHH)

Concentrated Nitric Acid 70% (see Appendix for Risk assessment and COSHH)

Method

A subsample of each sample (0.5g-1g) was accurately weighed into 50ml conical flasks. Each sample was prepared in triplicate. The aqua regia was made up by adding 50ml of 70% nitric acid to150ml of 32% hydrochloric acid. 10 ml of the aqua regia solution was added to each flask and left overnight in a fume cupboard. The samples were then heated on a hotplate for approximately 1½ hours. During this time the flasks were carefully rearranged every 30 minutes to ensure any variability of the temperature of the hotplate did not affect the final results. After the initial 1½ hours of heating, the samples were left to cool for approximately 1 hour. A further 5ml of aqua regia was added and the samples were boiled again for 30 minutes and then cooled. Once cooled the samples were filtered through Fisherbrand paper (FB59311) into 50ml volumetric flasks, using ultra pure water to flush out the conical flasks. Once filtered each volumetric flask was made up to 50 ml with

ultra pure water. Each of these flasks was decanted into 3x15ml tubes ready for loading into the ICP-OES machine.

Blank samples were prepared using ultrapure water. The standards solution was a mix of two standards;100mg/l multi-element for Ca Mg Fe Na K Zn & Cu (Fisher Scientific, Primar Ms, ISO 9001) and 1000mg/l phosphorus (Spex Certiprep) and made up to concentrations of 25ppm (12.5ml multi-element + 1.25 phosphorus in 50ml), 20ppm (10ml multi-element + 1 ml phosphorus in 100ml), (7.5ml multi-element + 0.75 ml phosphorus in 50ml) and 5ppm (2.5ml multi-element + 0.25 ml phosphorus in 50ml).

4. RESULTS

4.1 Proximate Analysis

The results of the proximate analysis are given in Table 4.1. All raw data can be found in appendix I. As the proximate data was performed using single analyses (except for DM) and as such, this exploratory data does not require statistical analysis. Mineral content analysis data are the results of a single analysis.

Table 4.1 Proximate composition of invertebrates on dry matter basis (DMB)

Species	DM (%) mean± SD	Crude Fat (%DMB)	Crude Protein (%DMB)	Ash (%DMB)
field cricket – control	27.58 ±0.56	23.69	68.25	4.32
field cricket – normal diet	29.66 ±2.91	22.61	66.50	5.66
field cricket - tadpole diet	31.80 ±0.96	32.20	50.75	3.75
brown cricket	27.73 ±1.32	23.44	62.48	4.06
firebrat	30.65 ±0.61	22.81	73.50	4.96
Argentinean cockroach	40.37 ±2.47	34.88	54.25	3.91
fruit beetle - adult	45.69 ±0.91	30.80	50.75	2.63
fruit beetle - larvae	23.41 ±1.82	16.24	45.50	14.13
bean weevil	50.08 ±2.71	32.24	71.25	1.88
giant African land snail – with shell	30.92 ±1.48	3.25	N/D	52.62
giant African land snail - without shell	21.34 ±1.43	6.75	40.25	10.12
compost worm	14.76 ±0.71	8.02	N/D	9.41
vestigial winged fruit fly	29.59 ±0.08	14.50	N/D	4.80
giant fruit fly	29.48 ±0.68	20.59	N/D	3.34
tropical woodlouse	44.36 ±0.45	25.00	N/D	18.07
dermestial beetle larvae	36.20 ±0.07	29.90	N/D	3.02

N/D- Not determined

The dry matter component of each sample was variable, from 14.76% (earthworm) to 50.80% (bean weevil). The highest DM value of the field crickets was 31.80% (Diet B). The proximate composition of the field crickets, the control group and the groups fed diet A and B are represented graphically in figure 4.1.

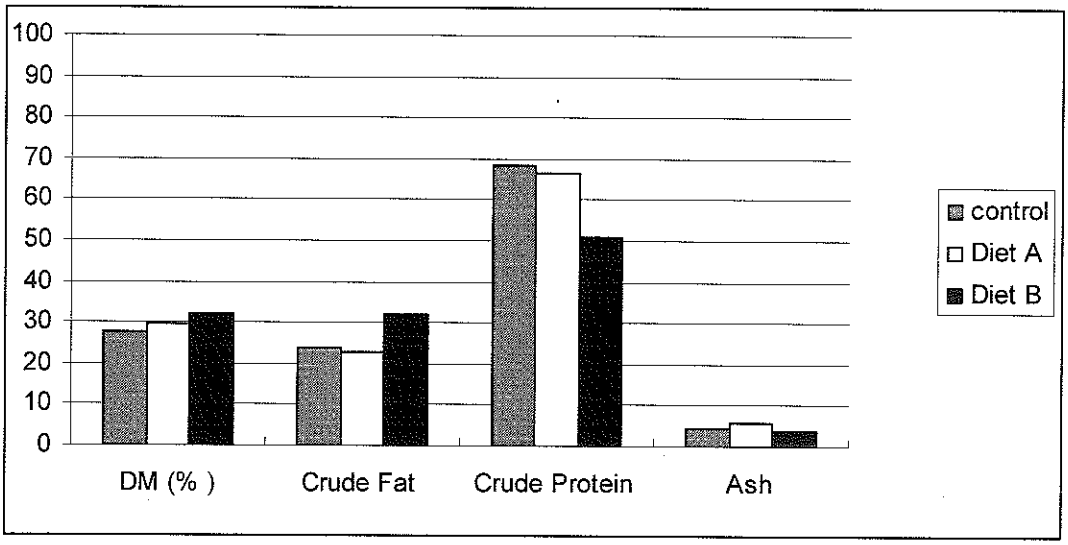

Figure 4.1 Proximate compositions of dry matter, fat, protein and ash of *Gryllus bimaculatus* fed on 3 different diets

The lipid content of the invertebrates sampled was variable with the lowest value of 3.25% (giant African land snail, with shell) and highest value of 34.88% (Argentinean cockroach DMB). Of the crickets the highest amount of fat was found in the crickets fed diet B (32.20% DMB) then the control group (23.69%) with the lowest amount found in the group fed diet A (22.61% DMB). Crude protein was the major component of all of the species it was analysed for in this study (>40% DMB). The lowest protein composition (40.25% DMB) was found in the giant African land snail (without shell) and the highest protein composition (73.5% DMB) was found in the firebrat. Ash values ranged from 1.88% DMB (bean weevil) to 52.62% DMB (giant African land snail, with shell). Amongst the field cricket groups, the group fed diet A had the highest

ash content (5.66%) and the lowest ash content was found in those fed diet B (3.75%).

5. DISCUSSION

The aim of this study was to determine the proximate and mineral compositions of field crickets fed on two different diets and of the other invertebrates used as prey for the captive amphibian populations at Durrell Wildlife Conservation Trust. As the proximate analysis data are the result of single analyses no significance testing between diets was performed. Unfortunately the results for the mineral analysis were outstanding at the time of writing this report.

The proximate composition of invertebrates varies greatly from species to species. The dry matter content of invertebrates found in the literature, ranges from 9.9% DM for bloodworm, *Chironomus* sp. (Bernard & Allen, 1997) to 95.2% DM for the palm weevil (Omotoso & Adedire, 2007). The dry matter (DM) content of the bean weevil as determined in this study (50.08%) is much lower than that of the palm weevil reported by Omotoso & Adedire (2007). The DM content of field and brown crickets in this study (27.58%-31.80%) is similar to that reported for the house cricket (*Acheta domesticus*), 26.8%-31% (Barker *et al.*, 1998; Bernard & Allen, 1997). The DM content of the Argentinean cockroach (40.37%) is similar to the value given for the American cockroach (38.7%) by Bernard & Allen (1997). Ademolu *et al.* (2004) studied the effect of diet on the proximate composition of snails (without shells). In this study DM content ranged from 17.7%-27.2%. This compares well with the DM content of 21.34% determined for snails (without shells). As it would be expected the DM content of snails analysed with shells was higher at 30.92%. The compost worm had the least amount of DM (14.76%) of all the

invertebrates analysed with 14.76%. This value is much lower than the 24.2% DM content of the earthworm as given by Barker *et al.* (1998) and the 20% DM content as given by Bernard & Allen (1997). The two species of fruit fly have very similar DM contents at 29.59% (Vestigial winged fruit fly, *Drosophila melanogaster*) and 29.48% (Giant fruit fly, *Drosophila hydei*). These values are similar to that reported for *Drosophila melanogaster,* 32.9% by Barker *et al.* (1998) and 29.6% by Bernard & Allen (1997). The DM content of the adult fruit beetle is considerably higher (45.69%) than that of the soft bodied larval form (23.41%) and is most likely caused by the large exoskeleton of the adult beetle. The remaining species with which no suitable comparisons that can be found in the literature are the firebrat (30.65% DM), tropical woodlouse (44.36% DM) and dermestial beetle larvae (36.20% DM).

The lipid content of the field crickets was highest (32.20%) in the group fed diet B. The control group (23.69%) and the group fed diet A (22.61%) had a similar lipid content to that of the brown cricket, (23.44%). These values compare favourably with those of the house cricket, 22.8% DMB given by Barker *et al.* (1998) but are higher than those reported for the same species (*Acheta domesticus*) of 13.8% DMB by Bernard & Allen (1997). The lipid content of the Argentinean cockroach (34.88% DMB) is slightly higher than the value given for the American cockroach (28.4%) by Bernard & Allen (1997). Larval or nymph forms of invertebrates usually have higher lipid content than their adult forms (Bernard & Allen, 1997; Finke, 2002; Finke, 2007 and Omotoso & Adedire, 2007). This was not found to be the case for the fruit beetle in this study. The adult beetle had a lipid content of 30.80%

DMB, nearly double the content of the larval form (16.24% DMB). Analysis of the lipid content of the giant African land snails without the shell predictably resulted in a higher value than that of the snail with the shell (6.75% DMB without shell, 3.75% DMB with shell). The lipid content of the snails as determined in this study is higher than that found by Ademolu *et al.* (2004). Using a similar species (*Archachatina marginata*), Ademolu *et al.* (2004) reported lipid contents as ranging from 1.18%-1.70% DMB. The bean weevil's lipid content (32.24% DMB) compares well with that of 31.4% DMB given by Banjo (2006) for the palm weevil (*Rhynchophorus phoenicis*) and is substantially lower than the 52.4% DMB reported by Omotoso & Adedire (2007) for the same species. The compost worm had a lipid content of 8.02% DMB and this lower than the values reported for earthworms by Barker *et al.,* (1998) of 10.6% DMB and of 17.7% DMB Bernard & Allen (1997). The fruit fly species had different lipid contents with the most found in the giant fruit, 20.59% DMB. The vestigial winged fruit fly had a lipid content of 14.50% DMB, this was only slightly higher than the 12.6% DMB reported by Bernard & Allen (1997) and slightly lower than 17.9% DMB reported by Barker *et al.,* (1998). The remaining species, firebrat, tropical woodlouse and dermestial beetle larvae had lipid contents of 22.81% DMB, 25.00% DMB and 29.90% DMB respectively.

Crude protein was fairly high in all species it was analysed for. Of the field cricket groups perhaps surprisingly, given the known positive impacts diet can have on nutritional composition (Ademolu *et al.* 2004; Hatt *et al.*, 2003 and Oonincx & van der Poel, 2009) the control group had the highest protein value

of 68.25% DMB. Slightly lower was the group fed diet A (66.50% DMB) and the group fed diet B had substantially lower value with a crude protein content of 50.75% DMB. The brown cricket had a crude protein content of 62.48% DMB. These values compare well with the values given by Bernard & Allen (1997) and Barker *et al.,* (1998) of 64.9% DMB and 64.4%DMB respectively for the house cricket. The snail (without shell) had a crude protein content of 40.25% DMB which is lower than the values reported by Ademolu *et al.* (2004). In that study, crude protein values of snails ranged from 47.54% to 87.94% DMB. The adult fruit beetle had a slightly higher crude protein content than the larval form (adult 50.75% and larva 45.50% DMB). The crude protein content of the Argentinean cockroach was 54.25% DMB and this is similar to that reported for the American cockroach, 53.9% DMB (Bernard & Allen, 1997). The crude protein content of the bean weevil (71.75% DMB) was substantially higher than the values reported for the palm weevil by Banjo (2006) and Omotoso & Adedire (2007) which are 28.4% and 8.4% DMB respectively. The firebrat had the highest protein content with 73.50% DMB.

Of the field cricket groups the highest ash content was found in the group fed diet A (5.66% DMB). The control group had ash content of 4.32% DMB and the lowest ash content was found in the group fed diet B. The brown cricket had an ash content of 4.06% DMB. These values compare favourably with the ash values given by Bernard & Allen (1997) and Barker *et al.,* (1998) of 5.7% DMB and 5.1% DMB respectively for the house cricket. The ash content of the Argentinean cockroach was 3.91% DMB and this is only slightly higher than that reported for the American cockroach, 3.3% DMB (Bernard & Allen, 1997).

The ash content of the fruit beetle larvae (14.13% DMB) was considerably higher than that found in the adult beetle (2.63% DMB) and deserves further investigation. The ash content of the bean weevil (1.88% DMB) fell in between the values reported for the palm weevil by Banjo (2006) and Omotoso & Adedire (2007) which are 2.7% and 1.4% DMB respectively. The ash content of the snails analysed with shells was considerably higher (52.62% DMB) than that of the snails analysed without shells (10.12% DMB). This is to be expected as the shell of air breathing land snails is composed of approximately 97% calcium carbonate (by weight) (Heller & Magaritz, 1983). The ash content of the compost worm was fairly high at 9.41% DMB. This was nearly twice the amount given for earthworms (5.0% DMB) by Bernard & Allen (1997) yet it is much lower than the 24.9% DMB given for earthworms by Barker *et al.,* (1998). Of the fruit flies, the vestigial winged fruit fly had the highest ash content with 4.8% DMB. This compares well the values for ash given by Bernard & Allen (1997) and Barker *et al.,* (1998) of 4.5% DMB and 5.2% DMB respectively for the vestigial winged fruit fly. The giant fruit fly had an ash content of 3.34% DMB. The tropical woodlouse had a high ash content of 18.07% DMB and such a high mineral content warrants further investigation.

The proximate compositions of each of the field cricket groups are shown in figure 4.1. From this graph it can be seen that neither diet A nor diet B resulted in an increase of all the nutrients analysed when compared with the control group. The DM content was higher in the groups fed diet A and B. The crickets fed diet A had a lower lipid and crude protein content and higher ash

content than the control group. The crickets fed on diet B had a higher lipid content and lower crude protein and ash content than the control group. However, due to the use of a single analysis for the proximate compositions no testing of the significance of these differences could be carried. Although the methods used are universally accepted as standard methods for determining proximate compositions of all types of food this cannot rule out the possibility of inaccurate or anomalous results that may occur as a result of a single analysis. Although not touched upon in this study, a more complete nutritional analysis should also perhaps determine vitamin concentrations.

5. CONCLUSION

Overall the results compared well with the available data. The use of a single analysis to determine the proximate compositions of the invertebrates supplied by Durrell was the major limitation of this study. This meant that no statistical significances between the field cricket diets could be determined. The results indicate that the diets fed to the field cricket does impact on the nutritional value of the cricket. They also show however that neither diet caused an increase in all the nutrients analysed, with diet A increasing the ash content and diet B increasing the lipid content. Interestingly the control group had higher protein content than both of the other two groups.

In the absence of species specific information regarding nutritional requirements, formulating appropriate amphibian diets is extremely difficult. Successful husbandry requires integrating knowledge of basic biological characteristics with a sound understanding of the ecology and behaviour of a species.

Although mineral analysis results are not included in this report it is hoped that the information provided in this study is still useful when formulating the diets of not just the amphibian but all the insectivores kept at Durrell. Knowledge of the nutritional composition of live invertebrate prey can prove useful with regard to identifying any possible nutrient excesses or deficiencies before they occur. The constant messages found in the literature concerning the use of live invertebrate prey are that they should be supplemented to counteract

known deficiencies (in particular calcium) and that the provision of a variety of prey species is essential.

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APPENDIX I
Data-Dry Matter

id	pot wt	sample wt	tot. wt	tot. dry wt	DM	%DM	Av. %DM	St. dev.	% Water
A1	6.395	10.362	16.757	9.199	2.804	27.060	27.580	0.560	72.940
A2	6.412	10.189	16.601	9.221	2.809	27.569			72.431
A3	6.395	11.327	17.722	9.488	3.093	27.306			72.694
A4	6.395	10.325	16.720	9.319	2.924	28.320			71.680
A5	6.395	11.188	17.583	9.428	3.033	27.109			72.891
A6	6.395	12.004	18.399	9.708	3.313	27.599			72.401
A1	6.412	15.313	21.725	10.780	4.368	28.525			71.475
A2	6.357	17.164	23.521	11.017	4.660	27.150			72.850
B1	6.395	11.967	18.362	10.137	3.742	31.269	29.660	2.906	68.731
B2	6.395	11.272	17.667	9.884	3.489	30.953			69.047
B3	6.395	10.483	16.878	9.650	3.255	31.050			68.950
B4	6.403	11.993	18.396	9.970	3.567	29.742			70.258
B5	6.395	10.578	16.973	9.667	3.272	30.932			69.068
B1	6.404	21.402	27.806	11.236	4.832	22.577			77.423
B2	6.403	14.747	21.150	10.850	4.447	30.155			69.845
B3	6.401	10.411	16.812	9.587	3.186	30.602			69.398
C1	6.395	13.019	19.414	10.572	4.177	32.084	31.800	0.958	67.916
C2	6.377	11.839	18.216	10.067	3.690	31.168			68.832
C3	6.352	11.906	18.258	10.258	3.906	32.807			67.193
C4	6.395	12.377	18.772	10.405	4.010	32.399			67.601
C5	6.395	13.484	19.879	10.615	4.220	31.296			68.704
C1	6.352	15.290	21.642	11.347	4.995	32.668			67.332
C2	6.377	18.917	25.294	12.085	5.708	30.174			69.826
D1	6.395	13.109	19.504	9.836	3.441	26.249	27.728	1.323	73.751
D2	6.393	10.391	16.784	9.230	2.837	27.302			72.698
D3	6.408	11.678	18.086	9.478	3.070	26.289			73.711
D4	6.374	12.465	18.839	9.912	3.538	28.383			71.617
D1	6.374	14.583	20.957	10.360	3.986	27.333			72.667
D2	6.393	14.574	20.967	10.747	4.354	29.875			70.125
D3	6.408	19.084	25.492	11.878	5.470	28.663			71.337
E1	6.395	10.339	16.734	9.521	3.126	30.235	30.645	0.610	69.765
E2	6.395	10.392	16.787	9.566	3.171	30.514			69.486
E3	6.395	11.041	17.436	9.907	3.512	31.809			68.191
E4	6.402	12.568	18.970	10.195	3.793	30.180			69.820
E5	6.354	14.734	21.088	10.787	4.433	30.087			69.913
E1	6.354	14.553	20.907	10.873	4.519	31.052			68.948
E2	6.402	18.083	24.485	11.943	5.541	30.642			69.358
F1	6.395	10.388	16.783	10.499	4.104	39.507	40.372	2.468	60.493
F2	6.397	12.739	19.136	11.619	5.222	40.992			59.008

F3	6.398	9.311	15.709	10.222	3.824	41.070			58.930
F4	6.398	12.729	19.127	11.355	4.957	38.943			61.057
F1	6.389	18.514	24.903	13.539	7.150	38.619			61.381
F2	6.390	19.806	26.196	15.372	8.982	45.350			54.650
F3	6.429	14.696	21.125	12.032	5.603	38.126			61.874
G1	6.395	12.335	18.730	11.907	5.512	44.686	45.688	0.914	55.314
G2	6.397	12.217	18.614	12.009	5.612	45.936			54.064
G3	6.395	12.337	18.732	12.115	5.720	46.365			53.635
G4	6.419	12.513	18.932	12.099	5.680	45.393			54.607
G1	6.405	13.279	19.684	12.315	5.910	44.506			55.494
G2	6.397	13.261	19.658	12.475	6.078	45.834			54.166
G3	6.416	16.077	22.493	13.988	7.572	47.098			52.902
H1	6.395	14.040	20.435	9.516	3.121	22.229	23.412	1.823	77.771
H2	6.361	12.713	19.074	9.310	2.949	23.197			76.803
H3	6.395	14.937	21.332	10.018	3.623	24.255			75.745
H4	6.402	14.124	20.526	9.390	2.988	21.155			78.845
H1	6.416	19.247	25.663	10.876	4.460	23.172			76.828
H2	6.416	19.415	25.831	11.554	5.138	26.464			73.536
I1	6.395	9.286	15.681	11.186	4.791	51.594	50.084	2.711	48.406
I2	6.395	11.984	18.379	12.211	5.816	48.531			51.469
I3	6.351	11.146	17.497	11.781	5.430	48.717			51.283
I4	6.429	9.862	16.291	11.173	4.744	48.104			51.896
I5	6.395	10.374	16.769	11.384	4.989	48.091			51.909
I1	6.349	15.279	21.628	14.833	8.484	55.527			44.473
I2	6.429	17.026	23.455	14.946	8.517	50.023			49.977
J1	6.395	18.653	25.048	11.781	5.386	28.875	30.915	1.481	71.125
J2	6.414	20.480	26.894	12.815	6.401	31.255			68.745
J3	6.389	17.854	24.243	11.785	5.396	30.223			69.777
J1	6.414	21.104	27.518	13.351	6.937	32.871			67.129
J2	6.390	20.962	27.352	12.962	6.572	31.352			68.648
K1	6.395	12.271	18.666	8.981	2.586	21.074	21.338	1.426	78.926
K2	6.395	17.311	23.706	9.807	3.412	19.710			80.290
K3	6.395	18.588	24.983	10.175	3.780	20.336			79.664
K4	6.377	20.534	26.911	11.123	4.746	23.113			76.887
K1	6.389	9.413	15.802	8.503	2.114	22.458			77.542
L1	6.395	12.551	18.946	8.341	1.946	15.505	14.764	0.713	84.495
L2	6.395	12.640	19.035	8.175	1.780	14.082			85.918
L3	6.395	11.689	18.084	8.204	1.809	15.476			84.524
L4	6.395	11.112	17.507	7.994	1.599	14.390			85.610
L5	6.372	12.068	18.440	8.206	1.834	15.197			84.803
L1	6.378	24.352	30.730	9.771	3.393	13.933			86.067
M1	6.377	2.183	8.560	7.024	0.647	29.638	29.585	0.076	70.362

M2	6.377	1.920	8.297	6.944	0.567	29.531			70.469
N1	6.395	5.160	11.555	7.891	1.496	28.992	29.476	0.684	71.008
N2	6.395	3.418	9.813	7.419	1.024	29.959			70.041
O1	6.391	2.677	9.068	7.570	1.179	44.042	44.362	0.453	55.958
O2	6.391	2.012	8.403	7.290	0.899	44.682			55.318
P1	6.401	2.676	9.077	7.371	0.970	36.248	36.198	0.071	63.752
P2	6.401	2.534	8.935	7.317	0.916	36.148			63.852

Data-Ash

Sample id	pot wt	sample wt	tot. wt	tot. wt after ashing	sample wt after ashing	% Ash
A	32.050	4.167	36.217	32.230	0.180	4.320
B	34.750	3.200	37.950	34.931	0.181	5.656
C	34.946	2.772	37.718	35.050	0.104	3.752
D	34.717	2.608	37.325	34.823	0.106	4.064
E	33.829	3.894	37.723	34.022	0.193	4.956
F	29.337	3.889	33.226	29.489	0.152	3.908
G	34.431	5.125	39.556	34.566	0.135	2.634
H	35.587	3.552	39.139	36.089	0.502	14.133
I	35.566	3.825	39.391	35.638	0.072	1.882
J	36.475	4.814	41.289	39.008	2.533	52.617
K	32.209	2.658	34.867	32.478	0.269	10.120
L	37.322	2.498	39.820	37.557	0.235	9.408
M	16.819	0.584	17.403	16.847	0.028	4.795
N	17.346	0.779	18.125	17.372	0.026	3.338
O	15.083	1.140	16.223	15.289	0.206	18.070
P	17.976	0.926	18.902	18.004	0.028	3.024

Data-Lipid

Sample id	flask+granules	sample	total wt	total wt after extraction	% crude fat
A	100.074	3.571	103.645	100.920	23.691
B	94.440	4.104	98.544	95.368	22.612
C	92.592	3.317	95.909	93.660	32.198
D	88.387	3.063	91.450	89.105	23.441
E	94.533	2.679	97.212	95.144	22.807
F	102.725	3.804	106.529	104.052	34.884
G	99.781	4.286	104.067	101.101	30.798
H	99.993	4.416	104.409	100.710	16.236
I	94.733	4.191	98.924	96.084	32.236
J	89.608	4.921	94.529	89.768	3.251
K	90.067	2.473	92.540	90.234	6.753
L	88.270	1.372	89.642	88.380	8.017

M		90.688	0.269	90.957	90.727	14.498
N		91.397	0.709	92.106	91.543	20.592
O		91.301	0.316	91.617	91.380	25.000
P		93.525	0.398	93.923	93.644	29.899

Data-Protein

ID	sample wt (g)	vol of acid used in sample titration (ml)	vol of acid used in blank titration (ml)	molarity of titration acid (M)	% N	% crude protein
A	0.5	4.1	0.2	1	10.92	68.25
B	0.5	4	0.2	1	10.64	66.5
C	0.5	3.1	0.2	1	8.12	50.75
D	0.5	35.9	0.2	0.1	9.996	62.475
E	0.5	4.4	0.2	1	11.76	73.5
F	0.5	3.3	0.2	1	8.68	54.25
G	0.5	3.1	0.2	1	8.12	50.75
H	0.5	2.8	0.2	1	7.28	45.5
I	0.5	4.3	0.2	1	11.48	71.75
J						
K	0.5	2.5	0.2	1	6.44	40.25
L						
M						
N						
O						
P						

APPENDIX II
Risk Assessments & COSHH

Soxhlet Extraction

Activity	Hazard	Control
Soxhlet extraction; use of glassware; extraction apparatus, anti-bumping granules, flasks	Breakage/injury	Wear safety glasses and nitrile gloves. Take care when handling glassware
Adding pet ether to flask	Chemical;irritant,flammable, harmful vapours.	Pet ether added in fume hood. Wear safety glasses and nitrile gloves. COSHH assessment available for pet ether.
Heating in Soxhlet apparatus	Burning	Ensure correct assembly of apparatus. Take care when handling apparatus
Heating on hotplate to evaporate pet ether	Chemical;irritant,flammable, harmful vapours. Risk of burns: hotplate	Pet ether evaporated in fume hood. Wear safety glasses and nitrile gloves. COSHH assessment available for pet ether.

Soxhlet Extraction
COSHH Assessment Form

Department:	**ARES**	*Assessment Date:*	**22**	***01***	***10***
Location/Building:	*Labs Brackenhurst*	*Review Dates:*			
Room Number:		*Ref:*			

Activity *Soxhlet extraction*

Substances Used (If Biological Agent include classification)	*Hazard**	*By***	*WEL*	*Storage Unit Quantity*	*Amount in Use*
Petroleum Ether 40/60%	**R11**;Highly flammable, **R38**;Irritating to skin **R51**;Toxic to aquatic organisms. **R53**; May cause long-term adverse effects in the aquatic environment. **R65**; Harmful: may cause lung damage if swallowed. **R67**; Vapours may cause drowsiness and dizziness. **XN**;Harmful. **F**;Highly Flammable **N**;Dangerous to the environment	Inhalation Ingestion Contact	**TWA:** 600 ppm VLE; 1796 mg/m3 VLE **STEL:** 750 ppm VLE; 2242 mg/m3 VLE		

** Very toxic, corrosive, harmful, irritant....etc.*
***Inhalation, Ingestion, Contact.*
WEL – Workplace Exposure Limit

Health Effects

Eye: Causes eye irritation.
Skin: Prolonged and/or repeated contact may cause irritation and/or dermatitis. Exposure may cause irritation characterized by redness, dryness, and inflammation.
Ingestion: Aspiration hazard. Causes gastrointestinal irritation with nausea, vomiting and diarrhoea. May cause central nervous system depression, characterized by excitement, followed by headache, dizziness, drowsiness, and nausea. Advanced stages may cause collapse, unconsciousness, coma and possible death due to respiratory failure. Aspiration of material into the lungs may cause chemical pneumonitis, which may be fatal.
Inhalation: Inhalation of high concentrations may cause central nervous system effects characterized by nausea, headache, dizziness, unconsciousness and coma. High vapour concentrations may cause drowsiness.
Chronic: Prolonged or repeated skin contact may cause dermatitis.

Exposure

How many persons are involved in the activity?				
Who may be exposed	Staff	Students	Others	
Is the activity repeated simultaneously?			Yes	No
If Yes how many times is activity repeated				
Duration of activity				
Is the activity repeated within an 8hour period?			Yes	No
If a WEL is specified for any substance used, what is the worst case exposure during the activity				

Control Measures

Handling: Wash thoroughly after handling. Use only in a well-ventilated area. Ground and bond containers when transferring material. Avoid contact with eyes, skin, and clothing. Empty containers retain product residue, (liquid and/or vapour), and can be dangerous. Keep away from heat, sparks and flame. Avoid ingestion and inhalation. Prevent build up of vapours to explosive concentration. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose empty containers to heat, sparks or open flames.

Storage: Keep away from heat, sparks, and flame. Keep away from sources of ignition. Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances. Flammables-area.

Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.

Personal Protective Equipment

Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin: Wear appropriate protective gloves to prevent skin exposure.

Clothing: Wear appropriate protective clothing to minimize contact with skin.

Emergency Procedures:

First Aid Requirements

Eyes: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid immediately.
Skin: Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
Notes to Physician: none given

Spills/Leaks

Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Avoid runoff into storm sewers and ditches which lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Remove all sources of ignition. A vapour suppressing foam may be used to reduce vapours.

Based on the information included in this risk assessment. The risks associated with the specified activity are adequately controlled.

Signature..... *Position*.....

Kjeldahl

Activity	Hazard	Control
Kjeldhal analysis; use of glass ware, tubes/flasks	Risk of breakage/injury	Wear safety glasses and nitrile gloves. Take care when using glassware
Adding conc. Sulphuric acid to digestion tube	Risk of burns: acid	Use pump dispenser to reduce contact. Acid added in fume hood. Wear safety glasses and nitrile gloves. COSHH assessment available for sulphuric acid.
Boiling acid and sample in heater at 450°C	Risk of burns: acid/heater	Digestion tube is self contained and used in fume hood Wear safety glasses and heat resistant gloves when handling tubes.
Adding water to hot digested sample	Exothermic reaction from adding water to acid	Use extreme care and add digested sample to water. Use fume hood. Wear safety glasses and nitrile gloves.
Collection of ammonia in boric acid	Chemical	Wear safety glasses and nitrile gloves. Take care when filling flask with acid. COSHH assessment available for boric acid.
Titration with hydrochloric acid	Risk of burns: acid	Wear safety glasses and nitrile gloves. Take care when filling burette, use funnel and beaker to prevent drips. COSHH assessment available for hydrochloric acid.

Kjeldahl

COSHH Assessment Form

Department:	**ARES**	*Assessment Date:*	**22 01 10**
Location/Building :	*Labs Brackenhurst*	*Review Dates:*	
Room Number:		*Ref:*	

Activity *Kjeldhal analysis*

Substances Used (If Biological Agent include classification)	*Hazard**	*By***	*WEL*	*Storage Unit Quantity*	*Amount in Use*
Sulphuric acid 90-98%	**R 35;** Causes severe burns **C;** Corrosive	Inhalation Ingestion Contact	**TWA:** 1 mg/m3 TWA **STEL:** 3 mg/m3 STEL		

** Very toxic, corrosive, harmful, irritant....etc.*
***Inhalation, Ingestion, Contact.*
WEL – Workplace Exposure Limit

Health Effects

Eye: Causes severe eye burns. May cause irreversible eye injury. May cause blindness. May cause permanent corneal opacification. The severity of injury depends on the concentration of the solution and the duration of exposure.
Skin: Causes skin burns. The severity of injury depends on the concentration of the solution and the duration of exposure.
Ingestion: May cause severe and permanent damage to the digestive tract. Causes gastrointestinal tract burns.
Inhalation: May cause irritation of the respiratory tract with burning pain in the nose and throat, coughing, wheezing, shortness of breath and pulmonary oedema. Causes chemical burns to the respiratory tract. Inhalation may be fatal as a result of spasm, inflammation, oedema of the larynx and bronchi, chemical pneumonitis and pulmonary oedema. Because its vapour pressure is negligible, it exists in the air only as a mist or spray. Exposure may impair lung function and cause mucostasis (reduced mucous clearance).
Chronic: Prolonged or repeated skin contact may cause dermatitis. Prolonged or repeated inhalation may cause nosebleeds, nasal congestion, erosion of the teeth, perforation of the nasal septum, chest pain and bronchitis. Prolonged or repeated eye contact may cause conjunctivitis. Effects may be delayed. Workers chronically exposure to sulphuric acid mists may show various lesions of the skin, tracheobronchitis, stomatitis, conjunctivitis, or gastritis. Occupational exposure to strong inorganic acid mists containing sulphuric acid is carcinogenic to humans.

How many persons are involved in the activity?				
Who may be exposed	Staff	Students	Others	
Is the activity repeated simultaneously?			Yes	No
If Yes how many times is activity repeated				
Duration of activity				
Is the activity repeated within an 8hour period?			Yes	No
If a WEL is specified for any substance used, what is the worst case exposure during the activity				

Control Measures

Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Do not allow water to get into the container because of violent reaction. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Discard contaminated shoes. Use only with adequate ventilation. Do not breathe spray or mist. Do not use with metal spatula or other metal items. Inform laundry personnel of contaminant's hazards.

Storage: Do not store near combustible materials. Keep container closed when not in use. Store in a cool, dry, well-ventilated area away from incompatible substances. Do not store near alkaline substances. Store protected from moisture. Ideally, sulfuric acid should be stored in isolation from all other chemicals in an approved acid or corrosives safety cabinet.

Exposure Controls, Personal Protection
Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits. Use a corrosion-resistant ventilation system.
Personal Protective Equipment
Eyes: Wear chemical splash goggles and face shield.
Skin: Wear neoprene gloves, apron, and/or clothing. Viton gloves are recommended.
Clothing: Wear appropriate protective clothing to prevent skin exposure.

Emergency Procedures:

First Aid Requirements

Eyes: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical aid immediately.

Skin: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid immediately. Wash clothing before reuse..

Ingestion: If swallowed, do NOT induce vomiting. Get medical aid immediately. If victim is fully conscious, give a cupful of water. Never give anything by mouth to an unconscious person.

Inhalation: POISON material. If inhaled, get medical aid immediately. Remove victim to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.

Notes to Physician: Monitor arterial blood gases, chest x-ray, and pulmonary function tests if respiratory tract irritation or respiratory depression is evident. Treat dermal irritation or burns with standard topical therapy. Effects may be delayed. Do NOT use sodium bicarbonate in an attempt to neutralize the acid.

Spills/Leaks

Avoid runoff into storm sewers and ditches which lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Carefully scoop up and place into appropriate disposal container. Provide ventilation. Do not get water inside containers. Cover with dry earth, dry sand, or other non-combustible material followed with plastic sheet to minimize spreading and contact with water.

COSHH Assessment Form

Department:	**ARES**	*Assessment Date:*	**22**	***01***	***10***
Location/Building:	*Labs Brackenhurst*	*Review Dates:*			
Room Number:		*Ref:*			

Activity *Kjeldhal analysis*

Substances Used (If Biological Agent include classification)	*Hazard**	*By***	*WEL*	*Storage Unit Quantity*	*Amount in Use*
4% Boric Acid Solution with Indicator	*None listed*				

** Very toxic, corrosive, harmful, irritant....etc.*
***Inhalation, Ingestion, Contact.*
WEL – Workplace Exposure Limit

Health Effects

Eye: May cause eye irritation.
Skin: May cause skin irritation. May be absorbed in harmful amounts through injured skin
Ingestion: May cause circulatory system failure. May cause central nervous system depression, characterized by excitement, followed by headache, dizziness, drowsiness and nausea. Advanced stages may cause collapse, unconsciousness, coma and possible death due to respiratory failure. Boric acid poisoning begins with nausea, vomiting and diarrhoea. There is a red skin rash followed by extensive exfoliation not only in areas of rash but also of mucous membranes. Other symptoms may include weakness, headache, restlessness & kidney injury
Inhalation: May cause respiratory tract irritation. May be absorbed through the lungs.
Chronic: Chronic poisoning by boron compounds, borism, may be little more than dry skin and mucous membranes, followed by appearance of a red tongue, patchy alopecia (hair loss), cracked lips, and conjunctivitis. Infants and young children are more susceptible to boric acid poisoning than adults. Prolonged absorption of boron compounds may cause anorexia, vomiting, mild diarrhoea, skin rash, alopecia, convulsions, weakness, confusion, menstrual disorders, and anaemia.

Exposure

How many persons are involved in the activity?				
Who may be exposed	Staff	Students	Others	
Is the activity repeated simultaneously?			Yes	No
If Yes how many times is activity repeated				
Duration of activity				
Is the activity repeated within an 8hour period?			Yes	No
If a WEL is specified for any substance used, what is the worst case exposure during the activity				

Control Measures

Handling: Wash thoroughly after handling. Wash thoroughly after handling. Use with adequate ventilation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation.

Storage: Store in a cool, dry, well-ventilated area away from incompatible substances.

Exposure Controls, Personal Protection
Engineering Controls: Good general ventilation should be sufficient to control airborne levels.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to minimize contact with skin.

Emergency Procedures:

First Aid Requirements
Eyes: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical aid.
Skin: In case of contact, flush skin with plenty of water. Remove contaminated clothing and shoes. Get medical aid if irritation develops and persists. Wash clothing before reuse.
Ingestion: If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical aid.
Inhalation: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
Notes to Physician: Treat symptomatically and supportively.

Spills/Leaks
Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container.

Based on the information included in this risk assessment. The risks associated with the specified activity are adequately controlled.

Signature..... *Position*.....

COSHH Assessment Form

Department: **ARES**

Assessment Date: **22 01 10**

Location/Building : *Labs Brackenhurst*

Review Dates:

Room Number:

Ref:

Activity *Kjeldhal analysis*

Substances Used (If Biological Agent include classification)	*Hazard**	*By***	*WEL*	*Storage Unit Quantity*	*Amount in Use*
Hydrochloric acid 0.1M	**R34**;Causes severe burns. **R37**;Irritating to respiratory system. **C**;Corrosive	Inhalation Ingestion Contact	**TWA:** 1 ppm TWA (gas and aerosol mists); 2 m TWA (gas and aerosol mists) **STEL:** 5 ppm STEL (gas and aerosol mists); 8 mg/m3 STEL (gas and aerosol mists)		

** Very toxic, corrosive, harmful, irritant....etc.*
***Inhalation, Ingestion, Contact.*
WEL – Workplace Exposure Limit

Health Effects

Eye: Causes severe eye burns. May cause irreversible eye injury.
Skin: Contact with liquid is corrosive and causes severe burns and ulceration. The severity of injury depends on the concentration of the solution and the duration of exposure.
Ingestion: Causes severe digestive tract burns with abdominal pain, vomiting, and possible death. May cause corrosion and permanent tissue destruction of the oesophagus and digestive tract.
Inhalation: Causes severe irritation of upper respiratory tract with coughing, burns, breathing difficulty, and possible coma. Inhalation of a mist of this material may cause respiratory tract irritation. Inhalation may be fatal as a result of spasm, inflammation, oedema of the larynx and bronchi, chemical pneumonitis and pulmonary oedema.
Chronic: Chronic inhalation may cause effects similar to those of acute inhalation. Repeated exposure may cause erosion of teeth. Repeated exposure to low concentrations of HCl vapour or mist may cause bleeding of nose and gums. Chronic bronchitis and gastritis have also been reported.

Exposure

How many persons are involved in the activity?			
Who may be exposed	Staff	Students	Others .....
Is the activity repeated simultaneously?			Yes / No
If Yes how many times is activity repeated			
Duration of activity			
Is the activity repeated within an 8hour period?			Yes / No
If a WEL is specified for any substance used, what is the worst case exposure during the activity			

Control Measures

Handling: Use only in a well-ventilated area. Avoid breathing dust, vapour, mist, or gas. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Do not ingest or inhale. Use caution when opening.
Storage: Store in a cool, dry place. Store in a tightly closed container. Do not store in metal containers. Store away from alkalies.
Exposure Controls, Personal Protection
Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.
Personal Protective Equipment
Eyes: Wear chemical splash goggles and face shield.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.

Emergency Procedures:

First Aid Requirements
Eyes: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid immediately.
Skin: Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
Notes to Physician: none given

Spills/Leaks
Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Avoid runoff into storm sewers and ditches which lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Provide ventilation. Spill may be carefully neutralized with lime (calcium oxide, CaO).

Based on the information included in this risk assessment. The risks associated with the specified activity are adequately controlled.

Signature..... *Position*.....

ICP Preparation Risk Assessment

Activity	Hazard	Control
Adding aqua regia (mixture of HCL & HNO_3 in ratio 3:1)to flask	Risk of burns: acid	Use pump dispenser to reduce contact. Aqua regia added in fume hood. Wear safety glasses and nitrile gloves. COSHH assessment available for hydrochloric and nitric acids.
Boiling aqua regia and sample on hot plate at ca. 70°C (3 times)	Risk of burns: acid/hotplate	Aqua regia and sample boiled in fume hood. Wear safety glasses and nitrile gloves. COSHH assessment available for hydrochloric and nitric acids.

ICP-Prep
COSHH Assessment Form

Department:

Assessment Date:

Location/Building:

Review Dates:

Room Number:

Ref:

Activity

Substances Used (If Biological Agent include classification)	*Hazard**	*By***	*WEL*	*Storage Unit Quantity*	*Amount in Use*
Nitric acid, 20-70%	**R 35;** Causes severe burns **C;**Corrosive	Inhalation Ingestion Contact	**TWA:** 2 ppm TWA; 5.2 mg/m3 TWA **STEL:** 1 ppm STEL; 2.6 mg/m3 STEL		

** Very toxic, corrosive, harmful, irritant....etc.*
***Inhalation, Ingestion, Contact.*

Health Effects

Eye: Causes severe eye burns. Direct contact with liquid may cause blindness or permanent eye damage.
Skin: Causes skin burns. May cause deep, penetrating ulcers of the skin. Concentrated nitric acid dyes human skin yellow on contact.
Ingestion: May cause severe and permanent damage to the digestive tract. Causes gastrointestinal tract burns. May cause perforation of the digestive tract. May cause systemic effects.
Inhalation: Effects may be delayed. Causes chemical burns to the respiratory tract. Inhalation may be fatal as a result of spasm, inflammation, oedema of the larynx and bronchi, chemical pneumonitis and pulmonary oedema. Aspiration may lead to pulmonary oedema. May cause systemic effects. May cause acute pulmonary oedema, asphyxia, chemical pneumonitis, and upper airway obstruction caused by oedema.
Chronic: Exposure to high concentrations of nitric acid vapour may cause pneumonitis and pulmonary oedema which may be fatal. Symptoms may or may not be delayed. Continued exposure to the vapour & mist of nitric acid may result in a chronic bronchitis, & more severe exposure results in a chemical pneumonitis. The vapour & mists of nitric acid may erode the teeth, particularly affecting the canines & incisors.

How many persons are involved in the activity?			
Who may be exposed	Staff	Students	Others .....
Is the activity repeated simultaneously?			Yes / No
If Yes how many times is activity repeated			
Duration of activity			
Is the activity repeated within an 8hour period?			Yes / No
If a WEL is specified for any substance used, what is the worst case exposure during the activity			

Control Measures

Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Do not breathe dust, mist, or vapour. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Avoid contact with clothing and other combustible materials. Discard contaminated shoes. Do not use with metal spatula or other metal items. Use only with adequate ventilation or respiratory protection.
Storage: Do not store near combustible materials. Do not store in direct sunlight. Keep container closed when not in use. Store in a cool, dry, well-ventilated area away from incompatible substances. Keep away from metals. Store away from alkalines. Separate from organic materials. Inspect periodically for damage or evidence of leaks or corrosion.

Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits. Use a corrosion-resistant ventilation system.

Personal Protective Equipment

Eyes: Wear chemical splash goggles and face shield.
Skin: Wear butyl rubber gloves, apron, and/or clothing.
Clothing: Wear appropriate clothing to prevent skin exposure.

Emergency Procedures:

Eyes:
Get medical aid immediately. Do NOT allow victim to rub eyes or keep eyes closed. Extensive irrigation with water is required (at least 30 minutes).
Skin: Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Destroy contaminated shoes.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Do NOT use mouth-to-mouth resuscitation. If breathing has ceased apply artificial respiration using oxygen and a suitable mechanical device such as a bag and a mask.
Notes to Physician: Treat symptomatically and supportively.

Based on the information included in this risk assessment. The risks associated with the specified activity are adequately controlled.

COSHH Assessment Form

Department:

Assessment Date:

Location/Building :

Review Dates:

Room Number:

Ref:

Activity

Substances Used (If Biological Agent include classification)	*Hazard**	*By***	*WEL*	*Storage Unit Quantity*	*Amount in Use*
Hydrochloric acid 32-38% solution	**R34**;Causes severe burns. **R37;**Irritating to respiratory system. **C;**Corrosive	Inhalation Ingestion Contact	**TWA**: 1 ppm TWA (gas and aerosol mists); 2 mg/m3 TWA (gas and aerosol mists) **STEL:** 5 ppm STEL (gas and aerosol mists); 8 mg/m3 STEL (gas and aerosol mists)		

Health Effects

Eye: May cause irreversible eye injury. Vapour or mist may cause irritation and severe burns. Contact with liquid is corrosive to the eyes and causes severe burns.
Skin: Contact with liquid is corrosive and causes severe burns and ulceration. The severity of injury depends on the concentration of the solution and the duration of exposure.
Ingestion: Causes severe digestive tract burns with abdominal pain, vomiting, and possible death. May cause corrosion and permanent tissue destruction of the oesophagus and digestive tract.
Inhalation: May be fatal if inhaled. May cause severe irritation of the respiratory tract with sore throat, coughing, shortness of breath and delayed lung edema. Causes chemical burns to the respiratory tract. Causes corrosive action on the mucous membranes.
Chronic: Prolonged or repeated skin contact may cause dermatitis. Repeated exposure may cause erosion of teeth. Repeated exposure to low concentrations of HCl vapor or mist may cause bleeding of nose and gums. Chronic bronchitis and gastritis have also been reported.

Exposure

How many persons are involved in the activity?				
Who may be exposed	Staff	Students	Others	
Is the activity repeated simultaneously?			Yes	No
If Yes how many times is activity repeated				
Duration of activity				
Is the activity repeated within an 8hour period?			Yes	No
If a WEL is specified for any substance used, what is the worst case exposure during the activity				

Control Measures

Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Contents may develop pressure upon prolonged storage. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Discard contaminated shoes. Keep away from strong and metals. Use caution when opening. Do not use with metal spatula or other metal items. Do not breathe vapor or mist. Use only with adequate ventilation or respiratory protection.
Storage: Store in a cool, dry, well-ventilated area away from incompatible. Corrosives area. Do not store in metal containers. Store from alkalines. Separate from oxidizing materials.
Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits. Use a corrosion-resistant ventilation system.
Personal Protective Equipment
Eyes: Wear chemical splash goggles and face shield.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin

Emergency Procedures:

First Aid Requirements

Eyes: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical aid immediately.

Skin: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid immediately. Wash clothing before reuse.

Ingestion: If swallowed, do NOT induce vomiting. Get medical aid immediately. If victim is fully conscious, give a cupful of water. Never give anything by mouth to an unconscious person.

Inhalation: POISON material. If inhaled, get medical aid immediately. Remove victim to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.

Notes to Physician: Do NOT use sodium bicarbonate in an attempt to neutralize the acid.

Spills/Leaks: Avoid runoff into storm sewers and ditches which lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Isolate area and deny entry. Provide ventilation. Spill may be carefully neutralized with lime (calcium oxide, CaO). A vapour suppressing foam may be used to reduce vapours. Approach spill from upwind.

Based on the information included in this risk assessment. The risks associated with the specified activity are adequately controlled.

Signature..... *Position*.....

APPENDIX III

Ethical Review Form for Scientific Procedures (Animals)

School of Animal, Rural and Environmental Sciences
Nottingham Trent University

1. **Name of Applicant** Chris Young

2. **Position (eg. BSc year 3 Zoo Biology)** BSc Yr 3 Animal Biology

3. **Contact details (email/telephone)** n0167089@ntu.ac.uk
   Mob. Tel. 07588495345 Home tel. 01827 700525

4. **Purpose of project application (eg. BSc year 3 dissertation, PhD thesis, or paper for publication)** BSc Yr 3 dissertation

5. **Name of supervisor/member of staff responsible**
   Dr Emily Clarke

6. **Details of planned method/procedure, including explanation of how the 5 freedoms will be maintained. Use a separate sheet if necessary.** Proximate Analysis - Protein (Total N) Kjeldahl method Crude fat content using Soxhlet extraction with pet. ether Fibre will be determined using neutral detergent fibre. Ash: muffle furnace incineration. Mineral analysis determined by ICP/MS. All samples frozen at Dumell prior to shipping to Brackenhurst.

7. **Statement of ethical review**

I have read the Ethical Review Procedure of the School of Animal, Rural and Environmental Sciences and agree to maintain the 5 freedoms as set out by the Farm Animal Welfare Council. I understand that studies will not be allowed to take place until ethical approval has been obtained, and that permission will be withdrawn and the study cancelled if there is a breach of these conditions.

Signatures:

Applicant..... Date..10/11/09.....

Supervisor Dr Emily Clarke Date..12/01/10.....