Chapter 1 General Introduction

1.1 Background

Continuous growth in human population over the decades has lead to greater pressure on the existing animal protein sources. Poultry, pork, beef, lamb and fish are considered the major sources of meat protein for humans. These sources are currently declining due to persistent drought, diseases, natural disasters, high cost of feed, and low productivity of local breeds and this decline in productivity is most evident in some developing countries (Copper and Knower, 1991). It is well established that the consumption of animal products which contain a high fat content could result in several pathological conditions such as elevated blood cholesterol levels and coronary heart disease. Finding new meat sources which have low fat to solve the shortage of meat protein is a major challenge to the food industry worldwide.

In recent years, research into the nutritional value of molluscs has attracted much attention. The main reason for this interest is the large amount of molluscs available globally. Molluscs constitute at least 80000 species and they are considered the second largest phylum in the animal kingdom (Adeyeye, 1996). Molluscs have soft bodies and are found in different ecosystems throughout the world. They are predominantly found in aquatic systems but some are terrestrial (Adeyeye, 1996). The mollusc includes univalve organisms such as snails and slugs, bivalves such as mussels, oysters and cockles, and cephalopods such as octopus, squid and cuttlefish (Copper and Knower, 1991). Snails play an important role in nature with diverse functions and can act as sensitive indicators of environmental change (Adeyeye, 1996). Snail meat is a nutritionally sound food. (Ademolu *et al.*, 2004) reported that land snail meat is low in fat, rich in protein and many other nutrients required for a healthy and well balanced diet. Therefore, snails are harvested for food in many parts of the world, and are considered a delicacy in France, Korea, Japan and China (King and Bruce, 1949; Mohan, 2002; Qun *et al.*, 2004; Xia *et al.*, 2007). Molluscs contain high amounts of essential elements. For example, both land snails and marine shellfish including mussels, scallops and oysters, are rich source of calcium, sulphate, sodium, zinc and iron (Hughes *et al.*, 1980; Karakoltsidis *et al.*, 1995; Sidwell *et al.*, 1978; Vlieg *et al.*, 1991). Hence, there is the potential for using the under-utilized snail meat from aquatic resources to help fill the gap in current animal protein shortage in some countries that suffer from protein shortage.

Most of the currently available information regarding the nutritional value of snails has been from studies which were carried out on land snails, namely *Archachatina* sp., *Achatina* Sp., *Limicolaria* Sp., *Oreohelix* sp., *Helix* sp. (Fagbuaro *et al.*, 2006; Gomot, 1998; Hamzat *et al.*, 2002; Milinsk *et al.*, 2003; Milinsk *et al.*, 2006b; Rees and Hand, 1993), and other molluscs such as *Perna* sp., *Atrina* sp., *Ostra* sp., *Pecten* sp., *Mutilus* sp., *Pstra* sp., *Tellina* sp. (Anselland and Trevallion, 1967; Blackmore, 1969; Karakoltsidis *et al.*, 1995; Orban *et al.*, 2002; Vlieg, 1988). Such information for marine snails is scarce. This is particularly important, since New Zealand, as an island with rich marine sources has potentially a large amount and diverse species of marine snails. The present study was designed to assess the nutritional aspects of *Cookia sulcata*, an abundant marine snail found in the New Zealand coast.

Some studies have determined the chemical composition and nutritional value of selected land snails and marine shellfish. However, comparatively little work has been published on the potential toxicological effects of marine snail meat consumption. In this project trace elements (mainly heavy metals) and organochlorine pesticides levels in *C. sulcata* were determined to examine the toxicological safety of the meat.

1.2 Hypothesis

The hypothesis of the current study is that *C. sulcata* is a nutritionally viable and a safe source of food without heavy metal and organochlorine contaminations.

1.3 Objectives

The objectives of this study are:

· To determine the nutritional value of snail meat, *C. sulcata.*

· To investigate the toxicological aspects of *C. sulcata* with emphasis on heavy metal and organochlorine contaminants.

Chapter 2: Literature Review

2.1 Overview of *Cookia sulcata*

2.1.1 Description of Turbinidae Family

Cookia sulcata is a marine snail that belongs to the phylum of mollusc. The genus of *Cookia* is found within the class of gastropoda, family of *Turbinidae* and Subfamily of *Astraeiane* (Minson, 1972; Powell, 1979). The common name of this snail is Cook's Turban because it was collected by scientists sailing with Captain Cook. The family of *Turbinidae* include other snails such as *Modelia granosa* and *Astraea heliotropium*, which are commonly known as granose turban and circular saw, respectively (Crowe, 1999; Morley, 2004; Penniket, 1982). A famous member in the same family, *Turbo smaragdus* is called cat's eye and is often gathered for food by the Maori (Crowe, 1999).

The family of *Turbinidae* have a spirally growing, externally calcified, rigid operculum that completely fills the aperture of the shell when the soft parts are withdrawn (Vermeij and Williams, 2007). The operculum has an important protective function (Vermeij and Williams, 2007).

2.1.2 Description of *Cookia sulcata*

Cookia sulcata is found in many parts of New Zealand (North, South, Stewart and Chatham Islands). *C. sulcata* lives along ledges or among seaweed but is frequently found under rocks or along beaches on the open coast at low tide (Morley, 2004). This snail feeds on algae and seaweed (Crowe, 1999; Morton *et al.*, 1968). The size of an adult snail varies from about 65 to 80 mm in height and 65-90 mm in width (Powell, 1979). *C. sulcata* has a shell which is heavy, solid and strong, helping it to survive being ripped from the rocks by crashing waves and tossed around by the tide (Crowe, 1999). The shell is spirally coiled forming a protoconch with up to four whorls, and has a rounded to sub-angulated periphery with a strong and arcuated shell surface which varies with the rounding (Minson, 1972; Powell, 1979). The spiral structure inclines forward and is overridden by sharp spiral cords. The whole shell is crossed by fine lamellose growth striate (Minson, 1972; Powell, 1979). These fine striae are rarely visible on mature shells as layers of calcified material form a coating up to about 6 mm thick on the top and sides of the shell (Minson, 1972; Powell, 1979). The spire of the shell is often encrusted with white, calcareous algae (Morley, 2004) which turn into a red colour as the snail grows (Figure 2-1). The surface of the shell is often worn off, showing the silver

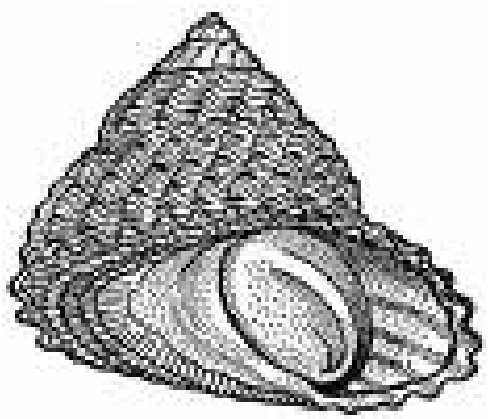
layer underneath (Crowe, 1999). The interior is white and is sealed off by an operculum (Morley, 2004). The large oval shelly operculum is an oblong plate with several spiral ridges, which is marked rather like the winding of a human ear (Dell, 1965).

In New Zealand, Maori people consume the meat and use the shells for making tiny detailed wood-carving and for making fish-hooks (Crowe, 1999). The shells of this snail have a commercial value after being polished and carved into exquisite spoons or mounted in gold or silver, set with jewels and treasured as ceremonial goblets.

The whole body of the *C. sulcata* after removal from the shell, consist of viscera, head, foot and a muscle. The edible tissues of *C. sulcata* are the foot, muscle and some parts of the visceral hump (Figure 2-2). The visceral hump parts (usually found further inside the shell) are white with some tinges of green. The foot is partly black and partly dark green in colour.

Figure 2-1: Schematic representation of *C. sulcata* shells (A) showing the deposition of coating layers on the shell during growth (B) of *C. sulcata* samples collected from Te Oka bay for this study

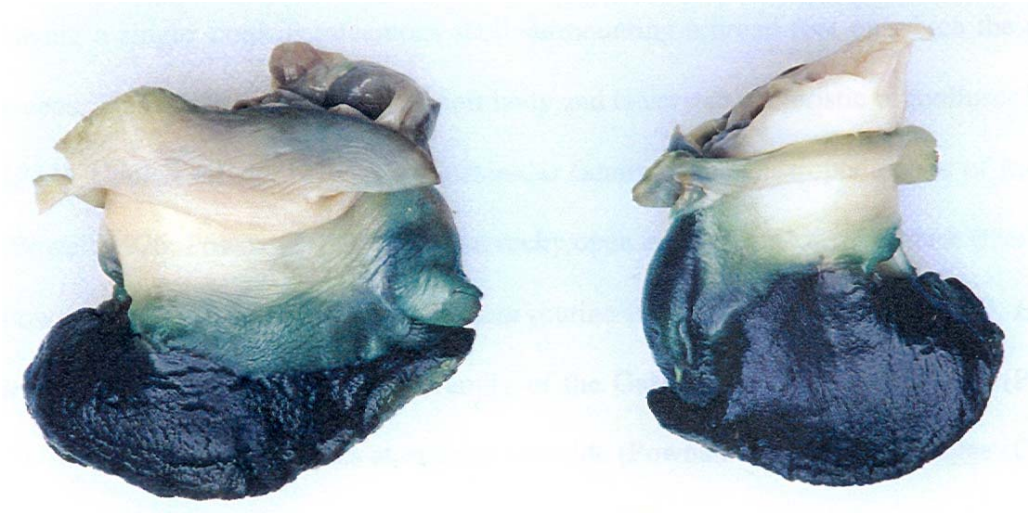
(A; *http://www.teara.govt.nz/NR/rdonlyres/A78BD490-DF0E-42B4-8977-9C50FF7509B3/23953/McLinv1236.gif***)**



A

B

Figure 2-2: The edible part of *C. sulcata* (Ding, 2000)



2.2 Nutritional aspects of snail meat

Snails are the largest groups of mollusc. Mollusc is the largest animal group after the arthropods (Powell, 1979). The mollusc includes univalve organisms such as snails and slugs; bivalves such as mussels, oysters and cockles; and cephalopods, such as octopus, squid and cuttlefish (Copper and Knower, 1991). Some molluscs are used as human food among which oysters, mussels and some snails are the most popular (Miletic *et al.*, 1991). Humans have consumed snails as food for thousands of years and edible snails have had a place on the menu in many European countries, especially France where snails are consumed in the greatest quantities (Milinsk *et al.*, 2003). Similarly, many Asian countries harvest snails for food and they are considered a delicacy in Korea and Japan (King and Bruce, 1949; Mohan, 2002; Qun *et al.*, 2004; Xia *et al.*, 2007). Commercial production of edible snails has been considered for years especially in China where snail production has developed because of the favourable weather and environmental conditions (Xia *et al.*, 2007).

In the following section, a summary of the chemical composition of land and marine snails is presented and the relative composition of snail meat compared with other marine molluscs and common commercial meats is discussed.

2.2.1 Proximate composition

a) Land snails

Land snails are collected commercially and considerable literature on basic information of their composition is available (Table 2-1). Snail species of *Helix* spp., *Archachatina*, spp., *Achatina* spp. have been studied the most. Snail meat is high in protein with a range of 8-20% (fresh weight basis) which equates to around 60 - 70 % on dry weight basis, depending on the snail type (Adeyeye, 1996; Fagbuaro *et al.*, 2006; Gomot, 1998; Hamzat *et al.*, 2002; Milinsk *et al.*, 2003; Milinsk *et al.*, 2006b). In contrast, snail meat is low in fat. Usually the fat content in snail meat is between 0.5% and 5% on fresh weight basis, which equates to 3% - 20% of dry weight basis. The most dominant component in snail meat is the moisture, which ranges between 70 - 85% of the fresh meat weight (Gomot, 1998; Udoh *et al.*, 1995). The ash content was reported to be low as well. For example, it has been reported that the ash content of snail approximates to 0.5 - 2.7% (fresh weight basis), which equates to 2 - 13.4% on dry weight basis (Table 2-1). Carbohydrates content has not been regularly reported in the studies but based on the available data, it can be estimated to be in the range of 0.2- 4.4% (fresh weight basis) (Hamzat *et al.*, 2002; Milinsk *et al.*, 2003; Milinsk *et al.*, 2006b; Udoh *et al.*, 1995).

Table 2-1: Proximate composition of Land snails

Species	Proximate composition (%FW)					References
	Protein	**Lipids**	**Moisture**	**Ash**	**Carbohy drate**	
Archachatina sp.	20.3-20.8	1.2-1.4	74.3-78.0	1.4	<0.4	Adeyeye, (1996); Fagbuaro *et al.*, (2006)
	39.7-43.3*	N/A	N/A	0.5-0.6*	N/A	(Hamzat *et al.*, 2002)
Helix. Sp.	7.3-18.4	0.4-2.7	72.8-87.6	0.7-2.7	0.4-1.7	Adeyeye, (1996); Gomot, (1998); Milinsk *et al.*, (2003); Milinsk *et al.*, (2006b); Wiesre and Schuster, (1975)
	51.4-72.5*	3.1-10.8*	N/A	8.8-13.4*	N/A	Gomot, (1998)
Achatina sp.	9.9-19.3	1.4-4.2	77.5-89.2	1.3-2.1	0.4-4.4	Adeyeye, (1996); Fagbuaro *et al.*, (2006); Watt and Merrill, (1975)
Limicolaria sp.	17.5-18.7	1.2-4.3	71.2-78.7	1.1-1.4	0.2	Claeys and Demeyer, (1986)
	51.4*	9.7*	N/A	11.8*	27.1*	Udoh *et al.*, (1995)

*= the percentage on dry matter basis FW=fresh weight N/A=not available

b) Marine snails

Very few studies have been carried out to investigate the composition of marine snails (Table 2-2). Although the marine species differ from the land snails in terms of diet and the living environment, the proximate composition of marine snails is not vastly different from land snails. The protein and lipid contents are slightly lower than land snails, 8-16% and 1.4-8% of fresh weight, respectively, whereas the moisture content is similar (around 80% of the fresh weight). The ash and carbohydrate contents vary in different species of marine snails.

Table 2-2: Proximate composition of Marine snails

Species	**Proximate composition (%FW)**					**References**
	Protein	**Lipids**	**Moisture**	**Ash**	**Carbohydrate**	
Trobo sarmaticus	62.7-74.8*	3.2-6.7*	NA	4.7-7.6*	15.9-23.1*	McLachlan and Lombard, (1980)
Monodonta turbinata	51.2*	2.9*	82.8	N/A	N/A	Miletic *et al.*, (1991)
unknown	16.1	1.4	79.2	1.3	2	USDA, (2006)

N/A= not available *= the percentage on dry matter basis FW=fresh weight

Several factors contribute to the variability in the proximate composition of the snail meat. The variability due to snail species, size (age), sampling position (location) and season have been reported which lead to large differences in the proximate composition of meat among different land-snails species (Ansell, 1972; Ansell and Trevallion, 1967; Blackmore, 1969; McLachlan and Lombard, 1980; Miletic *et al.*, 1991; USDA, 2006).

c) Other marine molluscs

The proximate composition of other marine molluscs such as mussel and oyster vary widely, and thus it is difficult to establish a comparison with snails (both land and marine) and to find a general trend (Table 2-3).

Table 2-3: Proximate composition of some common marine molluscs consumed as food

Species	Proximate composition(%FW)					References
	Protein	Lipids	Moisture	Ash	Carbohydrate	
Perna canaliculus	11.9-13.9	2.1-2.2	78.2-80.9	1.7-2.2	3.4	1, 4
Atrina zelandica	14.8	0.5	81.0	1.3	2.4	1
Ostra edulis	12.9	3.0	79.4	1.6	3.1	1,3
Pecten novaezelandiae	14.3-15.4	0.7-1.3	78.7-82.4	1.6-1.9	2.7	1,4
Mutilus qalloprovinciallis	8.0-13.0 42.4-56.2*	1.0-2.0 5.8-13.4*	82.0-87.0	0.7-2.0 11.0-21.0*	2.0-5.0	2,3
Ostra edulis	11.0-15.0	0.2-1.0	82.0-88.0	1.0-1.6	2.0-3.0	1,3
Patella vulgate	N/A	10.5-53.3#	75.0-84.0	8.6-34.7*	N/A	5
Tellina tenuis	7.9-10.9	3.9-12.8*	71.0-83.0	5.8-27.7*	6.4-25.6*	6

1=Vlieg, (1988) 2=Orban *et al.*, (2002) 3=Karakoltsidis *et al.*, (1995) 4=Hughes *et al.*, (1980)
5=Blackmore, (1969) 6=Ansell and Trevallion, (1967)
N/A=not available #=ash free DM *= the percentage on dry matter basis FW=fresh weight

d) Commercial Meat

The average proximate composition of mutton, beef, lamb, pork, chicken and goat meat are highly variable with a range of 1.8 -23.9% for protein, 1.1-4.2% for fat, 44.4-78.6% for moisture and 0.6-3.2% for ash (Table 2-4). Moisture was the most predominant component in both domestic animal and molluscs, and molluscs contained higher moisture content than land based animals. The production of poultry, pork, beef, lamb which are considered the major sources of meat protein for human are currently declining due to persistent drought, diseases, natural disasters, high cost of feed, and low productivity of local breeds and this is most evident in some developing countries (Fagbuaro *et al.*, 2006). At the same time, a continuous growth in human population is occurring. This has led to a greater pressure on the existing animal protein sources. Hence, interest in snails as food has increased in the recent years. The higher end of scale for protein content in domestic animal meat was similar to land snails, and was slightly higher than marine snails and other molluscs. Therefore, in terms of protein quantity, snails potentially can be used as an alternative source of protein to overcome the shortage of land based animal meat products.

Many domestic meat products have a high fat content. Also the fats of domestic meats contain a high percentage of saturated fatty acids which are associated with several pathological conditions such as elevated blood cholesterol levels and vascular coronary heart diseases. In contrast, snails (both land and marine) and other marine molluscs contain much lower fat

content than land based animal meat. Also the fats of snails contain a high proportion of monoenes and long chain fatty acids which is discussed in more detail in section 2.2.3 (Table 2-8).

Table 2-4: Proximate composition of raw meat from common domestic animals

Species	Proximate composition (% FW)					References
	Protein	Lipids	Moisture	Ash	Carbohydrate	
Goat	19.8-24.0	2.9-16.1	68.3-78.7	0.9-1.7	N/A	Lee *et al.*, (2008; Paleari *et al.*, (2008)
Beef	22.5-24.0	1.1-6.9	44.4-74.9	1.2-1.4	N/A	Crosland *et al.*, (1995; Isaksson *et al.*, (1996); Seggern *et al.*, (2005); Smith and Williamson, (1986)
Chicken	20.4-22.7	2.8-9.2	69.7-74.3	0.3-1.3	N/A	Al-Najdawi and Abdullah, (2002); Crosland *et al.*, (1995)
Pork	15.0-23.1	1.7-22.1	59.2-73.9	1.0-3.2	N/A	Cheng and Sun, (2007; Crosland *et al.*, (1995)
Lamb	23.4-25.0	2.5-26.4	55.4-76.9	0.8-1.2	N/A	Badiani *et al.*, (1998); Crosland *et al.*, (1995); Kosulwat *et al.*, (2003); Lee *et al.*, (2008)

N/A = not available FW=fresh weight

2.2.2 Amino Acids

Proteins which make up many essential components in living cells are naturally made from 20 amino acids (Nollet, 2004). Once proteins are ingested, amino acids are released and absorbed by the body. Protein quality depends on its amino acid content and digestibility. Amino acids are generally supplied in food and/or may be synthesised through different metabolic pathways from other amino acids inside the organisms. Essential amino acids cannot be synthesised in sufficient quantities in the organisms and must be supplied in adequate amounts in the diet for optimal functioning of an organism. In addition, some amino acids are essential at certain stage of life such as during infancy and/or period of pregnancy (Nollet, 2004). Therefore, the quality as well as the quantity of a protein is equally important for any food. A simple method of determining amino acid compositions and comparing with a reference protein is commonly used to evaluate the protein quality. This method is called amino acid scoring (Whitney and Rolfes, 2005). Whole egg protein which is one of the most complete and digestible proteins has been used as a standard or reference protein for measuring protein quality since early 1970's. Egg protein is assigned a value of 100 and the quality of other food protein is compared with egg. The limiting amino acid is the one which

falls short compared with the egg. In addition, a new standard for the reference protein, the daily essential amino acid requirements for human, has been established by the Food and Agricultural Organisation of the United Nations (FAO) and the World Health Organisation (WHO) (FAO/WHO/UNU, 1981). Both whole egg protein and daily essential amino acid requirements were used as standards for protein quality measurement in this research.

Given the nutritional importance of amino acids, considerable information on the amino acid profiles of land and marine snails, as well as some marine molluscs and common meats are already available (Table 2-5, Table 2-6). Aspartic acid (asp) and glutamic acid (glu) are the major amino acids in marine molluscs, land snails and meats (beef, pork and lamb). Marine molluscs contained larger amounts of alanine (ala) and glycine (gly), compared with land snails and common meats. Molluscs (including land snails) and meats have similar amounts of lysine (lys) and leucine (leu) (Table 2-5, Table 2-6). Marine molluscs and meats from domesticated animals exhibited a high total amino acid compared with land snails. The total essential amino acids in marine molluscs was similar to the common meats (about 40%), but was 5-10% higher than land snails (Table 2-7). In terms of protein quality, both molluscs (marine and land snails) and meats (beef, pork and lamb) have a lower protein quality compared with egg protein because of the higher proportion of essential amino acids in eggs. Lysine is the predominant essential amino acid in marine molluscs, while histidine (his) is found most in both land snails and meats. Total amount of methonine (met) and cysteine (cys) are the limiting amino acids in marine molluscs, land snails and meats (Table 2-7). Essential amino acids in marine molluscs, land snails and common meats generally meet the daily requirement for human with the exception of the tryptophan requirement for infants and pre-schoolers (Table 2-7). Therefore, snails (both land and marine) can be used as a potential protein source.

Table2-5: Amino acid profiles of some marine molluscs

AA	Marine molluscs							
	Monodonta lineata	*Gibbula umbilicalis*	*Gibbula pennanti*	*Gibbula cineraria*	*Mya arenaria*	*Cardium edule*	*Tapes decussatus*	*Perna canalicus*
	g/100g protein							g/100g (FW)
Asp	10.5	8.0	9.1	11.6	9.3	N/A	N/A	1.3
Glu	10.7	10.9	10.8	10.1	13.0	N/A	N/A	1.7
Ser	4.9	5.9	6.2	5.5	4.3	N/A	N/A	0.7
His*	2.3	2.5	2.6	2.2	1.8	1.6	1.6	0.2
Gly	8.5	10.4	10.5	10.0	6.9	N/A	N/A	1.1
Thr*	5.3	5.0	5.4	5.8	4.4	4.5	3.2	0.5
Arg	5.7	5.5	5.9	6.0	7.0	5.6	4.0	1.0
Ala	8.1	7.4	7.9	8.6	6.8	ND	ND	0.6
Tyr	2.8	3.0	3.1	2.9	3.2	1.1	ND	0.4
Val*	5.8	5.3	5.9	6.1	4.2	4.5	3.2	0.5
Phe*	4.0	3.5	3.8	3.7	3.3	4.4	3.2	0.4
Ile*	4.9	4.4	5.3	4.7	4.5	4.5	5.4	0.5
Lys*	8.5	8.9	6.1	7.1	7.7	11.2	8.0	1.0
Leu*	7.6	8.5	7.3	6.5	6.5	9.0	8.2	0.8
Pro	6.3	7.3	3.8	3.9	3.4	N/A	N/A	0.5
Cys	1.8	1.2	2.7	1.3	ND	ND	ND	0.1
Met*	2.3	2.4	3.3	2.8	2.4	2.2	2.3	0.3
Trp*	N/A	N/A	N/A	N/A	1.2	1.2	1.1	N/A
TAA	100.0	100.0	99.7	98.8	89.9	49.8	40.2	11.6
TEAA	49.2	50.9	50.2	48.9	42.9	43.1	36.2	5.3
TEAA/TAA	0.49	0.51	0.50	0.49	0.48	0.87	0.90	0.46
References	Daguzan, (1983); Harvey, (1970); Hughes *et al.*, (1980); Mead and Kemmerer, (1953); Miletic *et al.*, (1991)							

AA= Amino acid TAA= total amino acids TEAA= total essential amino acids
ND=not detected *= essential amino acid N/A = not available
TEAA/TAA= total essential amino acids /total amino acids FW=fresh weight

Table 2-6: Amino acid profiles of some land snails and common meats

	Land Snail				Common meats			
AA	1	2	3	4	5	Beef	Pork	Lamb
	g/100g protein			% DM	g/100g Protein			
Asp	7.6-7.8	6.9	7.3	6.2	N/A	8.8	8.9	8.5
Glu	10.1-13.5	11.1	11.4	9.8	N/A	14.4	14.5	14.4
Ser	4.0-4.1	3.2	3.3	3.5	N/A	3.8	4.0	3.9
His*	3.6-3.7	3.0	4.7	2.2	0.96	2.9	3.2	2.7
Gly	5.1-6.5	5.2	4.6	1.8	N/A	7.1	6.1	6.7
Thr*	1.8-3.9	2.2	2.8	1.3	N/A	4.0	5.1	4.9
Arg	0.3	6.3	6.0	4.5	6.46	6.6	6.4	6.9
Ala	4.0-6.9	2.4	5.2	1.9	N/A	6.4	6.3	6.3
Tyr	2.6-5.9	2.5	2.9	5.4	N/A	3.2	3.0	3.2
Val*	4.1-7.6	3.3	7.1	6.1	2.97	5.7	5.0	5.0
Phe*	4.4-6.7	3.8	4.7	2.3	2.58	4.0	4.1	3.9
Ile*	5.2-7.3	4.5	3.9	2.2	3.15	5.1	4.9	4.8
Lys*	7.2-8.3	6.1	5.7	4.7	5.97	8.4	7.8	7.6
Leu*	6.9-11.5	5.6	8.1	4.9	3.43	8.4	7.5	7.4
Pro	4.0-5.6	3.0	3.6	2.4	N/A	5.4	4.6	4.8
Cys	0.5-2.0	0.9	1.1	N/A	N/A	1.4	1.3	1.3
Met*	1.8-2.3	1.4	2.0	5.6[a]	0.7	2.3	2.5	2.3
Trp*	ND	ND	ND	ND	ND	1.1	1.3	1.3
TAA	73.2-103.9	71.4	84.4	59.2	26.2	99.0	96.5	95.9
TEAA	35.0-51.3	29.9	39.0	23.7	19.8	41.9	41.4	39.9
TEAA/TAA	0.48-0.49	0.42	0.46	0.40	0.75	0.42	0.43	0.42
References	Adeyeye, (1996); Mead and Kemmerer, (1953); Crosland *et al.*, (1995); Udoh *et al.*, (1995)				Gibson *et al.*, (1993); Pearson and Dutson, (1990)			

1= *Limicolaria aurora* 2= *Archatina archatina* 3= *Archachatina marginata* 4= *Helix pomatia*
5= *Acbatina spp.* ND=not detected N/A = not available AA= Amino acid
*= essential amino acid TAA= total amino acids DM = dry matter
TEAA= total essential amino acids
TEAA/TAA= total essential amino acids /total amino acids

Table 2-7: Daily essential amino acids requirement for human

AA	**Daily requirement g/100g protein**			**Egg protein**	**AA score (%)**		
	Infant[1]	School-child[2]	Adult[3]		Marine molluscs	Land snails	Meats
His	1.4	/	/	2.3	90.7	167.4	127.5
Ile	3.5	3.7	1.8	5.4	89.5	96.8	91.4
Leu	8.0	5.6	2.5	8.6	89.0	93.3	90.3
Lys	5.2	7.5	2.2	7.0	117.4	97.5	113.3
Met + Cys	2.9	3.4	2.4	5.7	61.9	42.5	64.9
Phe + Tyr	6.3	3.4	2.5	9.3	64.5	95.4	76.7
Thr	4.4	4.4	1.3	4.7	102.1	56.9	99.3
Trp	0.85	0.46	0.65	1.7	68.6	N/A	72.5
Val	4.7	4.1	1.8	6.6	75.8	83.7	79.3
References	FAO/WHO/UNU, (1981)			Whitney and Rolfes, (2005)	Equated values from table 2-5, and table 2-6.		

/=There is no evidence for or against a His requirement for children or adult; N/A= not available
1=2-6 months old 2=10-12 years old 3= >18 years

2.2.3 Fatty acid composition

Over the years, some fat classes have been recognized as an unhealthy component of our diet. Many forms of fats which have a range of differing biological properties and physiological effects have been studied (Mills *et al*., 2005). Fatty acids (FA) may be saturated, monounsaturated (MUFA) or polyunsaturated (PUFA). These FA serve different functions in the human body. For example, short chain fatty acids provide energy and long chain FA are parts of the cell membrane structure (Berg *et al.*, 2007). Unlike some PUFA, SFA and MUFA are not necessary in the diet because they can be produced in the human body. Some PUFA are considered essential because animals and humans cannot synthesize them in the body, and therefore they must be obtained from diet (Caballero *et al.*, 2003). The carbon furthest from the carboxyl group is known as the omega (n)-carbon, and the position of the first double bond from the methyl group is known as omega (n)-x, where x is the carbon number on which the double bond occurs (Ruxton *et al.*, 2005). According to this classification, two PUFA, omega-3 and omega-6 (n3 and n6) fatty acids, are essential in the diet and therefore are very frequently reported in food.

Recently, n3, n6 and the ratio of n6 to n3 fatty acids have acquired enormous attention. Most interest has been on n3 fatty acids, which have a number of beneficial effects on human health and disease. Many studies demonstrate a beneficial role for n3 fatty acids in reducing the severity of atherogenesis, chronic heart disease, hypertension, inflammatory disease, and cancer (Mills *et al.*, 2005; Mozaffarian *et al.*, 2004; Rose and Connolly, 1999; Simopoulos, 1991; Simopoulos, 1999). Linolenic acid (LA) and alpha-linolenic acid (ALA) are the parent compounds of the ω6 and ω3 fatty acids, respectively for FA metabolism (Ruxton *et al*., 2005). LA can be converted to gamma-linolenic acid (GLA), which can be elongated to dihomo-gamma-linolenic acid (DGLA), and then further desaturated to adrenic acid (AA). In the case of n6 FA, ALA can be converted to eicosapentaenoic acid (EPA) by using the same enzyme as n3 FA. Omega 6 FA has different metabolic effects from n3. More potential factors leads to heart disease and inflammatory disease with n6 metabolism (Wakil, 1970). Since there are differing effects of n3 and n6 fatty acids on metabolism, the ratio of n6 to n3 FA is important (Caballero *et al.*, 2003).

Table 2-8: Fatty acid compositions of molluscs

FA[a] (%)	Octopus	Scallop	Mussel	Oyster	Land snail	Marine snail[1]
C14:0	3.0	0.8-3.8	1.5-7.0	1.2-5.3	0.3-0.7	2.3
C15:0	0.3	0.2-1.0	0.4-5.0	0.3-1.1	0.3	N/A
C16:0	27.0	10.2-26.3	10.1-25.5	17.6-20.8	6.7-10.3	18.1
C17:0	1.0	0.5-1.9	0.2-2.9	1.3-2.1	0.7-2.0	ND
C18:0	10.0	3.4-8.1	3.7-10.8	0.9-7.9	10.4-16.1	4.8
C20:0	*	<0.5	<3.6	*	0.8	5.3
C22:0	N/A	<2.1[2]	<0.1	N/A	6.3	N/A
C24:0	N/A		<0.8	N/A	0.8	N/A
C14:1	*	*	<6.4	<0.3	0.1	2.7
C15:1	<0.1	<0.2	<2.0	0.2-0.4	*	N/A
C16:1	2.0	1.1-5.5	2.7-12.6	1.6-8.7	0.4-3.2	5.4
C17:1	2.0	0.2-4.1	<2.1	0.4-1.5	0.3	N/A
C18:1	7.0	1.7-8.6	1.5-7.3	5.3-9.0	10.0-21.6	16.5
C20:1	3.0	1.1-3.8	1.0-5.4	3.4-8.2	0.9-3.6	ND
C22:1	5.0	0.1-2.5	<6.0	0.3-1.8	*	ND
C24:1	N/A	<0.3	<0.8	*	2.0	N/A
C14:2	N/A	N/A	N/A	N/A	N/A	N/A
C15:2	N/A	N/A	N/A	N/A	N/A 11.0-24.5	N/A
C18:2 n6	1.0	0.3-1.8	1.6-5.0	1.7-11.9	9.1-12.1	3.0
C20:2 n6	N/A	0.2-0.8	0.5-3.3	0.2-0.8	0.5	0.6
C22:2	N/A	0.2-0.8	<2.9	0.1-1.1	0.8-4.3	N/A
C18:3 n3	*	<1.9	<2.5	<4.4	*	1.9
C18:3 n6	N/A	<0.2	<4.6	0.1-1.1	0.3-0.6	N/A
C20:3 n6	N/A	<0.3	N/A	0.1-0.2	N/A	N/A
C22:3	N/A	0.5-0.6	1.1	N/A	N/A	N/A
C18:4 n3	*	0.4-5.2	<6.0	1.6-7.9	9.0-16.9	N/A
C20:4 n6	N/A	0.2-8.8	<5.8	1.3-3.5	3.5-5.3	N/A
C22:4 n6	*	0.5-3.7	<1.7	0.6-2.4	0.1-5.5	N/A
C20:5 n3	18.0	12.5-23.2	11.6-31.6	7.9-21.2	0.7-2.0	N/A
C22:5 n6	*	0.9-2.9	0.7-2.3	0.7-1.2	ND or 0.2	N/A
C22:6 n3	21.0	8.7-31.0	9.0-26.4	7.3-21.9	2.7-11.9	N/A
n3	39	30.6-54.7	25.5-46.8	15.9-46.9	13.6-48.8	1.9
n6	1.0	2.1-12.4	2.1-9.4	2.4-16.5	4.9-7.8[2]	3.6
n3:n6	39	3.8-15.4	4.6-21.9	2.5-11.0		1.9[3]
References	Karakoltsidis *et al.*, (1995)	appendix 2	appendix 1	appendix 3	appendix 4	Miletic *et al.*, (1991)

[1]= % is % FA in total lipids
[2]= total content of C20:0, C22:0 and C24:0 were less than 2.1%
[3] =the ratio of snail was 1/n3:n6
[a]=unknown fatty acids were not shown on the table
*= trace (less than 0.1%)
^= less levels of 18:3 were contained in 20:1
^^= less levels of 20:4 were contained in 22:1
N/A = not available
ND=not detected

Fatty acid content and profile in shellfish vary between and within species. The amount and the profile of FA in shellfish will depend on their feed, size (age), gender, reproductive status, geographic location and season (Narváez *et al.*, 2008; Orban *et al.*, 2002; Pirini *et al.*, 2007; Ventrella *et al.*, 2008; Pazos *et al.*, 1987; Silina and Zhukova, 2007). Fatty acids found in the oil of various seafood species differ from terrestrial animals in having a substantial portion of 20- or 22-carbon chains. These consist of 20- and 22-carbon monoenes and very long chain

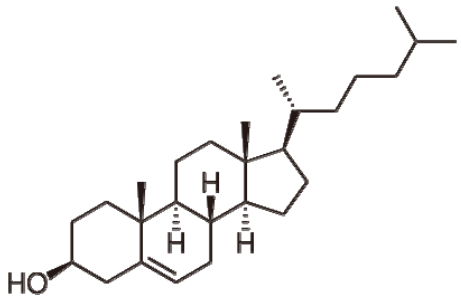
PUFA (Katvi, 2005). Table 2-8 summarises of the fatty acid composition of some common molluscs, including mussel, oyster, scallop, octopus, land snails, and some limited information on a marine snail. Saturated FA, C16:0 and C18:0 are the most predominant in shellfish. Snails, including land and marine ones, have the highest concentrations of C18:1, while other molluscs have higher C16:1 than C18:1. The two major PUFA in mussel, oyster, scallop and octopus were eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6), which are n3 FA, whereas snails had the largest amount of linoleic acid (LA C18:2) and arachidonic acid (AA C20:4), which are n6 fatty acids. Hence, the composition of PUFA in snails has more n6 than n3.

2.2.4 Other nutritionally important components

2.2.4.1 Cholesterol

Cholesterol belongs to the sterol group of fats (Figure 2-3). Cholesterol performs vital functions in the body such as being an important constituent of cell membranes contributing to the membrane fluidity and solute permeability. Cholesterol is also the precursor of steroid hormones, bile salts and vitamin D (Hames and Hooper, 2000).

Figure 2-3: Chemical structure of cholesterol
(http://commons.wikimedia.org/wiki/Image:Cholesterol.svg)



Although it plays a vital role in cell growth and development, the accumulation of cholesterol in certain tissues can lead to harmful effects. Increased concentration of cholesterol particularly low density lipoprotein cholesterol in the blood (hypercholesterolemia) has been found to be the major reason for coronary heart disease (Araujo *et al.*, 2007; Cui *et al.*, 2007; Gould *et al.*, 2007; Kosulwat *et al.*, 2003; Ware, 2008). This can damage the arteries especially coronary arteries that supply the blood to the heart. This can lead to accumulation of cholesterol-laden “plaques” in vessel linings, a condition called atherosclerosis. When

blood flow to the heart is impeded, the heart muscle becomes starved of oxygen causing chest pain. If a blood clot completely obstructs a coronary artery affected by atherosclerosis, a heart attack may occur which can lead to death (Orakzai *et al.,* 2008). Furthermore, a high blood cholesterol level is correlated with increased risk of diabetes, stroke and hypertension (Gould *et al.*, 2007; Law *et al.*, 2003; MRC/BHF, 2003; Sever *et al.*, 2003). Cholesterol is oxidized under certain conditions, such as thermal treatment, photocatalysis, or enzymatic oxidation. The oxidation process produces several hydroperoxide isomers which have been linked to several human diseases, such as atherogenesis, cytotoxicity, mutagenesis, and carcinogenesis (Guardiola *et al.*, 2002).

Table 2-9: Cholesterol contents of snails and some marine molluscs

Species		**Cholesterol Content**		**References**
		mg/100g FW	% total sterol	
Land Snails	*Helix* sp	86.6	87.2	Zhu *et al.*, (1994)
	Haplotrema sportella	118.4	91.0	
	Vespericola columbiana	116.0	86.0	
Marine snails	*Lunatia groenlandica*	98.0	N/A	Idler and Wiseman, (1971)
	Littorina littorea	56.0		
	Urosalpinx cinerea	98.5		
	Buccinum undatum	94.4		
	Neptunea decemcostrata	93.5		
	Busycon canaliculatum	85.5		
	Melampus lineatus	85.1		
Clam	N/A	218.0	42.5	Kritchevsky *et al.*, (1967); Lin *et al.*, (1982)
		190.0	36.7	
Oyster		150.0	41.4	
		140.0	57.4	
Crab		175.0	25.7	
Scallop	*Pectin* sp	N/A	34.1-68.0	Idler and Wiseman, (1971)

FW=fresh weight N/A= not available

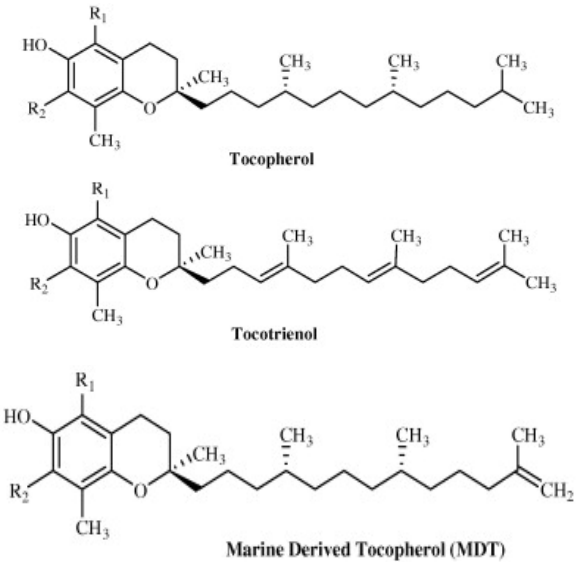
Many clinical trials have shown that lower blood cholesterol levels can reduce the incidence of heart attack (Araujo *et al.*, 2007; Cui *et al.*, 2007; Gould *et al.*, 2007; Kotseva *et al.*, 2008; Ware, 2008). Hence, it is useful to identify and limit the consumption of foods which are rich in cholesterol. Cholesterol is the major sterol in gastropods (Table 2-9). (Idler and Wiseman, 1971) investigated the sterols in 7 marine snails and found that more than 85% of the sterols were in the form of cholesterol in the 6 snail species while the remaining specie had about 56% of the sterols as cholesterol. Three land snails have been studied by (Zhu *et al.*, 1994) where they found that more than 87% of the sterols was cholesterol with an average of 100

mg cholestrol/100g fresh weight. Other marine molluscs like oyster, scallop and clam had higher (about 150-200 mg/100g fresh weight) cholesterol content than in land snails.

2.2.4.2 Vitamin E

Vitamin E was discovered as "factor X" by (Evans and Bishop, 1922) while investigating dietary factors that are essential for reproduction in rats. Later in 1924, their experiment was repeated and the substance was named vitamin E. Vitamin E is insoluble in water but is readily soluble in non-polar solvents (Ball, 2004). Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, the α-, β-, γ-, and δ-tocopherols and the α-, β-, γ-, and δ-tocotrienols, where α-tocopherol is the most abundant and important member (Figure 2-4). The vitamin E members are designated as α-, β-, γ-, and δ- according to the number and position of substituent methyl groups on the chromanol ring of tocopherols and tocotrienols (Ball, 2004). The tocotrienols have three double bonds in the phytyl side chain, whereas the tocopherols contain a trimethyltridecyl tail with three chiral centres which naturally occur in the *RRR* configuration (Zingg, 2007). Recently, a natural vitamin E which is called marine derived tocopherol (MDT) was found in some marine organisms (Figure 2-4), with a single unsaturated bond at the end of the side chain which is thought to be responsible for cold-water adaptation (Zingg, 2007).

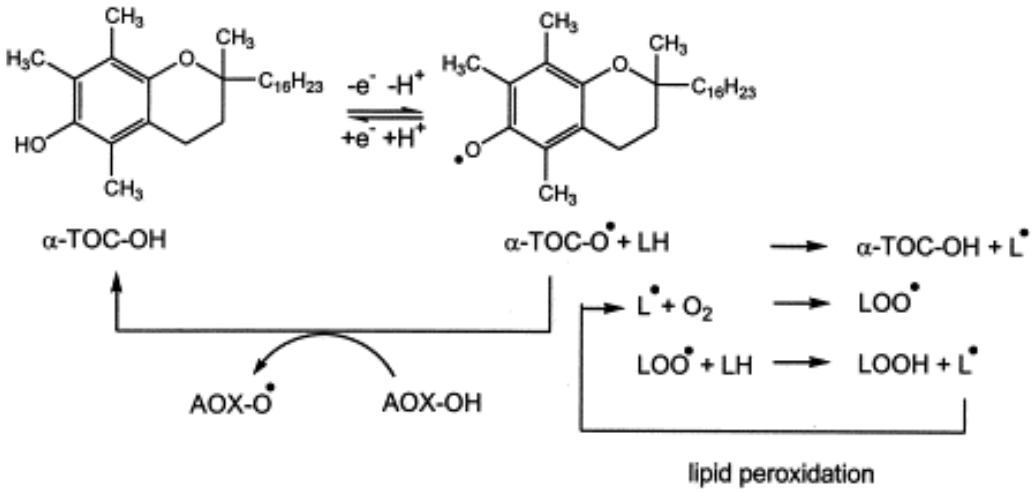
Figure 2-4: Vitamin E analogues (Zingg, 2007)



R_1	R_2	
CH_3	CH_3	α
CH_3	H	β
H	CH_3	γ
H	H	δ

Vitamin E is rich in plant products especially in plant oils such as sunflower and soybean oils and lesser amounts are found in animal tissues (Ball, 2004). Vitamin E has a significant antioxidant activity (Rietjens *et al.*, 2002). Vitamin E is the major antioxidant in the lipid environment, protecting against lipid oxidation, and the mechanism action is shown in Figure 2-5 (Rietjens *et al.*, 2002). Vitamin E may help against the aging, immune responses and counteract atherosclerosis (Azzi and Stocker, 2000).

Figure 2-5: Mechanism of the antioxidant activity of vitamin E (Rietjens *et al.*, 2002)



Since there is a large proportion of unsaturated FA, especially the long chain FA and essential FA in marine snails, the potential of vitamin E availability in *C. sulcata* to protect these FA from oxidation is biologically sound. Therefore, it is a worthwhile to study vitamin E concentration in *C. sulcata*.

2.3 Elements and potential toxic compounds in snail

2.3.1 Elements

Elements are found in minute amounts in living organisms but can cause detrimental effects if the concentrations are higher or lower than the physiological basal concentration. Elements are considered essential for normal development and growth of biological systems (Pais and Jones, 2000). A classification which was suggested by (Feiden, 1984), categorised elements into various categories based on the amount found in tissue rather than designating a specific function for essentiality (Table 2-10). Based on that definition, minerals can be categorized as structural elements [(e.g phosporous (P)], essential trace elements [the most important being

iron (Fe), copper (Cu), and Zinc (Zn), and toxic [(e.g lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg)].

Table 2-10: Classification of minerals (Feiden, 1984)

Classification			**Elements**
Bulk structural elements			C, H, O, P, S
Essential elements	Macroelements		Ca, Cl, K, Na
	Microelements (Trace elements)		Cu, Fe, Zn
Toxic elements	Ultratrace elements	Non-metals	As, B, F, I, Se
		Metals	Cd, Cr, Co, Pb, Mn, Mo, Ni, Sn, V

The essential elements include macroelements and microelements and are required to support adequate growth, reproduction, and maintain health throughout the life cycle, when all other nutrients are optimal (McDowell, 2003). The non-toxic elements have four broad functions: structural, physiological, catalytic, and hormonal or regulatory. The most obvious function of trace elements is to provide structural support. For examples: Ca, phosphate and magnesium contribute to the mechanical stability in bones and teeth. Moreover, P and S are essential compounds in muscle proteins which are the structural components of body tissues. Furthermore, Zn and P can also contribute to structural stability of the molecules and membranes which they are part of (McDowell, 2003).

Toxic elements which are also called heavy metals have a high atomic weight (Pais and Jones, 2000). Some of them are extremely toxic. They are non-biodegradable and therefore can accumulate in the environment over time, and they can be absorbed by plants or animal tissues. After absorption, heavy metals tend to bind to biomolecules such as proteins and nucleic acids, impairing their function (Yu, 2005). Toxic effects of heavy metals including growth rate depression, heavy-metal induced diseases and immune functions have been studied in detail (Ooik *et al.*, 2008; Pattee *et al.*, 2003; Yu, 2005). Heavy metals in aquatic systems originate from numerous sources, both natural processes (eg., weathering of rocks, atmospheric deposition) and anthropogenic activities (eg., storm-water runoff, industrial agricultural process and sewage discharges) (Florea and Büsselberg, 2006; Pattee *et al.*, 2003). Aquatic organisms exposed to heavy metals suffer from a decreased metabolic rate and a decreased ability to siphon food and the extent of toxic effect and the bioaccumulation of these metals will depend on these being bioavailable to the organisms (Pattee *et al.*, 2003;

Waldichuk, 1985). However, different species of marine organisms have different rates of bioaccumulation of toxic metals and the sites of accumulation within the organism may also differ from one species to another (Waldichuk, 1985).

Research on the trace elements of molluscs including land snails and marine shellfish are summarised in Table 2-11 and Table 2-12, respectively. The marine shellfish have a higher amount of both macro and trace elements than land snails except for Ca. Calcium concentrations were higher (around 260 mg/100g) in land snails (varied from 22 in *Limicolaria* sp. to 726 mg/100g in *Helix pomatia*) compared with marine shellfish (Adeyeye, 1996; Özogul *et al.*, 2005). However, the predominant element in marine shellfish was S with a mean value of more than 300 mg/100g (Vlieg *et al.*, 1991). The concentrations of Na, K and P in land snails are within a narrow range from 100 to 120 mg/100g. Similar concentrations are found in marine shellfish. In addition, the Mg content in both in land snails and marine shellfish are similar, with mean values of 44 and 50, respectively. Heavy metals concentrations differ among species and even within same species in marine shellfish. Information on heavy metals in land snails is limited.

Table 2-11: Essential and toxic element contents of land snails

	***Limicolaria* sp.**			***Archachatina marginata***	***Archacha* sp.**			***Helix* sp.**	***Helix pomatia***
Na	178*	54.0	65.0	196.0	182.0	53.0	61.0	N/A	91.0
K	533*	73.0	198.0	44.0	50.0	210.0	194.0	N/A	82.0
Ca	401*	22.0	209.0	212.0	189.0	208.0	205.0	319.0	726.0
Mg	771*	19.0	46.0	46.0	38.0	45.0	46.0	60.0	54.0
P	636*	183.0	154.0	1.0	216.0	123.0	131.0	91.0	105.0
S	N/A	N/A	4.0	N/A	N/A	2.0	3.0	N/A	N/A
Fe	ND*	4.7	9.5	9.3	5.0	9.4	9.4	1.3	1.7
Cu	N/A	0.2	ND	0.6	0.6	ND	ND	3.2	N/A
Zn	259.0*	1.2	1.5	1.4	1.9	1.7	1.8	1.1	1.4
Mn	N/A	0.6	0.4	0.4	0.4	0.4	0.4	N/A	0.2
Al	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
As	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cd	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Co	N/A	ND	ND	ND	ND	ND	ND	N/A	N/A
Cr	ND	ND	N/A	ND	ND	N/A	N/A	N/A	N/A
Ni	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pb	ND	N/A	ND	N/A	N/A	ND	ND	N/A	N/A
References	1	2	3	2	2	3	3	4	5

Na, K, Ca, Mg, P, S, Fe, Cu, Zn and Mn are expressed as mg/100g fresh weight
Al, As, Cd, Co, Cr, Ni, and Pb are expressed as µg/100g fresh weight
ND= not detected N/A= not available *=mg/100g dry weight basis
1 = Udoh *et al.*, (1995) 2=Adeyeye, (1996) 3 = Fagbuaro *et al.*,(2006)
4 = Gomot, (1998) 5= Özogul *et al.*, (2005)

Table 2-12: Essential and toxic element contents of shellfish

	Mussel			Scallop				Oyster
	1	2	3	4	5	6	7	8
Na	N/A	N/A	211.0	429.0	319.0	149.0	N/A	N/A
K	328.0	N/A	320.0	236.0	315.0	348.0	N/A	N/A
Ca	88.0	N/A	11.0	134.0	43.0	31.0	N/A	N/A
Mg	44.0	N/A	41.0	58.0	55.0	50.0	N/A	N/A
P	N/A	N/A	138.0	179.0	279.0	258.0	N/A	N/A
S	N/A	N/A	324.0	310.0	N/A	N/A	N/A	N/A
Fe	10.9	7.7	0.6	4.2	14.3	9.4	1.9	5.5
Cu	1.1	0.2	1.2	9.3	0.2	0.1	0.2	7.9
Zn	4.6	3.4	1.3	1.9	1.5	2.0	2.3	84.4
Mn	0.8	0.2	16	148	0.9	4.6	0.8	0.3
Al	N/A	2.9	N/A	N/A	N/A	N/A	ND	13.4
As	N/A	29.7	297	336	N/A	N/A	8.8	9.1
Cd	2.0	0.7	41	34	N/A	N/A	2.8	ND
Co	0.2	0.1	N/A	N/A	N/A	N/A	2.3	0.2
Cr	10.0	ND	11	13	N/A	N/A	0.1	0.1
Ni	7.0	0.5	ND	ND	N/A	N/A	ND	0.5
Pb	1.0	1.1	N/A	N/A	N/A	N/A	0.3	24.9
References	1 from Karakoltsidis *et al.*, (1995); 2, 7, 8 from Sidwell *et al.*, (1978); 3,4 from Vlieg *et al.*, (1991); 5,6 from Hughes *et al.*, (1980);							

Na, K, Ca, Mg, P, S, Fe, Cu, Zn and Mn are expressed as mg/100g fresh weight
Al, As, Cd, Co, Cr, Ni, and Pb are expressed as µg/100g fresh weight
ND= not detected N/A= not available *= on dry weight basis
1= *Mediterranean* 2= *Mytilidae* 3= *Atrina zelandica*;
4=*Perna canaliculu* 5= *Perna canaliculus* 6= *Pectan novaezealandiae*;
7= *Pectinidae* 8= *Ostreida*

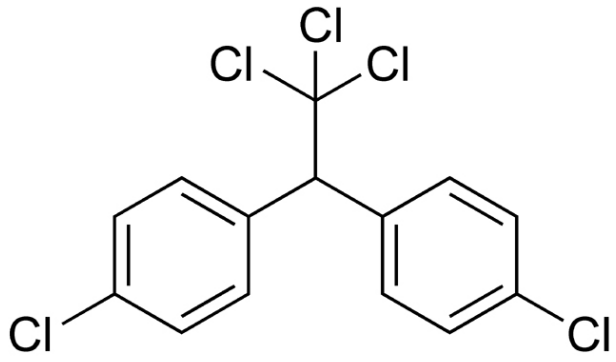
For quite a long time, assessment of heavy metal contamination in aquatic environment was based on four methods, namely analysis of water, sediment, biological material or a combination of all three (Milne, 1998). However, all these analysis contained weaknesses. Metal concentrations are often quite low in water, which makes the quantification both difficult and expensive. Furthermore, metal concentrations in sediments can be affected by a number of factors including sediment particle size, organic carbon content and high spatial variability. Hence, in recent years, aquatic organisms have been used as a biomonitor of heavy metal contamination in the coastal environment (Morrisey *et al.*, 1994; Phillips, 1977; Waldichuk, 1985). Aquatic organisms like shellfish are sedentary and cannot move from an environment that is becoming increasingly toxic from the heavy metals deposition (Kennedy, 1986; Smith and Williamson, 1986). Since *C. sulcata* is a common shellfish in New Zealand and has the advantage of being a sedentary animal, we believe it can potentially be used as a biomonitor for heavy metal contamination.

2.3.2 Organochlorine pesticides (OCPs)

Pesticides refer to any chemicals intended to prevent, deter, destroy, or otherwise impair the ability of pests to compete with desired organisms (Yu, 2005). Depending on the target, pesticides can be classified as insecticides, herbicides, fungicides, rodenticides, algaecides, and nematocides. Organochlorine pesticides are effective against insects. DDT (2,2-bis [p-chlorophenyl]-1,1,1-trichloroethane or dichloro-diphenyltichloroethane), aldrin, chlordane, dieldrin, endrin, lindane (BHC), hexachlorobenzene (HCB), and heptachlor are some examples (Yu, 2005). Organochlorine pesticides which are also called chlorinated hydrocarbons, are insecticides, and were the first commercial organic insecticides to be developed, especially the DDT (Yu, 2005). DDT which belongs to the family of organic halogen compounds was prepared by the reaction of chloral with chlorobenzene in the presence of sulphuric acid, and was first synthesized in 1874 (Figure 2-6). It was first used as an effective and a cheap insecticide in 1939 for controlling disease-carrying insects, such as mosquitoes (Bearsd, 2006; Ross, 2005). During the World War Ⅱ, DDT was used by soldiers to control the body lice that spread typhus. Later, it was used world-wide, including New Zealand to combat a large number of pests, including gypsy moth, potato pests, corn earthworm, grass grub and codling (Yu, 2005).

Figure 2-6: Chemical structure of DDT

(http://www.tc.umn.edu/~allch001/1815/pestcide/sim/ddt.jpg)

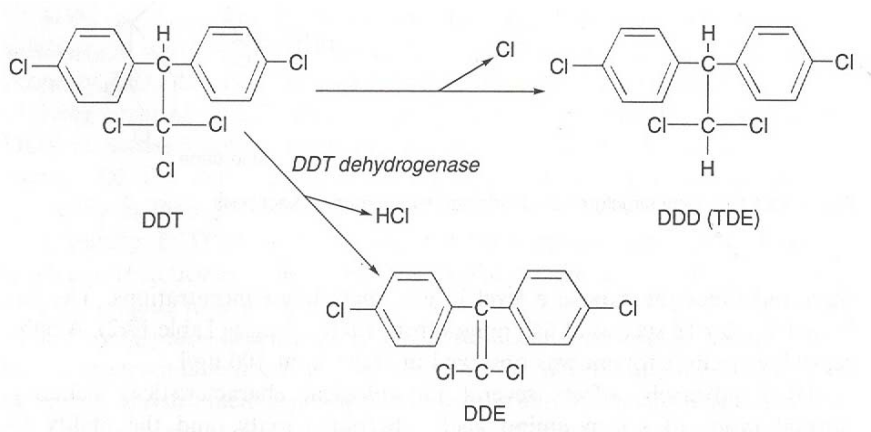


Organochlorine pesticides are fat soluble and can enter the food chain through the absorption by fat and accumulation in food. For example, OCPs can enter the aquatic systems through a number of ways, including run-off from non-point sources, industrial discharge and sewerage waste water and wet/dry deposition (Sarkar *et al.*, 2008). Moreover, OCPs can be transferred

globally through the atmosphere by an effect called "chemical distillation" (Hong *et al.*, 2008; Yang *et al.*, 2008). This can be explained by the nature of OCPs which are regarded as semi-volatile compounds. They can evaporate and travel long distances from warmer areas to cooler regions following condensation, deposition and accumulation.

Organochlorine pesticides have been considered as one of the most problematic chemicals due to their resistance to biodegradation, persistence, and bioaccumulation, and their adverse effects on wildlife, human and the environment in the last few decades (Zhou *et al.*, 2008). DDT can affect human health negatively. Thus, in the 1960s, DDT was limited or totally banned in industrialized countries where its environmental and hazardous impact became evident (Zhou *et al.*, 2008). DDT is stable to metabolic breakdown in the environment. However, it can degrade to DDE (ethyl 1,1-dichloro-2,2-bis(p-chlorophenyl) and DDD (ethane 1,1-dichloro-2,2-bis(p-chlorophenyl) (Figure 2-7). Some of the adverse effects of DDT and its metabolites on human health is its effects on the nervous system, interference with the endocrine system, and reproduction (Yu, 2005). DDT accumulation by disrupting the body's normal hormonal system is of great importance especially in pregnant women who can pass it to their infants via breast milk (WHO, 1972).

Figure 2-7: Metabolism of DDT (Yu, 2005)



In New Zealand, DDT was banned in 1960s. However, because of the characteristics of DDT, such as persistency and high lipophilicity, it is still present in the environment and tends to accumulate in adipose tissue of animals including marine organisms over a long period of time. As a result, fish and shellfish have been used as biological indicators of DDT contamination (Amaraneni and Pillala, 2001; Chen *et al.*, 2002).

It has been noted that the greatest risk of DDT to marine organisms was due to the contaminated sediment in beaches near agricultural fields that used DDT, which acted as long-term sinks of DDT and its metabolites (Yu, 2005). Many reports have shown that DDT and its metabolites could be directly toxic to marine organisms such as shellfish and fish at low concentrations (Kennish and Ruppel, 1998; Manirakiza *et al.*, 2002; Solly and Harrison, 1972; Tavares *et al.*, 1999). In the present study, the sampling area was around 200 meters away from a dairy farm, which might have the potential of long-term effects of having DDT in soil, thus rendering the collected samples to be potentially unfit for consumption. In addition, due to the animal ecological behaviour and being attached to rocks for extended periods, there is a potential to utilize *C. sulcata* as bioindicator for marine pollution. So it was important to determine the contamination by DDT and its metabolites in the experimental samples.

Chapter 3: Nutrition Evaluation of Snail Meat *Cookia sulcata*

3.1 Introduction

Poultry, pork, beef, lamb and fish are considered the major sources of meat protein for humans. These sources are gradually depleted by persistent drought, diseases, natural disasters, high cost of feed, and low productivity of local breeds, especially in some developing countries (Copper and Knower, 1991). The continuous growth of the earths population over the decades has led to a greater pressure on the existing animal protein sources. Also, it is well established that consumption of animal products which contain a high fat content particularly saturated fats could result in several pathological conditions including elevated plasma cholesterol levels and vascular coronary heart disease. This has gained momentum in the recent years and the search for alternative meat sources which contain little saturated fat and can contribute to solving the shortage of meat protein is ongoing. In this regard, utilization of new meat protein resources as an alternative to common animal protein sources has attracted much attention. In recent years, research into the nutritional values of molluscs has increased and the main reason for this interest is the large number of molluscs available since it is the second largest phylum in the animal kingdom (Copper and Knower, 1991).

Molluscs constitute at least 80000 species and members of this group are found throughout the world. They are predominantly found in aquatic systems, but some are terrestrial (Adeyeye, 1996). The phylum includes univalve organisms such as snails and slugs, bivalves such as mussels, oysters and cockles, and cephalopods such as octopus, squid and cuttlefish (Copper and Knower, 1991). Snails play important diverse roles in nature and are also sensitive indicators of environmental change (Adeyeye, 1996). In addition, Milinsk *et al.*, (2006a) and Ademolu *et al.*, (2004) reported that snail meat contained little fat but was rich in protein and contained many nutrients required in a healthy and well balanced diet. Snails are harvested for food in many parts of the world and they are considered a delicacy in France, Korea, Japan and China (King and Bruce, 1949; Mohan, 2002; Qun *et al.*, 2004; Xia *et al.*, 2007). Hence, there is potential for using the underutilised snail meat from aquatic resources to help fill the gap in current animal protein shortage.

Most of the available information regarding the nutritional content of snails has been from studies on land snails (Fagbuaro *et al.*, 2006; Hamzat *et al.*, 2002; Rees and Hand, 1993); (Gomot, 1998; Milinsk *et al.*, 2006a; Milinsk *et al.*, 2006b; Oudejans and Horst, 1974). Information for marine snails is scarce. New Zealand with potentially rich marine resources contains a large amount and diverse species of marine snails. Therefore, the present study was designed to assess selected nutritional aspects of *C. sulcata* (an abundant marine snail in New Zealand). Proximate analysis, amino and fatty acid profiles, vitamin E, and cholesterol level were determined in *C. sulcata* collected from Te Oka Bay near Christchurch (Figure 3-1).

3.2 Material and methods

3.2.1 Sample collection

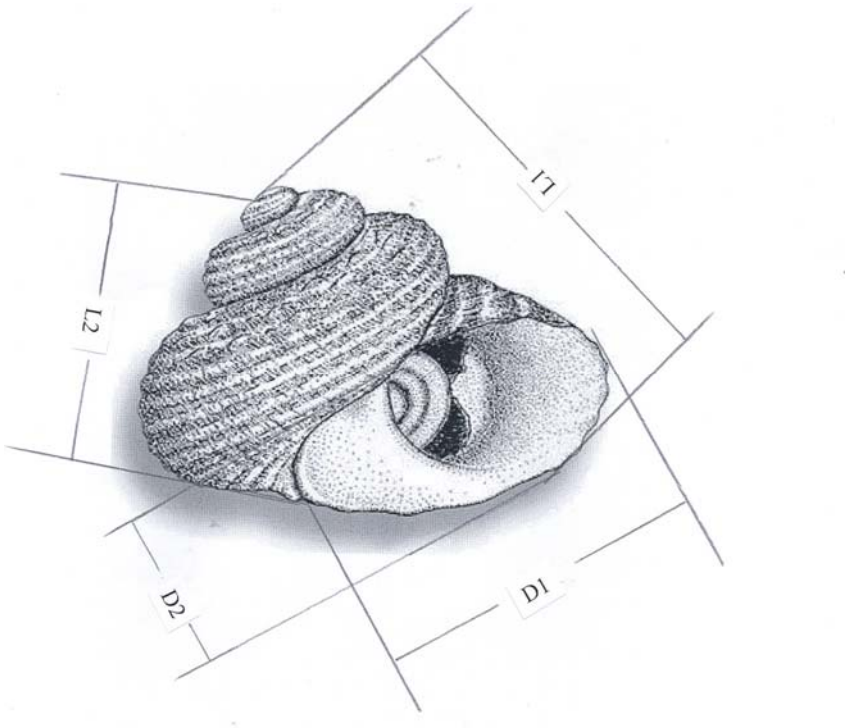
Snails were collected from Te Oka Bay (Figure 3-1) between middle tide and low tide level in late 2006 (n=10) and early 2007 (n=84). The live snails of different sizes were collected for both the nutritional and toxicological analysis and stored in a plastic pot filled with seawater and kept on ice during transport to the laboratory. The snails were frozen upon arrival at the laboratory and stored at -18 °C until analysis. Between 11 and 16 individual samples were used for each analysis. The snails were analysed individually unless stated otherwise in the method. Sex of the snails was not determined because this was not criterion for the consumption of snails, and also there was no expert available to identify the sex.

3.2.2 Size and weight measurements

On the day of measurement the snails were thawed overnight in a refrigerator at 4 °C and the dimensions of the animals, diameter 1 (D1), diameter 2 (D2), length 1 (L1), length 2 (L2) were determined (Figure 3-2). The snails were blotted dry, weighed and then cracked open to allow removal of the meat. The edible part (foot, parts of the visceral hump and muscle) was separated from the whole animal. The weight of the whole animal (with shell) and the weight of the edible part were measured to determine the yield.

Figure 3-1: The sampling site for *C. sulcata*.

Figure 3-2: The size measurement of *C. sulcata* shell



3.2.3 Sample preparation

For FA, vitamin E and cholesterol analysis, wet snail meat was used. Weighed portions of edible snail meat were freeze dried, re-weighed to measure the dry matter content (DM %) and determine the moisture loss during freeze drying. Freeze dried snail meat were individually transferred to glass tubes, sealed and stored in a refrigerator (4 °C) pending proximate and amino acid analysis.

3.2.4 Proximate analysis

Proximate composition analysis of individual snails was carried out as described in standard methods (AOAC, 2000).

3.2.4.1 Moisture analysis

Duplicate sub-samples (1.0 g each) from each sample were dried in the oven at 103 °C (±5 °C) for 48 hours. Weight loss was determined by the difference in the sample weight before and after drying and was converted to residual dry matter (rDM%). Moisture content was calculated by the difference from the total dry matter content [DM% × (rDM%/100)] and the moisture content was expressed as a % of the sample wet weight.

3.2.4.2 Crude fat analysis

Crude fat content was determined using Soxhlet extraction method (AOAC, 2000). Duplicate sub-samples (1.0 g each) from each snail were used and the fat content was expressed as a % of the sample wet weight.

3.2.4.3 Crude protein (total nitrogen) analysis

Crude protein content was measured using Kjeldhl digestion method (AOAC, 2000). Duplicate samples (0.5 g each) for each sample were used and the crude protein content was expressed as a % of the sample wet weight.

3.2.4.4 Ash analysis

Ash content was determined according to (AOAC, 2000). Duplicate samples (0.3 g each) for each sample were used and the ash content was expressed as % of the sample wet weight.

3.2.4.5 Carbohydrate analysis

Carbohydrate content was calculated by the difference of the sum of moisture, fat, protein and ash contents from 100%.

3.2.5 Amino acid analysis

3.2.5.1 Sample preparation

Freeze-dried samples were finely ground to pass`through a 0.5 mm screen using an Ultra Centrifugal Mill ZM 100 (Gmbh and Germany). Three preparation methods were used for different groups of amino acids.

Acid hydrolysis was used for analysis of amino acids: aspartic acid (Asp), glutamic acids (Glu), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), tyrosine (Tyr), valine (Val), phenylalanine (Phe), isoleucine (Ile), lysine (Lys), leucine (Leu), proline (Pro), taurine (Tau) (Gehrke *et al.*, 1985). Samples (0.1 ± 0.005g) were weighed in 50 ml round bottom flasks. Samples (0.1 ± 0.005g) were weighed individually in culture tubes and 5 ml 6N of HCl was added to each tube. 10.0 µl internal standard (0.5 M of amino-butyric acid) was added and mixed well by ultrasonication for 5 min. Then tubes were purged with nitrogen, sealed and heated under 110 ^{o}C for 20 hours in block heater. After cooling to room temperature tubes were washed with nanopure water (10 ml) and vacuum evaporated at 45 ^{o}C till dryness. Lastly, dried samples were re-dissolved with nano-pure water to 50 ml, filtered through 0.45 µm filter (LabServ Filtration, BIOLAB, Australia), and stored in vials at -20 ^{o}C till high performance liquid chromatography (HPLC) analysis.

Alkaline hydrolysis of sample was used for analysis of the amino acid, tryptophan (Trp) (Yust *et al.*, 2004). Samples (0.1 ± 0.005g) were weighted, dissolved in 3 ml 4.2 N NaOH, and incubated in an oven at 105 ^{o}C for 5 hours. After cooling to room temperature, each sample was neutralized to pH 6.5 using 12 N HCl, diluted to 25 ml with sodium borate buffer (pH=9.39), filtered through 0.45 µm filters (LabServ Filtration, BIOLAB, Australia), and stored in vials at -20 ^{o}C till HPLC analysis.

Oxidation was used for analysis of sulphur-containing amino acids, cysteine (Cys) and methionine (Met) (Gehrke *et al.*, 1985; Gehrke *et al.*, 1987; Toran *et al.*, 1996). A 5 ml performic acid, [made by mixing formic acid and peroxide (19:1 v/v) at room temperature for 1 hour was transferred into an ice bath 15 min before use] and 10 µl internal standards (0.5 M

norleucine) were added into each flask. Flasks were sealed and kept in an ice bath for 17 hours. Each sample was then dried in a vacuum evaporator at 40 ^{o}C after adding 0.75 ml hydrobromic acid (HBr). 15 ml of 6 N HCl was used to dissolve the samples again and flasks were refluxed in an oil bath at 115-120 ^{o}C for 23 hours under reflux. After cooling, samples were vacuum dried at 40 ^{o}C again, re-dissolved in nanopure water to 50 ml, filtered through the 0.45 μm filters (LabServ Filtration, BIOLAB, Australia), and stored in vials at -20 ^{o}C till high performance liquid chromatography (HPLC) analysis.

3.2.5.2 Amino acid detection

The amino acids analysis was performed on an Agilent 1100 series HPLC systems (Agilent Technologies, Waldbronn, Germany), which consisted of a degasser, binary HPLC pump, auto sampler, thermostat, fluorescence detector and a photodiode array detector, controlled by Chemstation Software (Version A 09.01). A Lichrospher 100 NH2 (250*4 mm, particle size 5 μm) coupled with a LiChroCART 4-4 guard column was used to separate the amino acids at a temperature of 40°C.

A mixture of buffer A (0.01 M of Na_2HPO_4 with 0.8% tetrahydrofuran, pH=7.5) and B (20% buffer A, 40% methanol and 40% acetonitrile) was used as the elution buffer for acid and alkaline hydrolysed samples at a flow rate of 1.2 ml/min with the gradient as shown in Table 3-1 for a total run time of 32 minutes. The detection of all the amino acids, except Cys and Met, was carried out using a fluorescence detector at wavelengths of 335 nm and 440 nm for excitation and emission, respectively for 18 minutes. After that, the detector was switched to a second channel at wavelengths of 260 nm and 315 nm for excitation and emission respectively for optimal detection of Trp (Sánchez-Machado *et al.*, 2008). For cysteine and methionine a mixture buffer of 0.02 M of CH_3COONa (pH=6.0) and methanol was used as elution buffers at a flow rate of 1.0 ml/min with the gradient shown in Table 3-2. The detection of Cys and Met was achieved using a fluorescence detector at wavelengths of 335 nm and 440 nm for excitation and emission, respectively for a total run time of 21 minutes.

Table 3-1: HPLC analysis gradient for elution of amino acids from acid and alkaline hydrolyzed samples

Time (min)	Buffer A[1]	Buffer B[2]
24	20%	80%
26	0%	100%
32	100%	0%

[1] : Buffer A= 0.01 M of Na_2HPO_4 with 0.8% tetrahydrofuran, pH=7.5

[2] : Buffer B= 20% buffer A, 40% methanol and 40% acetonitrile

Table 3-2: HPLC analysis gradient for elution of amino acids from oxidized samples

Time (min)	CH_3COONa solvent (0.02 M, pH=6.0)	Methanol solvent
0	66%	34%
5	40%	60%
17	0%	100%
21	66%	34%

3.2.5.3 Standards and quantification

Standard mixtures of Asp, Glu, Ser, His, Gly, Thr, Agr, Ala, Tyr, Val, Phe, Ile, Lys, Leu, Pro, Tau, Cys, Met and Try (Sigma Aldrich, Aus.) at concentrations of 25, 50, 100, 250, 500 µM were used to construct standard curves. Amino acid contents were identified and quantified by the comparison of the retention time and the peak areas of pure standards.

3.2.6 Fatty acid analysis

3.2.6.1 Sample preparation

Edible muscle tissues were thawed at 4°C overnight and lipid extraction was carried out using a modification method of Folch *et al*., (1957). A 4 g sample of snail muscle was homogenized with 90 ml of a chloroform-methanol mixture (1:2 vol/vol). The homogenate was filtered through 2 glass fibre filter papers and the retentate was washed with another 90 ml of chloroform-methanol mixture and 75 ml of phosphate buffer (pH 6). The mixture was kept at 4°C overnight to allow the separation of the lipid chloroform layer from the methanol-water

layer. The lipid-chloroform layer was transferred to a flask and the chloroform was evaporated in a rotary evaporator (40 °C). The residual oil was flushed with nitrogen and stored in a freezer at -20 °C until analysis.

Methyl esters were prepared by transmethylation using 1% sulphuric acid in methanol and n-hexane according to the method described by Hughes *et al*. (1980) with minor modifications. The extracted fat, (minimum 10 mg), was mixed with 1 ml internal standard (tridecanoic acid, 3mg/ml in heptane), then 2 ml of 1% sulphuric acid in methanol was added to the mixture, flushed with nitrogen and incubated at 50 °C for 16 hours. The non-lipid impurities were removed from the cooled tubes with 2ml of 5% NaCl and the methyl esters were extracted with 2 ml of hexane. The organic phase was transferred to a new tube and washed by 2ml of potassium bicarbonate (2%) and dried using anhydrous sodium sulphate. The volume of the hexane fraction was reduced to 1.5 ml in a stream of nitrogen and samples were kept at -20 °C until analysis by gas chromatography (GC).

3.2.6.2 Fatty acids detection

Fatty acid methyl esters were separated using a GC model 2010 (Shimadzu, Japan) equipped with an auto-sampler (AOC-20i, Shimadzu, Japan), a split/splitless injector, a flame ionization detector (FID), and a 30 m 0.25 mm ID JW-Innowax column with polar ethylene glycol stationary phase (0.25 μm film thickness). The GC was operated and controlled using GC Solution software (Version 2.300 SU4).

The oven temperature was initially held at 50 °C for 0.5 min, then heated to 150 °C at 20 °C/min, then to 250 °C at 2 °C/min, and held for 30 min. Post-run temperature was 240 °C for 5 min. The injector and detector temperatures were set at 230 and 250 °C, respectively. A 1 μl sample was injected (split ratio 30:1). The carrier gas was initially at 0.6 ml/min and a pressure of 55.7 KPa which was then ramped to 107 KPa at 4 KPa/min and then to 200 KPa at 7 KPa/min to reduce baseline drift caused by the temperature increase.

3.2.6.3 Standards and quantification

Tridecanoic acid (C13) (T0502-5G, Sigma Aldrich, Bellefonte, USA) at a concentration of 3 mg/ml was used as the internal standard. Fatty acids were identified by comparing the retention times of fatty acid methyl esters (FAME) with a standard of mixed known composition (Nu-Check GLC-68E, GLC-68F and GLC-411, Nu-Check, Elysian, USA). The

results were expressed as the percentage of the total FAME and mean value ± standard deviation.

3.2.7 Vitamin E analysis

3.2.7.1 Sample preparation

Vitamin E was measured in 15 snails as described by Ng *et al*. (2004). Muscle tissue (4 g of wet weight) was weighed and homogenized in 20 ml of ethanol containing 0.2% BHT, and 8 ml tissue homogenate was mixed with 0.2 ml of 60% KOH. The mixture was flushed with nitrogen, capped, vortexed, and saponified at 70 °C for 20 min. After cooling on ice, 8 ml of 20% NaCl was added to the mixture and then 12 ml hexane containing 10% (v/v) ethyl acetate was added for the extraction of vitamin E and cholesterol. The hexane phase was divided into two tubes, A and B (2:3 vol/vol), and dried under a stream of nitrogen and stored frozen at -20°C until analysis. Tube A was used for vitamin E determination, and tube B was used for cholesterol determination.

3.2.7.2 Vitamin E determination

Vitamin E was analysed using an Agilent 1100 series HPLC systems (Agilent Technologies, Waldbronn, Germany), which consisted of a degasser, a binary HPLC pump, an auto sampler, a thermostat, a fluorescence detector and a photodiode array detector, and was controlled by Chemstation Software (Version A 09.01). A Lichrospher 100 NH2 column (250*4 mm, particle size 5 μm) coupled with a LiChroCART 4-4 guard was used for the analysis of the samples. Column temperature was 40 °C for vitamin E analysis. A mobile phase of heptane: tert-butymethyether: tetrahydrofuran: methanol (79: 20: 0.98: 0.02 v/v/v/v) with a flow rate of 1.2 ml/min was used and vitamin E was detected using a fluorescence detector at wavelengths of 294 nm and 320 nm for excitation and emission, respectively.

Standard mixture of α-tocopherol, δ-tocopherol and γ-tocopherol (LB-37874, LB-98130, LB36642, Sigma Aldrich, Bellefonte, USA) at concentrations of 5, 12.5, 20, 25 μg/ml were used to construct a standard curve for tocopherols. Different tocopherols in *C. sulcata* were identified by comparing the retention times of the sample peaks with standards and the concentrations were calculated by comparison with the standard calibration curves.

3.2.8 Cholesterol analysis

3.2.8.1 Sample preparation

The sample preparation for cholesterol analysis was the same for vitamin E (section 3.2.7.1).

3.2.8.2 Cholesterol determination

Cholesterol was measured using a PU 8675 VIS spectrophotometer (Philips, UAS.). The dried lipid sample (as described in section 3.2.7.1) was dissolved in 5 ml of chloroform and 2 ml of Liberman-Burchard reagent (0.5 ml of sulphuric acid dissolved in 10 ml of acetic anhydride, kept on ice in dark) was added to develop the blue-green oxidation product. Absorbance was determined at 640 nm (Sabir *et al.*, 2003).

Cholesterol standard (10 mg) (Product No. 43011, BDH Chemicals Ltd, Poole, England) was dissolved in 10 ml chloroform, and 5 dilutions from this standard were used to construct a standard curve as shown in Table 3-3. Cholesterol concentrations in the samples were calculated from a standard calibration curve.

Table 3-3: Standard and blank solutions for cholesterol analysis

Reagents (ml)	Blank	Standard Concentrations				
		S1	S2	S3	S4	S5
Standard concentration solution	0	0.5	1	1.5	2	2.5
Chloroform	5	4.5	4	3.5	3	2.5
Liberman-Burchard reagent	2	2	2	2	2	2

S: Standard

3.2.9 Statistical analysis

Analysis of variance was used to test for the effect of snail size on snail compositions. The data were analyzed using the general linear model protocol in MINITAB (release 15). The significant difference between means was determined by Tukey Simultaneous Tests ($p < 0.05$).

3.3 Results and discussion

3.3.1 Size measurements and classification of snails

Four external measurements were made on the snails and shells, and the whole animal weight was used to generate a classification system that reflected the size and maturity of the snails

so that an understanding of the relationship between size/maturity and the nutritional and toxicological parameters could be developed. The measurements were diameter 1 (D1), diameter 2 (D2), length 1 (L1) and length 2 (L2) as shown in figure (3-2). The weight of the whole animal and the whole edible weight were also measured to determine the yield. The snails were divided into two groups, small (≤ 60g whole animal weight) and large (> 60g whole animal weight) (Table 3-4, Appendix 8). This classification gave two groups that differed significantly ($P < 0.001$) in the measured size parameters (D1, D2, L1 and L2). As expected, higher ($P < 0.001$) edible weight was found in large snails compared with small snails. However, the % yield of the snails was similar in both small and large snails (Table 3-4). Strong positive correlations were found between whole animal weight, edible weight and length measurements (Figure 3-3). This suggests that the edible portion can be predicted accurately from the animal weight or any of the size measured parameters using the equation reported in Figure 3-3. This is in contrast to the finding of Benniington, (1979) who found that the total weight was higher in older animals but the shell length and width remained relatively were constant.

The yield from *C. sulcata* (24%) was much lower than the yield reported for land snails, *A. marginata, A. archatina* and *Limicolaria sp.* which ranged between 37-45 % (Adeyeye, 1996) and for four *Helix* species in which the edible yield ranged from 58% to 65% (Gomot, 1998). The lower yield in *C. sulcata* is related to the significant build up of calcified material on the shell which can lead to a lower edible part relative to higher shell proportion. Also, (Gomot, 1998) suggested that naturally growing snails (such as in the present study) usually have a lower body weight: shell ratio compared with farmed snails.

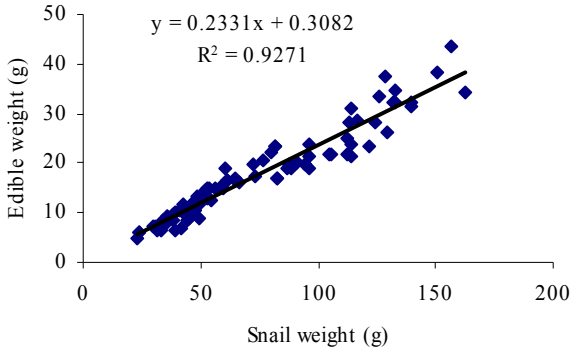
Table 3-4: Size measurements of *C. sulcata*

Group	Whole animal weight (g)	Diameter 1 D1 (cm)	Diameter 2 D2 (cm)	Length 1 L1 (cm)	Length 2 L2 (cm)	Whole edible weight (g)	Yield (%)
Small n=48	42.7±8.9a	53.1±4.2a	45.4±3.7a	48.6±4.6a	31.1±4.5a	10.2±2.7a	23.9±2.8
Large n=46	102.4 ± 27.2b	71.3±7.1b	59.8±7.3b	65.0±6.8b	41.3±5.2b	24.2±6.9b	23.6±3.0

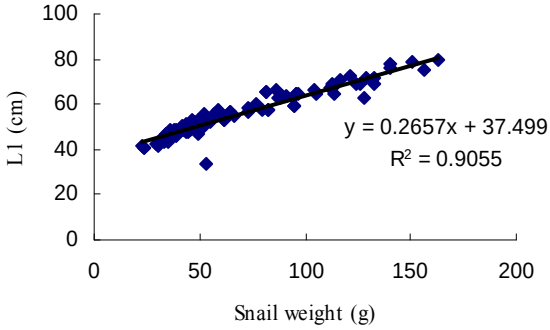
a/b=Within a column, values with different script letters are significant by different ($P<0.05$)

Figure 3-3: The relationship between (A) snail weight and its edible weight, (B) snail weight and L1, (C) snail weight and L2, (D) snail weight and D1, and (E) snail weight and D2

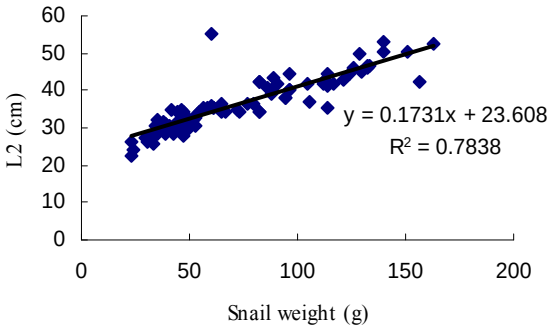
(A)



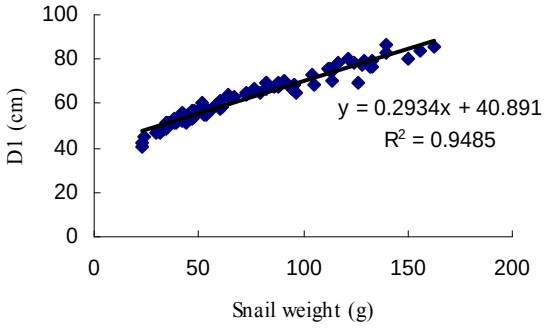
(B)



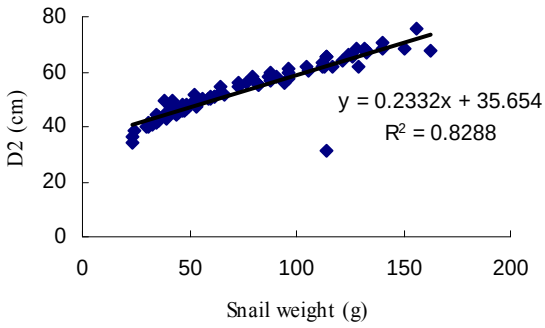
(C)



(D)



(E)



3.3.2 Proximate composition

The proximate analysis of *C. sulcata* is reported on dry and wet weight basis so that these results could be compared with other reported values for land and marine snails which are not consistently reported on either basis. The moisture content in the edible part of *C. sulcata* ranged between 75.5 to 80.0%. While this is higher than the normal range of moisture content reported for land-based meat producing animals (Table 2-4), it is within the range of average moisture content reported for land snails (Adeyeye, 1996; Fagbuaro *et al.*, 2006; Gomot, 1998; Hamzat *et al.*, 2002; Milinsk *et al.*, 2003; Milinsk *et al.*, 2006b)， marine snails (McLachlan and Lombard, 1980; Miletic *et al.*, 1991; USDA, 2006) and common marine molluscs (Table 2-3). The average protein content (% on fresh weight basis) was 17.5 ±0.9 and 17.6±1.5 % for small and large groups respectively, which equate to 78.8±1.8 and 79.6±2.3 % on dry weight basis. These values are higher than those reported for marine snails and common molluscs (Tables 2-2 and 2-3, respectively) but similar to the higher end of the scale for the values reported for land snails (Table 2-1) and domestic animals (Table 2-4). The lipid content of *C. sulcata* ranged from 0.7 % to 1.2 % on fresh weight basis which equates to 2.7 to 4.5 % on dry weight basis. This range is similar to the lower end of the scale for the lipid contents reported for land snails and marine molluscs including marine snails, but lower than lipid content reported for the meat from land based animals (Table 2-4). The ash and carbohydrate contents were within the range reported in Tables 2-1 to 2-4 for meat from snails, marine molluscs and domesticated animals.

While moisture, protein and carbohydrate content of snails were not affected ($P > 0.05$) by snail size, both fat and ash contents were significantly decreased ($P < 0.05$) with the increase in snail size (Table 3-5，Figure 3-4, 3-5). Similar observations for the lipid and ash contents were found in *H. aspersa* (Claeys and Demeyer, 1986) and *H. pomatia* (Gomot, 1998) but moisture and protein contents in those land snails were also affected by size. That is, moisture content increased while the protein content decreased with increase in snail size. Haniffa, (1982) showed that higher feeding rates are prevalent in young freshwater snail *Pila globosa* compared with intermediate or old snails, which might have contributed to the observed differences in fat and ash content in the present study. While the differences observed in fat and ash contents are significant, they are unlikely to have any nutritional impact.

Table 3-5: Proximate composition (Mean ± SD) of *C. sulcata*

Group	Moisture	Protein		Fat		Ash		Carbohydrate	
	% FW	%FW	%DW	%FW	%DW	%FW	%DW	%FW	%DW
Small (n=9) Mean (± SD)	77.7 (± 1.2)	17.5 (± 0.9)	78.8 (± 1.8)	1.0 a (± 0.2)	4.0 a (± 0.6)	2.1 a (± 0.2)	9.3 a (± 0.4)	1.7 (± 0.4)	8.0 (± 1.4)
Large (n=7) Mean (± SD)	78.0 (± 2.0)	17.6 (± 1.5)	79.6 (±2.3)	0.7 b (± 0.1)	3.1 b (± 0.3)	1.8 b (± 0.1)	8.4 b (± 0.3)	1.9 (± 0.5)	8.9 (± 2.2)

FW= fresh weight DW= dry weight

a/b=Within a column, values with different script letters are significant by different (P<0.05)

Figure 3-4: Effect of size on fat and ash concentrations in small and large *C. Sulcata*

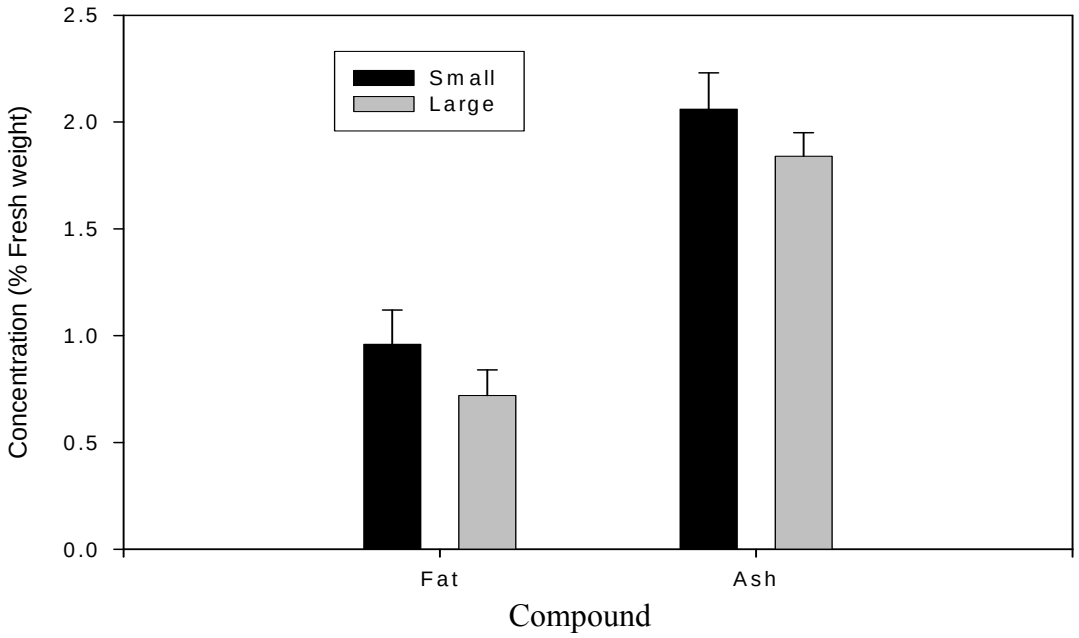
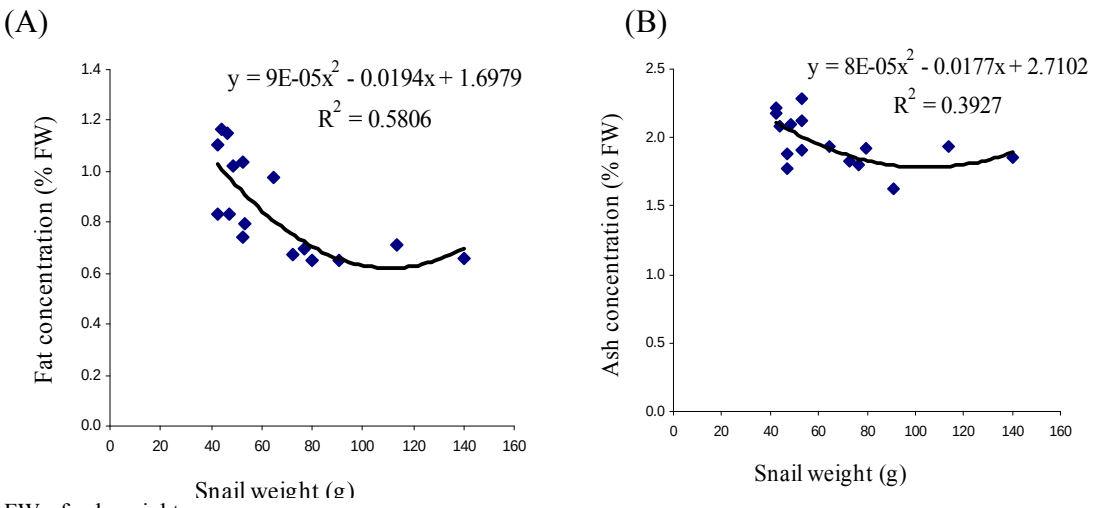


Figure 3-5: The correlation between the fat concentration and snail weight (A), and ash concentration and snail weight (B)



FW= fresh weight

3.3.3 Amino Acid composition

The amino acid composition for *C. sulcata* is shown in Table 3-6. *Cookia sulcata* contained all the amino acids naturally present in proteins. The major amino acids were Glu, Arg, Gly and Tau followed by Asp, Leu and Lys. The composition of the amino acids, Glu, Gly, Asp, Leu and Lys, were similar to those reported for marine molluscs (Daguzan, 1983; Harvey, 1970; Hughes *et al.*, 1980; Mead and Kemmerer, 1953; Miletic *et al.*, 1991), some land snails (Adeyeye, 1996; Mead and Kemmerer, 1953; Crosland *et al.*, 1995; Udoh *et al.*, 1995) and meat (beef, pork and lamb) from domesticated animals (Gibson *et al.*, 1993; Pearson and Dutson, 1990). It is worth noting that a significant variation in the amino acid profile of land snails does exist between species (Adeyeye and Afolabi, 2004)

The total amount of amino acid in *C. sulcata* (76.18 g/100 g dry weight basis) which equates to 95.13 g/100 g protein, was within the average content reported for other marine snails (Daguzan, 1983), and higher than other marine molluscs (Harvey, 1970; Hughes *et al.*, 1980; Miletic *et al.*, 1991), land snails and land-based meat producing animals (Table 2-6). The total essential amino acids in *C. sulcata* ranged from 26.0±1.1 to 25.5±4.5 % on dry weight basis, which equate to 28.3±2.0 to 30.0±6.8 g/100g protein (Table 3-6). This range was similar to the lower end of the range for total essential amino acids reported for land snails, and much lower than in marine molluscs including marine snails, and meats from land-based animals (Table 2-5, 2-6). Also Table 3-6 showed that there were no differences in the amino acid profile between large and small snails indicating that snail size did not affect the protein quality related to the snail size. Table 3-7 shows the essential amino acid profiles of *C. sulcata* compared with the reference protein of whole egg protein. The most limiting essential amino acids were the combined sulphur-containing Met and Cys, with an amino acid score of about 9.4 %. This was quite different to the amino acid scores of 61.9 %, 42.5 %, and 64.9 % respectively in marine molluscs, land snails and meats from land-based animals (Table 2-7). However, this does not mean that *C. sulcata* is not a good source of animal protein. The amount of essential amino acids in *C. sulcata* meets or exceeds those recommended daily requirement for young and adult humans (FAO/WHO/UNU, 1981). Being rich in lysine, *C. sulcata* may make an important contribution to protein of the diet as lysine is potentially limited in a predominantly cereal diet commonly consumed in many Asian and African countries

including China and Nigeria (Udoh *et al.*, 1995). One of the most interesting results found in the present study was that the level of tryptophan in *C. sulcata* (4.2±0.7 and 5.3± 1.3 g/100g protein for small and large groups, respectively) was much higher than the tryptophan from marine molluscs, land snails and domesticated animals (Table 2-7). The tryptophan content (g/100g protein and % AA score) of *C. sulcata* was much higher than in egg (Table 3-7).

Table 3-6: Amino acid profiles of small and large *C. sulcata*. The values are the mean ± SD and presented as dry weight and g/100 g protein.

AA	**Small Group (n=5)** Mean ± SD		**Large Group (n=7)** Mean ± SD	
	% dry weight basis	g/100g protein	% dry weight basis	g/100g protein
Asp	5.6±0.35	7.0±0.5	5.5±0.7	6.9±0.9
Glu	10.9±0.6	13.8±0.8	11.2±1.4	14.1±1.8
Ser	3.0±0.2	3.8±0.3	3.0±0.4	3.8±0.4
His*	1.1±0.1	1.3±0.1	1.1±0.2	1.4±0.2
Gly	7.8±1.0	9.8±1.2	7.6±1.1	9.6±1.3
Thr*	2.7±0.1	3.4±0.2	2.7±0.3	3.5±0.4
Arg	8.0±0.5	10.1±0.6	8.1±0.9	10.2±1.2
Ala	3.6±0.2	4.5±0.2	3.5±0.4	4.4±0.5
Tau	7.7±1.5	9.7±1.9	7.4±1.0	9.3±1.3
Tyr	1.8±0.1	2.3±0.1	1.8±0.2	2.3±0.3
Val*	2.0±0.1	2.5±0.1	2.0±0.2	2.5±0.3
Phe*	2.0±0.1	2.4±0.1	1.9±0.2	2.4±0.3
Ile*	2.0±0.1	2.6±0.1	2.0±0.3	2.5±0.4
Lys*	4.8±0.2	6.0±0.3	4.5±0.7	5.7±0.9
Leu*	4.4±0.2	5.6±0.2	5.1±2.3	6.5±2.9
Pro	4.1±0.4	5.1±0.5	3.8±0.6	4.8±0.7
Cys	0.2±0.1	0.2±0.1	0.2±0.1	0.3±0.1
Met*	0.3±0.1	0.3±0.2	0.2±0.1	0.3±0.1
Trp*	5.0±0.6	4.2±0.7	4.2±1.0	5.3±1.3
TAA	76.7±7.0	94.7±5.0	75.7±9.5	95.6±12.0
TEAA	26.0±1.1	28.3±2.0	25.5±4.5	30.0±6.8
(TEAA/TAA)%	33.8±0.9	29.9±4.0	33.7±2.1	31.2±2.2

AA= Amino acid TAA= total amino acids
TEAA= total essential amino acids *= essential amino acid
TE/TAA= total essential amino acids /total amino acids

Another interesting result was the very high Tau concentration in *C. sulcata*. There is no information available regarding Tau concentrations in snail's meat from previous studies. Taurine (2-aminoethane sulfonic acid), is usually considered as a β- amino acid. Previous studies showed that Tau has several biological functions in mammals. One clear biological function is the conjugation of taurine with bile acids, which

could promote lipid digestion and enhance fat adsorption (Laura, 2002; Redmond *et al.*, 1998; Wright *et al.*, 1986). Moreover, during the enterohepatic circulation of bile acids, some of the major primary bile acids may be degraded by the bacterial flora of the gut to secondary bile acids which have pyrogenic and inflammatory properties in man. Taurine can conjugate with these secondary bile acids as well as with various xenobiotics (Wright, 1986). Hence, not only are endogenously produced toxins, such as secondary bile acids, detoxified with Tau, but exogenous toxins can also be conjugated as well (Laura, 2002). Furthermore, a protective effect of Tau in human lymphoblastoid cell has been examined. Human lymphoblastoid cells grown in a biochemically defined medium are depleted of intracellular Tau and have a reduced rate of proliferation (Pasantes-Morales *et al.*, 1984). In addition, a number of health benefits have been found for Tau including protection for diabetes, antihypertensive effect, protection from alcohol toxicity, and growth and reproduction regulation (Hultman *et al.*, 2007; Markwell *et al.*, 1995; Quertemont *et al.*, 2000; Toshiro *et al.*, 1987).

Table 3-7: Amino acid scores of *C. sulcata*

AA	Egg protein (g/100g protein)	*C. sulcata*			
		Small	Large	Small	Large
		g/100g protein		AA score (%)	
His	2.3	1.3	1.4	58.3	59.6
Ile	5.4	2.6	2.5	47.6	47.0
Leu	8.6	5.6	6.5	64.5	75.2
Lys	7.0	6.0	5.7	85.9	81.4
Met + Cys	5.7	0.5	0.6	9.0	9.8
Phe + Tyr	9.3	4.7	4.6	50.8	49.7
Thr	4.7	3.4	3.5	72.3	73.6
Trp	1.7	4.2	5.3	249.4	312.4
Val	6.6	2.5	2.5	37.6	37.3
Reference	Whitney and Rolfes, (2005)				

3.3.4 Fatty acid composition

Cookia sulcata contained high amounts of SFA, with lesser amounts of MUFA and PUFA. The SFA were dominated by palmitic acid (C16:0) (27.3% and 22.6% for small and large snails, respectively), and stearic acid (C18:0) (8.1% and 9.6% for small and large snails, respectively). Oleic acid (C18:1) was the most predominant FA

among the MUFA with 8.8% and 8.0% for small and large snails, respectively. PUFA had prominent quantities of arachidonic acid C20:4 (11.4% in average), docosapentaenoic acid (C22:5 DPA) (8.0% in average) and EPA (5.45 in average).

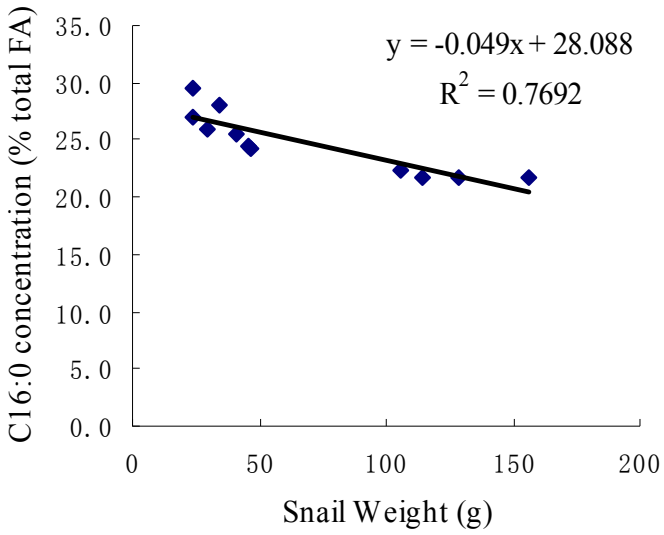
The FA profile of *C. sulcata* is quite different from that of land snails. The major FA detected in the muscle of *C. sulcata* were C16:0, C18:0, C18:1, C20:4 and C22:5 and these accounted to more than 60% of the FA. However, in land snails the dominant FA are C18:0, C18:1 and C18:2 which account for 50 to 60 % of the total FA depending on the diet, origin of the study and snail species (Milinsk *et al.* 2003, 2006; Özogul *et al*., 2005; Miletic *et al*., 1991). In addition, the presence of DHA is another unique feature for *C. sulcata* compared with land snails. Zhu *et al*., (1994) reported that lipid from land snails did not have DHA. Several of the reports confirmed this observation in different land snails (Özogul *et al*., 2005; Milinsk *et al*., 2003 and 2006). *C. sulcata* feeds on algae which are different from land snail diets and consequently some differences in the fatty acids composition could be expected. Milinsk *et al*. (2003) demonstrated that diet can affect the FA profile of *Helix aspersa maxima*. This suggests that environment and diet are the main factors that dictate the FA composition in snails. In addition, the high concentrations of C16:0 and C20:4 in *C. sulcata* as well as the general profile of other FA were similar to the FA composition of New Zealand marine molluscs as reported by Vlieg and Body (1988). EPA was at a much higher concentration than DHA in *C. sulcata*, while in other marine molluscs the DHA concentration was higher than EPA. The EPA and DHA concentrations of *C. sulcata* were lower than scallops, mussels, oysters and octopus (Table 2-8). The n6 content in *C. sulcata* is dominated by adrenic acid (C20:4), compared with other marine molluscs which generally have high quantities of C18:2 n6 and C18:3 n6 FA. These differences reflect by the range of diets on which wild mollusc species feed. Furthermore, *C. sulcata* had almost equal n3 and n6 FA amount, while other marine molluscs had a n3/n6 ratio varying from 2.5-39. The high n3/n6 ratio of other marine molluscs was dominated by high quantities of DHA and EPA, while lower DHA and EPA contents in *C. sulcata* were found. A possible reason is the different diets that these organisms consume. Kramer and Liston (1987) found that during the frozen storage of lipids hydrolysis occurred, which resulted in a decrease in lipids. It is noted that a relatively high percentage of unknown FA was found in *C.*

sulcata, which indicated that common commercial FA standards used were not successful in identifying all the FA in *C. sulcata.*

Apart from C16:0 and C18:3 n6, the snail size did not have a significant effect on the FA (Table 3-8). This was demonstrated by a strong correlation between the animal weight and the amount of C16:0 and C18:3 n6 found in the muscle lipid fraction (Figure 3-6).

Figures 3-6: The relationship between fatty acid of C 16:0 and snail weight (A) and fatty acid of C 18:3 and snail weight (B)

(A)



(B)

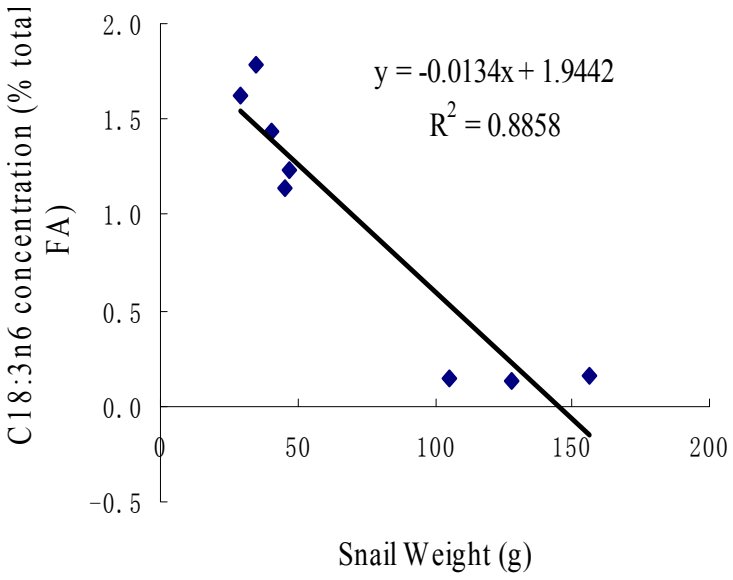


Table 3-8: Fatty acid profile of *C. sulcata* (% Fatty acids)

Fatty acid	Group (Mean±SD)	
	Small (n=7)	Large (n=4)
14:0	2.3±0.1	2.3±0.1
15:0	2.7±0.5	2.4±0.3
16:0	27.3±1.4 a	22.6±2.1 b
17:0	5.3±0.3	4.7±0.6
18:0	8.1±0.6	9.6±1.0
20:0*	0.4±0.4	0.3±0.1
21:0	ND	ND
22:0*	0.5±0.3	0.4±0.1
23:0	ND	ND
24:0*	ND	ND
14:1*	ND	ND
15:1*	ND	ND
16:1	0.7±0.4	0.9±0.4
17:1*	0.5±0.1	0.5±0.1
18:1	8.8±0.6	8.0±0.7
20:1*	2.1±0.1	1.0±0.2
C22:1	2.8±0.6	0.2±0.5
24:1(n9) *	ND	ND
C14:2*	ND	ND
C15:2*	ND	ND
C18:2	2.3±0.2	2.1±0.2
C20:2*	0.6±0.1	2.7±5.1
C22:2*	0.4±0.3	0.4±0.1
18:3(n3) *	0.5±0.4	1.0±0.4
18:3(n6) *	1.5±0.3 a	0.2±0.2 b
18:4(n3)	ND	ND
20:3(n3) *	ND	ND
20:3 (n6)	ND	ND
20:4(n6)	11.1±2.0	11.7±2.2
20:5(n3) EPA	4.9±0.7	6.0±0.8
22:4(n6) *	3.0±0.6	3.9±0.7
22:3	ND	ND
22:5(n6)	ND	ND
22:5(n3)	8.4±1.7	7.6±5.2
22:6(n3) DHA	0.6±0.2	0.6±0.2
Unknown	6.3±1.5	10.7±1.8
SFA	46.6±1.6	42.2±3.1
MUFA	14.9±0.9 x	10.8±1.4
PUFA	32.3±3.6	36.3±5.1
SFA/PUFA	1.4±0.5	1.2±0.6
n3	14.9±2.8	15.4±5.6
n6	14.2±2.5	15.8±3.0
n3/n6	1.1	1.0

a/b=Within a column, values with different script letters are significant by difference ($P<0.05$)
*=fatty acid was not detected in all the samples; ND=not detected

3.3.5 Vitamin E

Vitamin E was determined in *C. sulcata* (n=15), and all the vitamin E was identified as α-tocopherol only. *C. sulcata* contained vitamin E at a concentration of 2.16 and 3.71 mg/100g fresh weight in small and large snails (Figure 3-7; Appendix 13). The amount of α-tocopherol was higher in the larger snails ($P<0.05$) (Figure 3-8). It has been reported that marine invertebrates exposed to environmental pollutants are subjected to increased level of free radicals which will require antioxidants to counteract the toxicity of these radicals (Gorinstein *et al.*, 2003). A range of antioxidant defence mechanisms are present in molluscs such as enzymes activities [catalase, superoxide dismutase, glutathione (GSH) peroxidase] and antioxidant compounds (e.g. vitamin E and GSH) (Sheehan and Power, 1999) which appear to vary in different seasons. One possibility is that large snails are exposed to higher environmental levels of stressors (for a longer toxic period) than small snails and hence the higher concentration of vitamin E in larger snails. The level of α-tocopherol is considerably higher compared with those reported for *Mytilus galloprovincialis* (Orban *et al.*, 2002) but similar to those reported for *Perna viridis, Donax cuneatus and Meretrix meretrix* (Gopalakrishnan and Vijayavel, 2008).

Figure 3-7: The relationship between vitamin E content and whole animal weight

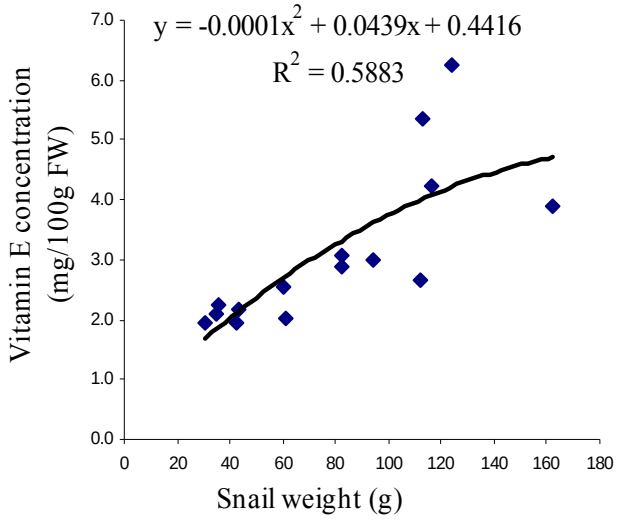
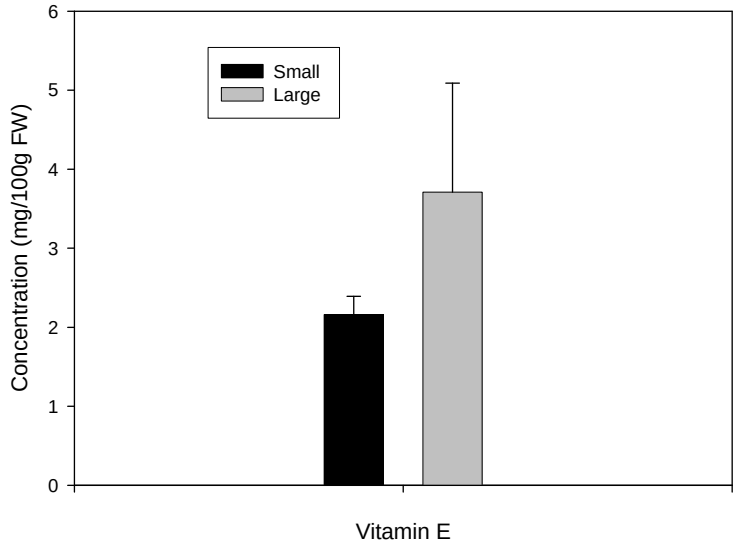


Figure 3-8: Effect of size on vitamin E concentrations in small and large *C. sulcata*



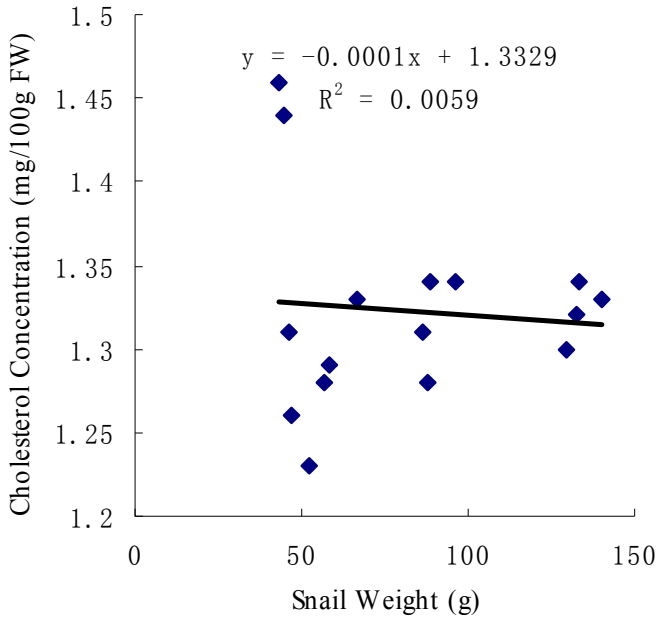
FW=fresh weight

3.3.6 Cholesterol

The average cholesterol concentration in *C. sulcata* was 1.33 mg/100g FW (Appendix 14.). Snail size did not affect the cholesterol level ($p > 0.05$) (Figure 3-9). *C. sulcata* contained much lower cholesterol concentration in the muscle compared with other more common molluscs such as oysters and scallops (Kritchevsky *et al.*, 1967; Zhu *et al.*, 1994). Furthermore, the cholesterol contents in land snails (Dignan *et al.*, 2004; Zhu *et al.*, 1994), several shellfish (Athar *et al.*, 2001), and a large array of fish (Osman *et al.*, 2001) varied between 30 and 200 mg/100 g, and the cholesterol concentration found in *C. sulcata* in the present study was within this range.

Several sterols have been found in marine and land molluscs (Idler and Wiseman, 1971; Kritchevsky *et al.*, 1967; Lin *et al.*, 1982; Zhu *et al.*, 1994) and most of the researchers agree that cholesterol is the major sterol in molluscs. However, new measuring techniques indicate that cholesterol levels of many shellfish are much lower than previously thought. In fact, molluscs were found to have a large amount of sterols, which have a similar chemical structure to cholesterol (Wahlqvist, 2004). Since many studies confirm that increasing dietary cholesterol results in higher blood cholesterol, the sterols appear to have a benefit because they inhibit the absorption of cholesterol when consumed at the same time (Wahlqvist, 2004).

Figure 3-9: The relationship between cholesterol content and snail weight



FW= fresh weight

Pork, beef and lamb have been used as major meat protein sources for years. However, these sources have declined recently because of persistent drought, disease, natural disasters, high cost of feed and continuous growth of the human population. Producing new meat sources which have low fat to solve the shortage of meat protein is a major challenge to the food industry worldwide. Seafood has been known as a good source of meat protein, which has been proved again in this study. *C. sulcata* contained a high protein concentration which was similar to red meat and superior to some other seafood. In addition, amino acids study showed that not only the quantity of protein in *C. sulcata* was high, but also its quality was good. Hence, *C. sulcata* could be useful as a protein source in the diet. Furthermore, *C. sulcata* also contained other nutrients such as Tau and Tryat a high level, which may make the incorporation of *C. sulcata* meat a useful addition to the diet and for more specialised dietary preparations. Lastly, the low fat content with relatively low cholesterol level of *C. sulcata* make this snail a suitable food source for consumption by the general population and especially by those who need low-fat and low-cholesterol diets.

Chapter 4: Macro, Trace and Toxic Elements, and OCPs Evaluation of *Cookia sulcata* Meat

4.1 Introduction

Seafood has been considered a nutritious food for decades and in some countries it constitutes the large proportion of the human diet. Seafood is high in protein and is considered a good source of some essential minerals, such as Fe and Zn (Katvi, 2005). Moreover, a number of PUFA, which play important functions including prevention role in coronary heart disease, obesity and certain human cancers, have been found in seafood oils (Mills *et al.*, 2005; Mozaffarian *et al.*, 2004; Rose and Connolly, 1999; Simopoulos, 1991; Simopoulos, 1999). In the last 30 years, aquaculture has developed around the world because of the significant increase in demand for seafood. New Zealand, a country with thousands of kilometres of coastline, has a diverse ecosystem with a large number of marine species. New Zealand has a large fish industry and seafood is its fourth biggest export-earner (Katvi, 2005).

An increase in industrial and agricultural activities can lead to increased production and usage of chemicals which can cause harmful effects on the environment (Fung *et al.*, 2004). Heavy metals are the second most important toxicants producing adverse effects in organisms and can also affect the environment and in particular the aquatic ecosystem (Fung *et al.*, 2004; Gregori *et al.*, 1992; Pattee *et al.*, 2003; Waldichuk, 1985). Another major pollutant of particular concern are the organochlorine pesticides (Fung *et al.*, 2004). These chemicals, especially DDT, made a significant contribution in pest control worldwide about 50 years ago (Bearsd, 2006; Ross, 2005). However, they were banned in developed countries and some developing countries due to their resistance to biodegradation, persistence and bioaccumulation and adverse effects on wild-life and humans, but their widespread distribution and extreme persistence continue to be a problem in some countries (Zhou *et al.*, 2008).

Organochlorine pesticides can enter aquatic systems, because they can be adsorbed to particulate matter and underwater sediments as well as being able to be absorbed, retained and accumulated in fatty tissues of seafood (Smith and Gangolli, 2002). Contaminants such as metals reach oceans through natural erosion, geochemical

cycles and human activity. Once in the marine environment, they can enter the food web where bioaccumulation and/or biomagnification can occur. Therefore, crustaceans, bottom feeders in these sea and seafood caught from coastal regions that are close to regions of high industrial and agriculture activities can be particularly at risk of contamination (Smith and Gangolli, 2002).The sampling site of this study was Te Oka Bay which is near a farm land close to Christchurch. These conditions raise the potential for pollution and contamination. Since minerals and OCPs can potentially accumulate in *C. sulcata*, toxic metal analysis and total organochlorine pesticides were characterized in this study for *C. sulcata*. Other minerals were also analysed as an overall part of the nutritional aspects of a study of this snail.

4.2 Material and method

4.2.1 Sample Collection

Sample collection was as described in Section 3.2.1

4.2.2 Size and weight measurement

Size and weight measurement of *C. sulcata* was as described in Section 3.2.2

4.2.3 Minerals (macro-, trace- and toxic-elements) analysis

10 samples of snail from 2006 and 20 from 2007 were classified into 2 groups: small (≤ 60 g whole animal weight) and large (> 60 g whole animal weight) were used for the analysis.

4.2.3.1 Sample digestion

Snail meat samples were dried and then prepared for digestion. A 0.5 g sample of snail meat was dissolved in 6 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%), and digested in a microwave digestion system (ETHOS SEL/ plus, Milestone Sorisole, Italy). Digestion conditions for the microwave system were 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, 8 min at 550 W, then venting for 8 min. The digested solution was filtered with filter paper (Whatman No. 5) and made up to 25 ml with deionized water. Fish protein certified reference material (DORM-3, National Research Council Canada) was used as a reference standard and digested under the same conditions.

4.2.3.2 Detection and quantification

AXIAL Varian 720 OES Series ICP (Inductively Coupled Plasma) Emission Spectrometer with a SP3 Auto Sampler (Varian, Inc. Palo Alto, USA) was used to detect and quantify the elements. Concentrations of elements in the sample were detected at the following absorption wavelengths (nm) shown on Table4-1. Elemental concentrations were calculated by comparison with a standard calibration curve which was generated by plotting the intensity of peak against known concentrations of the standard. The limits of detection are shown in Table 4-1.

Table 4-1: Absorption wavelengths and detection limits for mineral detection

Minerals	Absorption Wavelengths (nm)	Detection Limits (µg/kg)
Na	589.592	1.547
K	766.491	12.239
Ca	317.933	0.305
Mg	279.553	0.374
P	213.618	0.108
S	181.972	0.085
Fe	238.204	0.413
Cu	327.395	0.960
Zn	213.857	213.857
Mn	257.610	0.117
Al	167.019	0.770
As	188.980	1.504
Cd	214.439	1.224
Co	238.892	0.678
Cr	267.716	0.672
Ni	231.604	0.810
Pb	220.353	4.109

4.2.4 Organochlorine pesticides (OCPs)

The total OCPs in *C. sulcata* was analyzed commercially in an accredited laboratory (Hill Laboratories, Hamilton, New Zealand). Because of the expense of OCPs analysis the samples were pooled. The object of this analysis was to determine whether in general the contamination of OCPs in snail were low, medium or high.

Mixed standard solutions with different concentrations (0.1, 0.2, 0.5, 1.0, and 2.0 mg/L) were made from analytical grade of organochlorine compounds (environmental protection agency, USA). Sixteen snail meat samples were pooled into three groups of 20 g which was the minimum weight required for the analysis. Homogenous samples were prepared by mincing, chopping and/or blending of the pooled snail meat. The samples were subjected to sonication extraction, solid Phase extraction (SPE) cleanup, and Gel Permeation Chromatographyy (GPC) clean up. OCPs in *C. sulcata* samples were detected by gas chromatography mass spectrometry (GCMS) (Shimadzu GCMS-QP5050, Japan) with DB-5 M5 fused silica capillary coloum (30m, 0.25mm i.d., 0.25mm).

4.2.5 Statistical analysis

Analysis of variance was used to test for the effect of snail size on the mean concentrations of macro, trace and toxic elements. The data were analyzed using the general linear model protocol in MINITAB (release 15). Significant difference between means was determined by Tukey Simultaneous Tests ($p < 0.05$).

The results for OCPs are presented as mean value of the 3 pooled samples. Standard deviation was not calculated as the concentrations of OCPs were below the detection level.

4.3 Results and discussions

The results are expressed as mean mg/g and/or mg/kg fresh weight ± standard deviation values.

4.3.1 Mineral Compositions in *C. sulcata* and comparison with other shellfish

Mineral concentrations (macro-, trace- and toxic elements) in *C. sulcata* samples from 2006 (10 in total) and 2007 (20 in total) were investigated. There was no significant

annual variation (P>0.05) in mineral concentrations in snails. Therefore, two year's samples were separated into small (≤ 60g fresh weight) or large groups (>60g fresh weight).

In the following sections, the mineral concentrations in *C. sulcata* were expressed as mg/g fresh weight for macro metals and mg/kg fresh weight for trace and toxic metals and are shown in Table 4-2 and the importance and availability of minerals in *C. sulcata* compared with other shellfish will be discussed later.

Table 4-2: Mineral concentrations in small and large sized *C. sulcata.*

Minerals	**Small** n=15	**Large** n=15
Macro Elements (mg/g FW)		
Na	4.0 ± 0.3	4.1 ± 0.7
K	3.0 ± 0.4	2.8 ± 0.6
Ca	1.4 ± 1.2	0.7 ± 0.3
Mg	3.3 ± 0.1	3.1 ± 0.6
P	1.1 ± 0.1a	0.9 ± 0.1b
S	6.0 ± 0.7	5.5 ± 0.8
Trace elements (mg/kg FW)		
Fe	58.1 ± 14.7	74.1 ± 38.0
Cu	6.4 ± 3.0	7.8 ± 3.6
Zn	32.2 ± 5.2	38.2 ± 19.0
Toxic elements (mg/kg FW)		
Mn	1.3 ± 0.3	1.2 ± 0.6
Al	14.8 ± 7.8	15.9 ± 16.1
As	8.2 ± 1.9a	13.9 ± 7.2b
Cd	0.1 ± 0.04	0.2 ± 0.1
Cr	1.2 ± 0.5	1.0 ± 0.3
Ni	0.02 ± 0.02	0.05 ± 0.03
Co	0.2 ± 0.1	0.2 ± 0.1
Pb	<nl	<nl

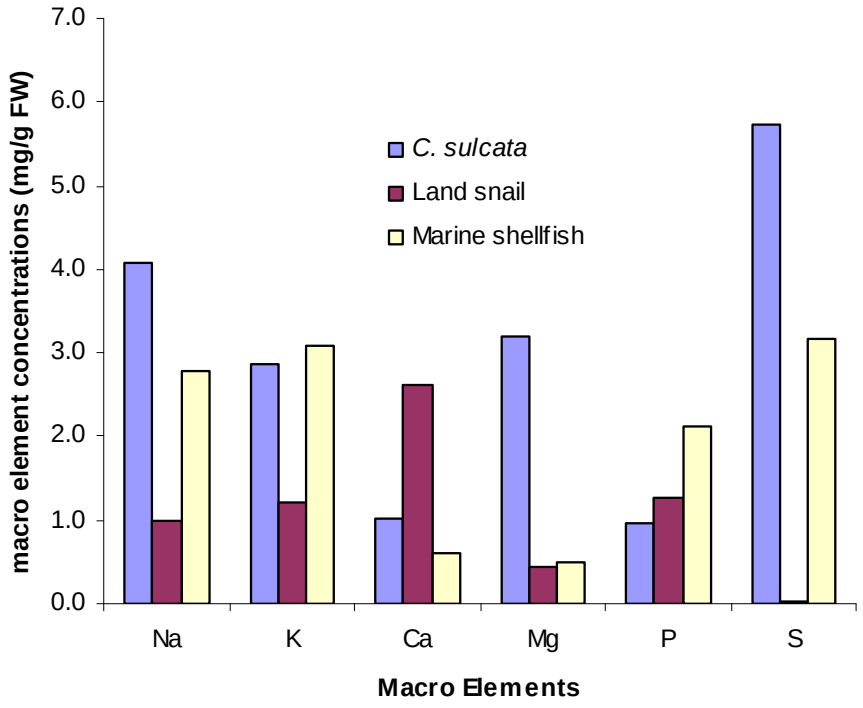
nl= below detection limit FW= fresh weight
a/b=Within a column, values with different script letters are significant by different (P<0.05)

4.3.1.1 Macro elements

The present study found that S was the elements with the highest concentration in *C. sulcata* (6.0±0.7, 5.5±0.8 mg/g FW basis for small and large snail respectively). The second highest amount of mineral concentration was Na with values 4.0-4.1 mg/g FW.

P was the macro element with lowest concentration in *C. sulcata.* The concentrations of Na, K, and Mg in large and small sized snails were very similar.

Figure 4-1: Comparison of macro element concentrations in *C. sulcata*, land snails and other marine shellfish.



Data for land snails and marine shellfish are from Table 2-11, Table 2-12 FW=fresh weight

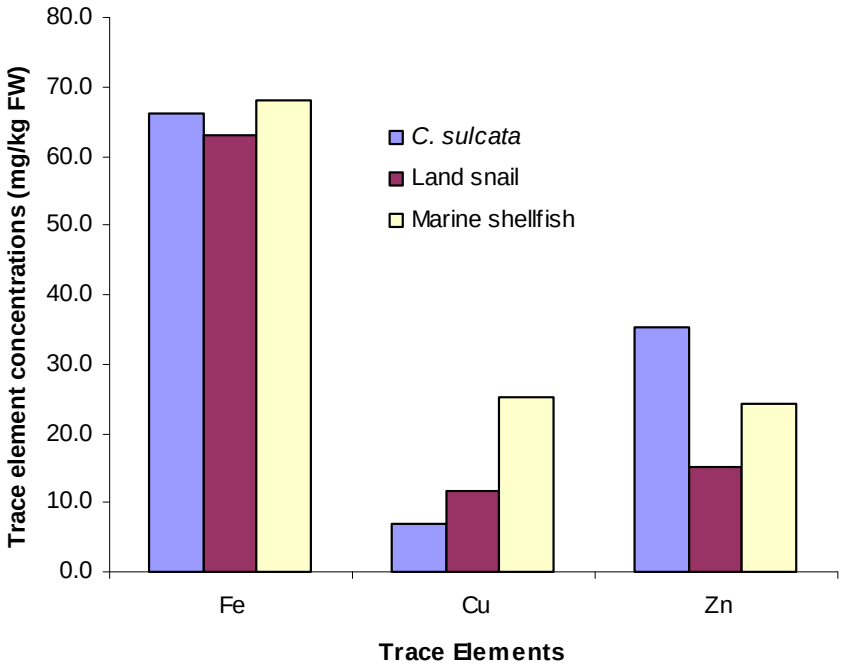
Figure 4-1 shows a comparison of macro element concentrations of *C. sulcata* with land snails and marine shellfish. Na concentration in *C. sulcata* (4.0-4.1 mg/g fresh weight) was extremely high compared to other marine shellfish (2.8 mg/g fresh weight) and land snails (1.0 mg/g fresh weight). *C. sulcata* also contained a high concentration of K (2.9 mg/g FW) compared to land snails (1.2 mg/g FW), but similar to the lower end of the range of the values reported for marine shellfish (Karakoltsidis *et al.*, 1995; Sidwell *et al.*, 1978; Vlieg *et al.*, 1991; Hughes *et al.*, 1980). The average content of Ca in both *C. sulcata* and other shellfish were much lower than that reported for land snails (1.0 and 0.6 mg/g FW compared to 2.6 mg/g FW). This may be due to the differing feeding habits in land snails compared to marine molluscs (Fagbuaro *et al*., 2006). The concentration of Mg in *C. sulcata* (3.2 mg/g FW) was extremely high compared with land snails (0.4 mg/g fresh weight) and marine shellfish (0.5 mg/g FW). P is an important element and most muscle protein such as

meats, fish and chicken are high in P. In this study, the P concentration in *C. sulcata* was less than that reported for macro-mineral land snails and marine shellfish. It can be seen clearly that S was predominant in both *C. sulcata* and other marine shellfish, and is much higher than the values reported in land snails. A possible reason for this is that S is a major component commonly found in seawater and is ubiquitous in crustacean tissues (Barrento *et al*., 2009).

4.3.1.2 Trace elements

In this study, among trace element, Fe concentration was the highest with values of 58.1±14.7 and 74.1± 38.0 mg/kg FW in small and large snails respectively, while Cu concentration was the lowest in *C. sulcata*, (6.4±3.0 and 7.8±3.6 mg/kg FW in small and large snails respectively). Average Zn concentration in *C. sulcata* varied from 32.2 to 38.2 FW in small and large snails respectively.

Figure 4-2: Trace element concentrations in *C. sulcata*, land snails and marine shellfish.



Data for land snails and marine shellfish is from Table 2-11, Table 2-12

FW:=fresh weight

The comparison of trace element concentrations in *C. sulcata* with land snails and marine shellfish is shown in Figure 4-2. The concentration of Fe in *C. sulcata* was

comparable to that found in land snails and marine shellfish (Udoh *et al.*, 1995; Adeyeye, 1996; Fagbuaro *et al.*, 2006; Gomot, 1998; Özogul *et al.*, 2005). It is noticeable that the mean concentration of Cu in *C. sulcata* was lower than the mean Cu content reported for land snail *Helix spp*. and marine shellfish, but higher than in other land snail species. Higher concentration of Zn was found in *C. sulcata* compared with other molluscs except oyster (Sidwell *et al.*, 1978).

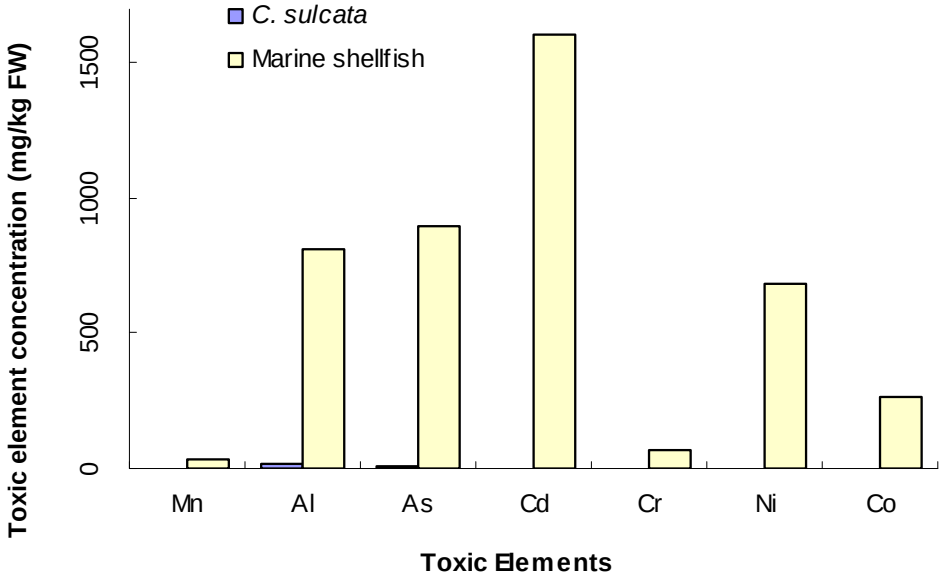
4.3.1.3 Toxic elements

Among the toxic elements, Al concentration in *C. sulcata* was the highest. The toxic element with the lowest concentration in *C. sulcata* was Ni with values of 0.02 ± 0.02 and 0.05 ± 0.03 mg/kg FW for small and large snails respectively. Similar concentrations of Co, Cr and Mn were found in small and large snails (Table 4-1). Pb was not detected in *C. sulcata.*

A comparison of distribution of toxic elements in *C. sulcata* and marine shellfish is shown in Figure 4-3. There is limited information of toxic metals in land snails (Table 2-12). The concentrations of toxic elements of *C. sulcata* were much lower than that reported for in other marine shellfish. There are many possible reasons: First of all, heavy metal concentrations differ among species and even with in the same species in marine shellfish. This clearly shows the role of generic and physiological factors on the toxic compositions of *C. sulcata*. For example, Mn concentration in *C. sulcata* was lower than in mussel and the lowest was in oyster. However, Mn concentration in *C. sulcata* was higher than the species of *Perna canaliculus* but lower than the species of *Artrinata pectinata*. Secondly, diet may affect the mineral contents. Ademolu *et al*., (2004) reported that different diets influenced the mineral compositions of African giant land snails (*Archachtina marginata*). They found that different nitrogen sources in the diets enhances snails' growth or limits food intake by snails, thereby resulting in different growth performance. Land snail, *Helix. Aspersa,* collected from wild populations showed the highest growth rate in soils richest in Ca and Mg, which demonstrated that vegetation and the nature of the environment influence the growth rate (Gomot, 1998; Gomot *et al.*, 1989). In addition, some natural factors may reflect the availability of minerals. For example, unusually high Fe, Zn and Cd were found in soft tissue of mussels which were collected from Kuril Island, north-west Pacific Ocean. The high accumulation of Fe and Zn in mussel was a reflection of the

hydrothermal activity at that site, and the elevated Cd concentration in mussel was associated with extensive upwelling, which brings deep waters laden with nutrients including Cd to the surface (Kavun *et al.*, 2002). However, the influence of environmental factor on toxic element accumulation by *C. sulcata* requires further study. This is especially true for those snails collected from wild populations because of the influence of vegetation.

Figure 4-3: Comparison of toxic element concentrations in *C. sulcata* and marine shellfish.



Data of marine shellfish is from Table 2-12

FW= fresh weight

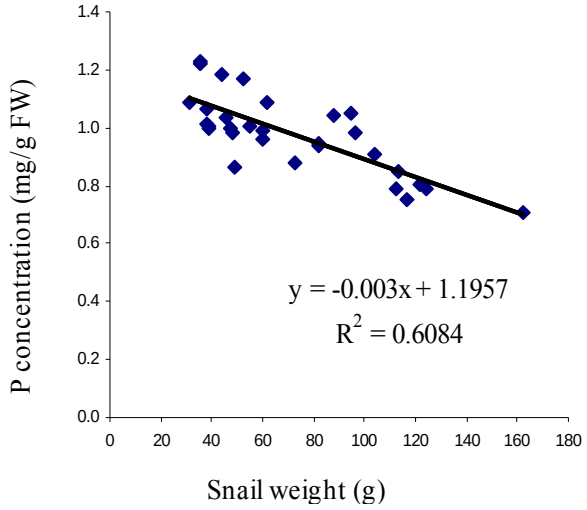
4.3.2 Animal size variation of minerals in *C. sulcata*

Only P and As content in *C. sulcata* were affected ($P < 0.05$) by the snail size (Figure 4-4). The remaining elements were not affected by animal size. Similar results were obtained by (Cevik *et al.*, 2008) who found that mineral concentrations of K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb in mussel did not depend on the mussel size. P concentration decreased as the animal size increased (Figure 4-4 A) whereas As concentrations increased with the increase in animal size (Figure 4-4 B). Several possible reasons might explain this. P exists naturally, and is more concentrated in the shell than the flesh (Adeyeye, 1996). Hence it is possible that in *C. sulcata* less P was concentrated in muscle of older snails. Also competitive inter-metal correlation may

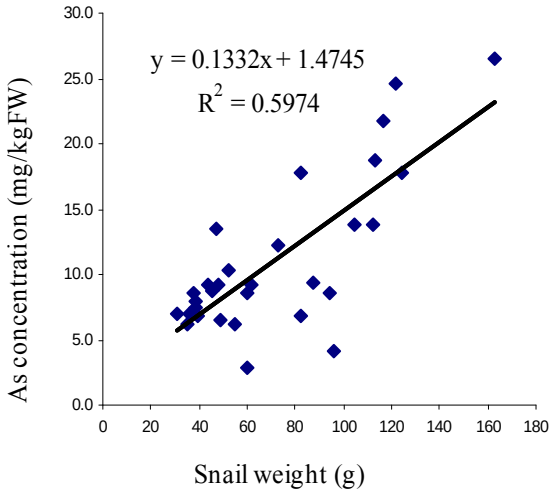
occur. For example, mussel tissue concentrations of Mn and P were lower as the tissue As increased (Hickey *et al.*, 1995). In *C. sulcata* with high As concentration, the second highest toxic element decreased the P concentration. However, a significant positive correlation between the size and As concentration in *C. sulcata* differed from other studies. Both Burger and Gochfeld, (2006) and Saavedra *et al.*, (2004) found that there was no significant positive relationship between mussel size and As concentrations. The differences may be explained by the feeding behaviour, location, and season all of which would affect the accumulation pattern of mineral in *C. sulcata* (Saavedra *et al.*, 2004).

Figure 4-4: The correlations between P concentration (A), As concentration (B) and whole animal weight

A



B



FW= fresh weight

4.3.3 Potential nutritional benefits and safety

The essential and beneficial minerals are Na, K, Ca, Mg, P, S, Fe, Cu, Zn and Mn. The Na concentration in *C. sulcata* was very high. High intake of Na can lead to Ca excretion and has been associated with hypertension (McDowell, 2003). The Tolerable Upper Limit (TUL) is about 6 g NaCl/day/person (WHO, 1990). This corresponds to about 1500g of snail meat, about 10 times the normal serving size (150g/day). Potassium is an essential mineral which has many functions including the role in transmission of nerve impulses and in carbohydrate and protein metabolism (McDowell, 2003). The recommended daily intake (RDI) of K is 4.7 g/day. So a normal serving size of snail meat (150g) would provide only 0.1 of RDI. When the amounts of Ca, Mg, P, and Mn in a typical serve of *C. sulcata* were compared with the RDA (Table 4-3), *C. sulcata* meat was not a very rich source of these elements. However, *C. sulcata* could be a very good source of Fe and Zn, since only 100-300 g of snail meat could exceed the RDI. The mineral Zn plays a vital role in immunity (McDowell, 2003). High Zn level in *C. sulcata* indicates that the meat from this snail can be used as a major Zn source for people who have a dietary Zn deficiency (Fagbuaro *et al*., 2006). Although Zn is beneficial too much Zn can suppress the immune system. The toxic level of Zn is 45 mg/day (Bekhit *et al*., 2009; WHO, 1999), which correspond to more than 1000g of snail meat. Therefore, consumption of *C. sulcata* with a normal meal serving size is safe. An adequate amount of Fe is important to keep the body healthy and for haemoglobin synthesis. In this regard, snail meat can contribute significantly to synthesis of haemoglobin and prevention of anaemia (Ademolu *et al*., 2004, Fagbuaro *et al*., 2006). Iron deficiency affects two million people in the world and occurs when demand for Fe is high such as during high menstrual loss and during pregnancy, or when the intake is quantitatively inadequate or when diet contains elements that render Fe unavailable for absorption (Board *et al.*, 1989). However, there is concern that Fe can be toxic and suppress the immune system. While there is no maximum level specified for Fe in the FSANZ (2004), the reported toxic level for human is more than 75 mg Fe /day (Finch and Monsen, 1972), which corresponds to at least 1135 g of snail meat, which is unlikely to be eaten in a day. *C. sulcata* is also a good source of Cu. About 400 g of *C. sulcata* meat per day is sufficient to achieve the RDI. Copper is a concern and also can be toxic at high concentrations and the maximum limit intake for humans is 10mg/day (Board *et al.*, 1989), which corresponds to approximately 1418 g of snail meat, which is in excess of a normal serving meal.

Table 4-3: Recommended daily allowances of mineral elements (Board *et al.*, 1989)

Elements	Recommended daily allowances (mg/day)		
	Infant <1 year old	Children 1-11 year old	Adult >11 year
Ca	600.0	800.0	800.0-1200.0
P	300.0-500.0	800.0	800.0-1200.0
Mg	30.0-60.0	300.0-400.0	280.0-350.0
Fe	10.0	1.0-10.0	10.0-15.0
Cu	0.4-0.7	1.0-2.0	1.5-3.0
Zn	5.0	10.0	12.5
Mn	0.3-1.0	1.3	2.0-5.0

One of the main objectives of this study was to investigate the toxicological aspects of *C. sulcata*, and to establish whether any of the metal levels in the snail approached concentrations that are undesirable or toxic for human consumption. Toxic elements which were investigated in this study included Al, As, Cd, Co, Cr, Ni, Pb and Mn. Aluminium toxicity is recognised in patients with longstanding chronic renal failure (Meiri *et al*., 1993). There is no maximum level specified for Al, Co and Ni in the (FSANZ, 2004)). Arsenic is found in two forms in marine animals: organic and inorganic. Inorganic As is more toxic but contributes only a minor component of the total arsenic content in marine animals (Edmonds and Francesconi, 1993). A joint group of experts on the scientific aspects of marine pollution (GESAMP, 1986) reported that the inorganic As proportion constituted only about 5% in marine animals. The As concentration was high with mean values in small and large *C. sulcata* of 8.2 and 13.9 mg/kg respectively, and therefore exceeded the maximum level of As in molluscs (FSANZ, 2004). However, the mean inorganic As concentration in *C. sulcata* would account for only about 0.41 (small snails) to 0.69 (large snails) mg/kg, which is well below the maximum level of 2 mg/kg (FSANZ, 2004). Cadmium is toxic to many organisms. It can accumulate in the human body mostly in the kidneys and may cause kidney dysfunction, skeletal damage and reproductive deficiencies, (Board *et al.*, 1989). The concentrations of Cd was well below the 2 mg/kg maximum level recommended by FSANZ (2004) for molluscs. Chromium is not listed in FSANZ (2004) for molluscs probably because inhalation is the main route for carcinogenicity by the hexavalent form (+6) and most of the Cr available in biota exists in the less toxic trivalent (+3) form (Mertz, 1987). Chromium is required by the body in trace amounts and is a key co-factor for insulin activity (Board *et al.*, 1989). The Cr concentration in *C. sulcata* was well below the level of concern (13 mg/kg) reported by the US FDA for chronic seafood consumers (Bekhit *et al*., 2009). Pb concentration in *C. sulcata* was below the limit of detection (4.1 μg/kg), which is far below the maximum level (0.5

mg/kg) recommended by FSANZ (2004). The toxic level of Mn for humans per day (11 mg/day) corresponds to about 1000g of marine shellfish and is much higher than a serving size for normal meals.

4.3.4 Organochlorine pesticides

The organochlorine pesticides concentrations in *C. sulcata* are shown in Table 4-4. All the compounds including aldrin, BHC, chlordane, DDT and its metabolites, DDD and DDE, dieldrin, endosulfan and its sulphate; endrin and its breakdown products (endrin aldehyde and endrin Ketone); heptachlor and its metabolite (heptachlor epoxid), HCB, and nethoxychlor (a replacement of DDT) were below the detection limit (<0.0005 mg/kg). Total chlordane concentration was also less than the detection limit (<0.0020 mg/kg).

Table 4-4: Organochlorine pesticide concentrations (mg/kg wet weight) in *C. sulcata*

OCPs	Concentration (mg/kg)		
	1	**2**	**3**
Aldrin	<0.0005		
α-BHC	<0.0005		
β-BHC	<0.0005		
δ-BHC	<0.0005		
γ-BHC (Lindane)	<0.0005		
cis-chlordane	<0.0005		
trans-chlordane	<0.0005		
2,4′-DDD	<0.0005		
4,4′-DDD	<0.0005		
2,4′-DDE	<0.0005		
4,4′-DDE	<0.0005		
2,4′-DDT	<0.0005		
4,4′-DDT	<0.0005		
Dieldrin	<0.0005		
Endosulfan Ⅰ	<0.0005		
Endosulfan Ⅱ	<0.0005		
Endosulfan sulfate	<0.0005		
Endrin	<0.0005		
Endrin aldehyde	<0.0005		
Endrin Ketone	<0.0005		
Heptachlor	<0.0005		
Heptachlor epoxide	<0.0005		
HCB	<0.0005		
Nethoxychlor	<0.0005		
Total Chlordane	<0.0020		

The maximum concentrations of aldrin and dieldrin, BHC (except γ-BHC), chlordane, sum of DDT, DDE and DDD, sum of heptachlor and heptachlor epoxide, and lindane recommended

by FSANZ (2004)) were 0.1, 0.01, 0.05, 1.0, 0.1, 0.05, 1.0 (mg/kg), respectively. According to FAO/WHO (FAO/WHO, 1986), the acceptable daily intake of DDT is 0.2 mg/day. The total OCPs concentrations of in *C. sulcata* were well below 100 fold the concentration that would affect breeding success in marine species (Butler *et al.*, 1972).

In this research, the detection of heavy metal levels in environmental sediments was not carried out. This should be considered for future research to investigate the relationship between heavy metal levels in *C. sulcata* and the environmental sediments. Such information together with more biological information about the regulation of elemental burden in *C. sulcata* may help to shed some light on the possibility of using *C. sulcata* as a biomarker of exposure to toxic elements.

Generally, macro and trace element concentrations in *C. sulcata* were higher than in the land snails except for Ca and P, and similar to or higher than those of marine shellfish. *C. sulcata* appears to be a rich source of Fe, Zn and Cu (Table 4-2). The RDI of these minerals could be achieved with a normal meal serving size of (150g). Heavy metal contents of *C. sulcata* were markedly less than the toxic levels and at least 100 fold below the concentrations reported by researchers who have used shellfish as a biomonitor for heavy metal contamination due to their sedentary character (Boyden, 1974; Fagbuaro *et al.*, 2006; Milne, 1998; Spronk *et al.*, 1971). On the basis of results obtained in this research, it can reasonably be assumed that the concentration of heavy metals and OCPs are unlikely to have a negative impact on human health because of consumption of *C. sulcata*.

Chapter 5: General Discussion and Future Work

5.1 Introduction

To maintain normal weight and health in adults and growth in childhood requires a constant intake of oxygen, water, energy and nutrients (Mann and Truswell, 2007). Proteins are important fundamental structural and functional elements within every cell, and are intimately linked to the metabolism of energy and other nutrients. Generally dietary proteins can be provided from plants such as beans and from animal meats. Common meat such as beef, lamb, pork and fish are the major sources of meat protein for humans. However, these sources are declining. Continuous growth in human population and shortage of food sue to persistent drought, diseases, natural disasters, high cost of feed, and low productivity of local breeds (Copper and Knower, 1991) have led to greater pressure on the existing animal protein sources. Therefore, finding new meat sources is a major challenge to the food industry worldwide.

Research on the nutritional value of molluscs has attracted much attention recently. The main reason is the large amount of molluscs available globally. Molluscs include univalve, bivalve, and cephalopods (Copper and Knower, 1991). In general, the snail belongs to the univalves, and its meat is considered to be a nutritionally balanced food: low in fat, rich in protein and containing many other nutrients required for a healthy and well balanced diet (Ademolu *et al*., 2004). Hence, there is potential for using the under-utilized snail meat from aquatic resources to help fill the gap in current meat decreasing market. However, consumer acceptance of snail as a new meat source is a concern. The more nutritional information is available on snail, the better the understanding will be, and this can be passed on to the consumers. This will increase their willingness to try something new, decrease consumer fears, and thereby improve consumer acceptance.

With this in mind, first part of this research was carried out to investigate the nutritional status of *C. sulcata.* The information generated in this section was helpful to evaluate the nutritional value of *C. sulcata* compared with land snails, other marine shellfish, and meats from domestic animals. Following this, a toxicological evaluation of *C. sulcata* was carried out to ensure its safety for consumption.

5.2 Nutrition and food safety

Food sources from water have always been an important part of the human experience. Oceans have generally provided an abundance of food for humans to eat. The edible animal which are collectively called seafood include finfish, crustaceans and molluscs (Wahlqvist, 2004). Since the publication of pioneering studies demonstrating low death rates from CHD among Greenland Inuits and also Eskimos, interest in seafood consumption has expanded dramatically (Mozaffarian *et al.*, 2004; Wahlqvist, 2004). Scientific information including evidence from animal experimental, observational, and clinical studies on the health benefits of consuming seafood is accumulating.

Firstly, seafood is a good protein source. This research has shown that *C. sulcata* contains a significant amount of protein. The protein content of *C. sulcata* was higher than that reported for marine molluscs, and was at the high end of the ranges for values reported for meat from domestic animals (Anthony *et al.*, 1983; Crosland *et al.*, 1995; Hughes *et al.*, 1980; Lee *et al.*, 2008; Sidwell *et al.*, 1978; Smith *et al.*, 1989). The quality of *C. sulcata* protein was also good. By assessing quality compared to standard egg protein *C. sulcata* had similar quality to other marine molluscs and traditional domestic meat. One serve can generally meet the daily requirement for adults (Adeyeye, 1996; Crosland *et al.*, 1995; FAO/WHO/UNU, 1981; Gibson *et al.*, 1993; Mead and Kemmerer, 1953; Udoh *et al.*, 1995; Whitney and Rolfes, 2005).

Secondly, seafood had only very small amount of fat. *C. sulcata* had a fat concentration between 0.7 and 1.0 % FW. This was similar to other marine molluscs but lower than the fat concentration reported for meat from domestic animals. It is well known that humans can manufacture some types of FA in the body, but must obtain those essential (n3 and n6) for good health from the diet. Omega 3 fatty acids are best found in seafood and plants such as soybean and canola. Omega 6 fatty acids are also found in different types of seafood to a variable extent. However, plant foods are usually a better source of n6 fatty acids. Many studies have suggested a beneficial role of n3 fatty acid in prevention of cardiovascular disease, cardiac arrhythmias, and inflammation including arthritis, improvement of blood flow, lowering of cholesterol level and blood pressure, and maintenance of body weight (Mills *et al.*, 2005; Mozaffarian *et al.*, 2004; Rose and Connolly, 1999; Simopoulos, 1991; Simopoulos, 1999). Studies have identified a wide range of PUFA, especially 2 essential long

chain n3 PUFA in seafood: EPA and DHA. *C. sulcata* contained a relatively high proportion of SFA, with less MUFA and PUFA. This was different from the FA profiles of other marine molluscs. The total n3 FA was much lower, while the total n6 FA was much higher in other marine molluscs. Nowadays, the diet of affluent societies has emphasised n6 type and this has resulted in the ratio of the n3 to n6 being low. This can lead to an unbalanced diet and may cause many negative effects on human health (Wahlqvist, 2004). The n3:n6 FA ratio in *C. sulcata* was about 1, while ratios in other marine molluscs ranged from 2.5 to 39. In addition, *C. sulcata* contained about twice as much cholesterol as other seafood (Hughes *et al.*, 1980; Vlieg, 1988; Vlieg and Body, 1988; Wahlqvist, 2004). For a long time, shellfish have been recognized as a food high in cholesterol (Idler and Wiseman, 1971; Kritchevsky *et al.*, 1967; Lin *et al.*, 1982; Zhu *et al.*, 1994) and there has been reasonable agreement among many of the researchers that cholesterol is the major sterol in molluscs. However, new measuring techniques indicate that cholesterol levels of many shellfish are much lower than previous thought. In fact most of the sterols in molluscs are compounds other than cholesterol (Wahlqvist, 2004). In addition, the presence of MUFA in *C. sulcata* can partly reduce this problem as MUFA can contribute positively to manitaining the plasma level of cholesterol at a lower level (Wahlqvist, 2004).

Thirdly, *C. sulcata* is a good source of Fe, Zn and Cu. Seafood in general is a rich source of Fe and Zn (Table 2-11, Table 2-12). Iron is an essential element for all forms of life. The best-known function of Fe is as a part of the proteins haemoglobin and myglobin, which participate in oxygen transport. Iron impacts the immune systems, and also plays a major role in the function of enzymes which participate in an assortment of other biological processes including synthesis of neurotransmitters, peroxide conversions, purine metabolism, FA synthesis, DNA synthesis, and nitric oxide production (DiSilvestro, 2005). The richest Fe source for humans include red meat, egg yolk, dried legumes, cocoa, cane molasses, and parsley. Seafood is also a good Fe source, with the Fe content in seafood being about a third to a half that in red meat (Wahlqvist, 2004). The Fe concentration in *C. sulcata* was comparable with other marine molluscs. Zinc is a moderately abundant element and is required for the functioning of several enzymes. Also Zn can stabilize the structure of various other molecules such as non-enzyme proteins, certain hormones and cell membrane (DiSilvestro, 2005). In addition, Zn is an essential trace element for proper functioning of the immune system and has shown to be effective in improving growth in humans. Animal products, meats, and some seafood are rich sources of Zn (DiSilvestro, 2005). Zinc

concentration in *C. sulcata* was much higher than in other marine molluscs. *C. sulcata* had similar or superior nutritional value to traditional protein sources including red meats and other seafood. Hence, meat from *C. sulcata* can be promoted and recommended for use as an alternative source of nutritional food. *C. sulcata* may even have an advantage over the other marine molluscs due to its high protein content. In addition, it could be utilized for the special dietary applications of Fe and Zn due to the presence of high concentration of Fe and Zn in *C. sulcata.*

In addition, another difference between fish and shellfish is the geographical distribution and location. Compared with fish, shellfish are relatively non-mobile (mussels and oysters) or less mobile (crab and shrimp) (Sioen *et al.*, 2009) . This certainly makes them more easily influenced by the environment they live in. With the increasing seafood consumption to achieve health and nutritional benefits a conflict situation may occur. Increasing seafood intake may be desirable, but a higher consumption may also lead to an increased intake of potentially harmful environmental contaminants, which can in some cases possibly lead to toxicological health concerns (Sioen *et al.*, 2009). Many researches have reported the toxic levels of heavy metals in marine shellfish including mussel, scallop and oyster (Karakoltsidis *et al.*, 1995; Sidwell *et al.*, 1978; Vlieg *et al.*, 1991). Heavy metal concentrations were analysed to determine the safety of consuming *C. sulcata*. Results indicated that none of the toxic elements exceeded the maximum levels recommended by FSANZ. In addition, 24 OCPs components were measured in *C. Sulcata* and the OCPs levels were below the detection limit.

New Zealand is an island nation. Its inland and coastal waters support fish and shellfish in abundance. New Zealand's seafood resources are small in international terms. New Zealand generates less than 2% of world-wide seafood production. However, seafood is New Zealand's fourth biggest export-earner, behind dairy, meat and forestry (Katvi, 2005). In the past 20 years, New Zealand's seafood industry has sharply increased in value from about NZ$ 500 million to NZ $1355 million. With the enhancement of product-value using modern techniques and expansion of the aquaculture sector, the seafood industry forecast a growth in annual exports to reach NZ$2 billon by 2010 (NZ Seafood Industry Council, 2006). The top three export species are mussels, hoki and rock lobster (NZ Seafood Industry Council, 2006). Species such as orange roughy, squid and paua are also predominant contributors. Asian countries such as Korea, China and Hong Kong are the main export markets. Although many benefits of consumption of seafood have been proven, and the seafood industry has developed

dramatically, consumption of seafood by the general public in NZ is still low. Unlike many other island nations, seafood was not a major part of the diet of most New Zealanders in the twentieth century. Fish consumption has traditionally been low, as New Zealanders have relied on meat, especially beef, lamb and chicken as sources of protein until quite recently. According to the reports between 2003 and 2005 (NZ Seafood Industry Council, 2006), nearly 88% of New Zealanders consumed seafood at least once a month, and only less than half (45%) had seafood at least once a week. The British background of many New Zealanders might help to explain this. In contrast to the bland diet available to working-class British in mid-twentieth century, New Zealand was a protein paradise. Many migrants enjoyed a ready supply of beef and lamb, and eating meat at almost every meal was the dietary aspirations of many nineteenth and twentieth-century migrant. However, it is only in the last 30 years that seafood has been accepted as a possible regular food source by the British migrant although the Maori communities have consumed fish as a regular protein source. In addition, the cost of fish often plays an important role in its consumption, especially for those on a restricted budget. For example, although it is common knowledge among consumers that salmon is a very good source of PUFA, the high retail price of salmon prevents regular consumption. A few, who are more knowledgeable about nutrition and health may choose an alternative n3 PUFA source such as nuts which have a more affordable price, while most will still stay at a red meat meal. .

5.3 Research limitations and future work

This thesis will serve as a pilot study on the nutritional information of *C. sulcata* in NZ. There is a need to extend to other New Zealand non-commercial shellfish, in order to identify nutritionally suitable shellfish that are available in New Zealand. *Turbo smaragdus* which is normally called cat's eye, is well known and widely utilized by New Zealanders, especially Maori. However, *C. sulcata* which is a member in the same family has not so far been accepted as a food and a good source of protein by many people in New Zealand. As a result, comparison of nutritional status of *C. sulcata* and *Turbo smaragdus* would be useful in the future.

In this study, only a small number of snails (10 to 16 in total) were used for each analysis. In hindsight, it would have been beneficial to have used a larger number of snails for each size (small and large) (up to 25-30 each size/analysis) to obtain more representative data.

Moreover, it is well known that a number of factors contribute to the variability in the composition of snail meat including species, size (age), sex, sampling location, and season (Ansell, 1972; Ansell and Trevallion, 1967; Blackmore, 1969; McLachlan and Lombard, 1980; Miletic *et al.*, 1991; USDA, 2006). Samples were collected during a short period from the same site to minimize variability since this thesis did not account for those sources of variability such as location and season, except animal size, due to cost and time constrains. Furthermore, it is believed that cholesterol concentration in *C. sulcata* has been overestimated in this research by using the method described in chapter 3 since more than 90% of sterol in snails was of the cholesterol type and/or similar chemical structure to cholesterol. An alternative method might be used in future studies to identify the differing sterols and more accurately estimate cholesterol. Similarly the method chosen for FA analysis can quantify FA in *C. sulcata*. However, modifications are recommended that would reduce the percentage of unknown FA.

There is potential for commercial farming of *C. sulcata.* Katvi (2005) indicated that farmed fish had a higher nutritional value than those in the wild. This may be the case for *C. sulcata* and would increase the potential for its use. When snails are used as food, the shell is a large waste product since it constitutes more than 70% of the whole snail weight. However, allantion which is found in the snail shell has been widely used as a cosmetic ingredient (DiSalvo, 2002).

There is large body of research that have used shellfish as a biomonitor for heavy metal contamination due to their sedentary character (Boyden, 1974; Fagbuaro *et al.*, 2006; Milne, 1998; Spronk *et al.*, 1971). Therefore, a study of the relationship between heavy metal contaminations in *C. sulcata* and ambient sediments from different locations is suggested. In addition, some animals such as the mud snail of *Amphibola* can regulate their internal metal burdens, enabling metal levels to be maintained at an acceptable concentration (Milne, 1998). More biological information of *C. sulcata* regarding its possible regulation of internal metal burdens is desirable. Hence, the possibility of utilization of *C. sulcata* as a biomonitor could continue to be expanded.

Chapter 6: References

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Appendix 1: Proximate composition of land snails

Species	Proximate content (%)					References
	Protein	**Lipids**	**Moisture**	**Ash**	**Carbo**	
Archachatina marginata	39.7-43.3*	ND	74.3-78.0	0.5 -0.6	N/A	Hamzat *et al.*, (2002)
Helix aspersa	10.5 65.2*	1.6 10.0*	83.7	1.5 9.4*	N/A	Gomot, (1998)
Helix aspersa maxima	8.1 58.0*	1.5 10.8*	85.8	1.2 8.8*	1.7 13.8*	
Helix lucorum	10.7-11.9 60-68.2*	0.6-1.5 3.4-9.7*	82.1-84.0	1.4-2.3 9.2-13.4*	N/A	
Helix pomatia	7.3-12.8 55.0-72.5*	0.4-1.1 3.1-7.0*	82.3-86.8	1.8-2.2 11.7-12.6*	N/A	
unknown	16.1	1.4	79.2	1.3	2.0	WattandMerrill, (1975)
Achatina sp.	9.9	1.4	89.2	2.1	4.4	
*Helix pormatia (*raw foot*)*	NM	NM	72.8-80.7	NM	N/A	Wiesre and Schuster, (1975)
Helix pormatia	12.3	0.7	84.9	1.9	N/A	Gomot, (1998)
Helix pormatia	12.6	0.5	84.9	1.8	N/A	Gomot, (1998)
Helix aspersa	9.9-12.0	0.5-0.7	83.3-87.6	1.2-2.7	0.4-0.5	Claeys and Demeyer, (1986)
Limicolaria aurora	51.4*	9.7*	71.2	11.8*	27.1*	Udoh *et al.*, (1995)
Archachatina marginata	20.8	1.2	76.6	1.4	N/A	Adeyeye, (1996)
Achatina sp.	14.5	4.2	79.9	1.3		
Limicolaria sp.	17.5	4.3	77.9	1.1		
Archachatina marginata Pfeiffer	20.6	1.4	76.6	1.4	0.01	Fagbuaro *et al.*, (2006)
Archachatina Marginata Philippi	20.3	1.2	76.7	1.4	0.4	
Achatina achatina	19.3	1.4	77.5	1.3	0.4	
Limicolaria spp.	18.7	1.2	78.7	1.4	0.2	
Helix aspersa maxima	9.5-12.6	0.5-2.7	77.5-79.9	0.7-0.9	NM	Milinsk *et al.*, (2006b)
Helix aspersa maxima	14.8-18.4	0.9-1.3	74.8-76.2	1.0-1.2	NM	Milinsk *et al.*, (2003)

N/A = not available Carbo = carbohydrate *= dry matter

Appendix 2: Proximate composition of some common Marine molluscs consumed as food

Species	Proximate content (%)					References
	Protein	Lipids	Moisture	Ash	Carbo	
Perna canaliculus	11.9	2.1	80.9	1.7	3.4	Vlieg, (1988)
Atrina zelandica	14.8	0.5	81.0	1.3	2.4	
Ostra edulis	12.9	3.0	79.4	1.6	3.1	
Pecten novaezelandiae	15.4	1.3	78.7	1.9	2.7	
Mutilus qalloprovinciallis	8.0-13.0	1.0-2.0	82.0-87.0	0.7-2.0	2.0-5.0	Karakoltsidis *et al.*, (1995)
Ostra edulis	11-15	0.2-1.0	82-88	1.0-1.6	2.0-3.0	
Mutilus qalloprovinciallis	42.4-56.2*	5.8-13.4*	ND	11.0-21.0*	N/A	Orban *et al.*, (2002)
Perna canaliculus	13.9	2.2	78.2	2.2	N/A	Hughes *et al.*, (1980)
Pecten novaezelandiae	14.3	0.7	82.4	1.6	N/A	
Patella vulgata	ND	10.5-53.3#	75.0-84.0	8.6-34.7*	N/A	Blackmore, (1969)
Tellina tenuis	7.9-10.9	3.9-12.8*	71.0-83.0	5.8-27.7*	6.4-25.6*	Ansell and Trevallion, (1967)

#=ash free DM ND = not detected N/A = not available Carbo = carbohydrate *= dry matter

Appendix3: Proximate composition of common meat

Species	Proximate content (%)					References
	Protein	Lipids	Moisture	Ash	Carbohydrate	
goat	23.4	5.0	68.3	1.7	N/A	Lee *et al.*, (2008)
	17.6	2.9	78.6	0.9	N/A	Paleari *et al.*, (2003)
	19.7	3.8	75.4	1.1	N/A	Paleari *et al.*, (2008)
beef chuck and round	ND	6.9	72.3	1.3	N/A	Seggern *et al.*, (2005)
ground beef	18.1-21.7	6.2-21.7	59.6-72.9	ND	N/A	Isaksson *et al.*, (1996)
beef steak	22.5-23.9	1.1-1.6	73.3-74.9	1.2-1.4	N/A	Smith *et al.*, (1989)
beef chuck and round	ND	6.9	72.3	1.3	N/A	Seggern *et al.*, (2005)
beef aitch bone	1.8-2.5	40.3-42.0	44.4-46.6	0.6-0.8	N/A	Crosland *et al.*, (1995)
Boneless chicken	20.4-22.7	2.8-9.2	69.7-74.3	0.3-1.3	N/A	Al-Najdawi and Abdullah, (2002)
pork	21.9-23.1	1.7-1.8	71.5-73.9	2.6-3.2	ND	ChengandSun, (2007)
pork shoulder	16.9-17.5	12-14.8	65.9-69.9	1-1.1	ND	Crosland *et al.*, (1995)
pork chines	15.0-16.9	21.7-22.1	59.2-61.3	1.3-1.6	ND	
lamb neck and ribs	15.6-16.3	21.4-26.4	55.4-60.7	0.9-1.0	ND	
lamb	15.89-19.39	10.44-24.14	65.21-69.76	0.78-1.06	ND	Kosulwat *et al.*, (2003)
ram lamb	20.00-20.40	2.53-2.67	76.49-76.89	1.11-1.13	ND	Badiani *et al.*, (1998)
lamb	23.39	4.56	68.96	1.17	ND	Lee *et al.*, (2008)

ND = not detected N/A = not available

Appendix 4: Fatty acid profile of mussel (% FA)

FA	1			2			3
C14:0	7.0	1.5	3.0-5.6	6.2	5.5	3.5-6.1	1.5
C15:0	5.0	0.6	1.1-1.4	0.8	0.9	0.6-1.1	0.4
C16:0	19.0	16.2	10.1-17.6	15.0	20.7	21.5-25.5	20.9
C17:0	1.0	2.9	0.4-1.9	1.2	1.1	0.2-2.0	2.4
C18:0	4.0	3.7	4.3-10.8	3.7	5.0	3.8-7.3	8.9
C20:0	*	N/A	N/A	total of	*	0.1-3.6	0.1
C22:0	N/A	N/A	N/A	C22:0,	N/A	<0.1	N/A
C24:0	N/A	N/A	N/A	C22:0 and C24:0 <0.3	N/A	0.2-0.8	N/A
C14:1	*	N/A	N/A	N/A	*	0.9-6.4	*
C15:1	2.0	N/A	N/A	N/A	0.6	0.1-1.0	0.2
C16:1	19.0	2.7	4.6-12.6	5.9	8.8	4.0-6.6	2.1
C17:1	0.3	N/A	N/A	0.5	2.1	*	1.0
C18:1	7.0	4.4	3.2-4.2	3.1	5.4	1.5-3.4	7.3
C20:1	1.0	5.1	1.8-2.7	1.0	5.4	2.5-4.9	4.8
C22:1	6.0	N/A	N/A	1.3	1.3	0.1-0.7	3.8
C24:1	N/A	N/A	N/A	0.8	N/A	0.1-1.0	N/A
C14:2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C15:2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C18:2 n6	3.0	2.0	0.6-1.4	1.6	2.1	1.6-5.0	1.6
C20:2	N/A	0.5-3.5	0.7-1.7	3.3	N/A	0.5-0.8	N/A
C22:2	N/A	0.6-2.9	0.1-0.3	N/A	N/A	ND	N/A
C18:3 n3	0.5	1.8	N/A	2.5	^	1.4-4.6	^
C18:3 n6	N/A	N/A	N/A	NM	N/A	ND	N/A
C20:3 n6	N/A	0.5	N/A	0.2	N/A	0.3-1.7	N/A
C22:3	N/A	1.1	N/A	N/A	N/A	N/A	N/A
C18:4 n3	*	2.0	0.6-2.9	4.5	N/A	1.0-6.0	N/A
C20:4 n6	N/A	4.1	1.0-5.8	1.3	^^	N/A	^^
C22:4 n6	*	0.5-1.1	N/A	N/A	0.7	0.2-0.8	1.7
C20:5 n3	15.0	13.0	17.4-31.6	15.9	16.6	12.0-21.2	11.6
C22:5 n6	1.0	1.3	N/A	0.7	1.0	1.5-2.3	2.0
C22:6 n3	9.0	26.4	9.7-19.5	21.4	14.4	15.1-19.7	20.1
n3	25.5	45.6	33.1-46.0	46.8	15.9	36.3-45.3	34.0
n6	3.0	7.2	2.1-7.4	9.4	2.4	5.1-7.9	3.1
n3:n6	8.5	6.3	5.1-21.9	5.0	6.6	4.6-8.9	11.0
References	Karakoltsidis *et al.*, (1995)	Pirini *et al.*, (2007)	Ventrella *et al.*, (2008)	Hughes *et al.*, (1980)	Vlieg and Body, (1988)	Taylor and Savage, (2006)	Vlieg and Body, (1988)

1= Mytilus galloprovinciallis; 2= *Perna canaliculus*; 3= *Atrinata pectinata*; 4=*Perna perna*
*= trace (less than 0.1%);
^= less levels of 18:3 were contained in 20:1; ^^= less levels of 20:4 were contained in 22:1
ND=not detected; N/A = not available

Appendix 5: Fatty acid profile of scallop (%)

FA[a]	1	1	2	3
C14:0	2.7	2.6	1.9-2.4	0.8-3.8
C15:0	0.7	0.7	0.9-1.0	0.2-0.8
C16:0	19.6	22.5	12.2-13.4	10.2-26.3
C17:0	1.5	1.9	0.9-1.1	0.5-1.2
C18:0	6.1	7.3	8.0-8.1	3.4-7.0
C20:0	total of C22:0, C22:0	0.1	N/A	0.2-0.5
C22:0	and C24:0 <0.3	N/A	N/A	N/A
C24:0		N/A	N/A	N/A
C14:1	N/A	*	N/A	N/A
C15:1	N/A	0.2	N/A	N/A
C16:1	2.6	4.5	2.6-2.2	1.1-5.5
C17:1	0.6	0.9	N/A	0.2-4.1
C18:1	5.4	8.6	4.3-5.2	1.7-5.5
C20:1	1.1	3.8	3.3-3.8	1.6-3.4
C22:1	2.2	1.9	0.1-0.4	2.5
C24:1	0.3	N/A	N/A	N/A
C14:2	N/A	N/A	N/A	N/A
C15:2	N/A	N/A	N/A	N/A
C18:2 n6	0.4	1.8	0.4-0.5	0.3-0.9
C20:2 n6	0.8	N/A	0.3-0.6	0.2-0.7
C22:2	N/A	N/A	0.2-0.8	N/A
C18:3 n3	1.9	^	0.3-0.4	0.5-0.8
C18:3 n6	N/A	^	N/A	0.2
C20:3 n6	*	N/A	N/A	0.3
C22:3 n6	N/A	N/A	0.5-0.6	N/A
C18:4 n3	5.2	N/A	0.4-0.7	1.3-2.4
C20:4 n6	0.9	^^	7.6-8.8	0.2-1.0
C22:4 n6	N/A	1.1	0.5-0.7	1.0-3.7
C20:5 n3	15.4	14.8	15.4-19.1	12.5-23.2
C22:5 n3	1.5	1.4	0.9-1.0	1.5-2.9
C22:6 n3	21.5	19.8	23.4-26.1	8.7-31.0
n3	46.8	34.6	40.6-47.4	30.6-54.7
n6	3.9	2.9	10.6-12.4	2.1-4.7
n3:n6	12	11.9	3.8	7.5-15.4
References	Hughes *et al.*, (1980)	Vlieg and Body, (1988)	Silina and Zhukova, (2007)	Pazos *et al.*, (1997)

1=*Pectan novaezealandiae*; 2=*Patinopecten yessoensis*; 3=*Pecten maximus*

*= trace (less than 0.1%); [a]=unknown fatty acids were not shown on the table;

^= less levels of 18:3 were contained in 20:1; ^^= less levels of 20:4 were contained in 22:1;

N/A = not available

Appendix 6: Fatty acid profile of oyster (%)

FA[a]	1			2
C14:0	1.2-2.6	2.9-3.8	5.3	2.6
C15:0	0.3-0.7	0.4-0.5	1.1	0.7
C16:0	19.8-20.8	17.6-19.2	28.5	19.2
C17:0	N/A	1.7-2.1	2.0	1.3
C18:0	0.9-2.3	7.3-7.9	5.9	5.1
C20:0	N/A	N/A	*	*
C22:0	N/A	N/A	N/A	N/A
C24:0	N/A	N/A	N/A	N/A
C14:1	N/A	N/A	0.3	*
C15:1	N/A	N/A	0.4	0.2
C16:1	1.6-2.2	3.6-8.7	5.5	5.0
C17:1	N/A	0.4-0.5	1.5	0.9
C18:1	5.3-7.5	6.2-6.3	9.0	6.3
C20:1	5.1-6.1	6.2-8.2	3.4	7.1
C22:1	N/A	0.3-0.4	1.4	1.8
C24:1	N/A	*	N/A	N/A
C14:2	N/A	N/A	N/A	N/A
C15:2	N/A	N/A	N/A	N/A
C18:2 n6	7.5-11.9	2.6-2.8	1.8	1.7
C20:2 n6	0.2-0.8	0.2	N/A	N/A
C22:2	0.1-1.1	N/A	N/A	N/A
C18:3 n3	3.1-4.4	2.6-3.0	^	^
C18:3 n6	0.1-1.1	0.2	^	^
C20:3 n6	0.1-0.2	N/A	N/A	N/A
C22:3 n6	N/A	N/A	N/A	N/A
C18:4 n3	3.3-7.9	1.6-1.8	N/A	N/A
C20:4 n6	1.3-2.2	2.9-3.5	^^	^^
C22:4 n6	1.6-2.4	N/A	0.6	1.4
C20:5 n3	10.7-12.1	7.9-8.3	8.6	21.2
C22:5 n3	0.7-1.2	1.0-1.1	1.0	0.9
C22:6 n3	17.7-21.9	8.4-10.0	7.3	12.8
n3	38.2-46.9	24.0-22.8	15.9	34.0
n6	14.6-16.5	7.2-6.8	2.4	3.1
n3:n6	2.5-3.2	3.4-3.5	6.6	11.0
References	Dridi *et al.*, (2007)	Abdulkadir and Tsuchiya, (2008)	Vlieg and Body, (1988)	

1=*Crassostrea gigas*; 2=*Ostrea sinuata*

*= trace (less than 0.1%); [a]=unknown fatty acids were not shown on the table; ^= less levels of 18:3 were contained in 20:1; ^^= less levels of 20:4 were contained in 22:1; N/A = not available

Appendix 7: Fatty acid profile of land snails (%)

FA[a]	1			1	2	3
C14:0	0.3-0.5	0.4-0.7	0.4	N/A	N/A	N/A
C15:0	N/A	N/A	0.3	N/A	N/A	N/A
C16:0	6.7-8.1	6.7-10.1	10.3	7.1	9.6	8.0
C17:0	0.9-1.0	0.7-0.8	2.0	N/A	N/A	N/A
C18:0	14.5-16.1	12.3-14.5	16.4	10.4	10.6	8.9
C20:0	N/A	N/A	0.8	N/A	N/A	N/A
C22:0	N/A	N/A	6.3	N/A	N/A	N/A
C24:0	N/A	N/A	0.8	N/A	N/A	N/A
C14:1	N/A	N/A	0.1	N/A	N/A	N/A
C15:1	N/A	N/A	*	N/A	N/A	N/A
C16:1	N/A	0.4-0.8	0.6	1.4	1.5	3.2
C17:1	N/A	N/A	0.3	N/A	N/A	N/A
C18:1	18.2-19.7	17.6-21.6	14.7	14.2	11.9	10.0
C20:1	2.7-3.4	1.6-2.5	1.9	2.2	0.9	3.6
C22:1	N/A	N/A	*	N/A	N/A	N/A
C24:1	N/A	N/A	2.0	N/A	N/A	N/A
C14:2	N/A	N/A	N/A	N/A	N/A	N/A
C15:2	N/A	N/A	N/A	N/A	N/A	N/A
C18:2 n6	17.9-21.6	19.4-24.5	13.5	15.7	14.5	11.0
C20:2 n6	N/A	N/A	9.1	12.1	10.5	9.1
C22:2	N/A	N/A	0.5	N/A	N/A	N/A
C18:3 n3	0.8-2.1	1.6-2.5	1.9	1.8	3.2	4.3
C18:3 n6	N/A	N/A	*	N/A	N/A	N/A
C20:3 n6	0.3-0.4	0.3-0.4	0.6	N/A	N/A	N/A
C22:3 n6	N/A	N/A	N/A	N/A	N/A	N/A
C18:4 n3	N/A	N/A	N/A	N/A	N/A	N/A
C20:4 n6	9.0-10.8	9.3-10.6	N/A	14.8	13.8	16.9
C22:4 n6	3.5-4.2	3.6-4.5	N/A	4.8	5.3	4.0
C20:5 n3	1.4-1.7	1.6-1.9	0.1	2.8	1.5	5.5
C22:5 n3	0.7-0.9	0.8-1.3	N/A	1.3	1.7	2.0
C22:6 n3	N/A	N/A	0.2	ND	ND	ND
n3	5.0-6.4	5.5-6.2	2.7	6.6	7.2	11.9
n6	32.0-35.0	33.1-38.5	13.6	50.3	48.8	45.9
n6:n3	5.0-7.1	5.5-6.9	4.9	7.8	6.9	5.2
Reference	Milinsk *et al.*, (2003)			Milinsk *et al.*, (2006b)	Özogul *et al.*, (2005)	Zhu *et al.*, (1994)

1=*Helix* sp.; 2=*Haplotrema sportella*; 3=*Vespericola columbiana*
*= trace (less than 0.1%); [a]=unknown fatty acids were not shown on the table;
N/A = not available; ND=not detected

Appendix 8: Size measurement of *C. sulcata*

Group	Sample ID.	WAW	D1 (cm)	D2 (cm)	L1 (cm)	L2 (cm)	WMW (g)
Small n=48	1	23.2	42.5	36.3	41.6	26.5	4.9
	2	23.4	40.3	34.2	40.8	22.7	6.2
	3	24.2	44.6	38.5	40.3	24.3	5.9
	4	29.5	47.0	40.2	42.3	27.2	7.4
	5	30.8	48.1	41.5	41.8	26.5	7.4
	6	31.2	46.7	40.0	44.3	27.2	6.6
	7	33.0	49.0	40.7	43.7	25.8	6.7
	8	34.3	49.7	42.4	47.3	29.1	8.5
	9	34.4	51.5	44.2	47.2	30.6	8.6
	10	34.8	48.2	41.8	43.7	28.2	7.6
	11	35.2	50.0	42.3	46.8	32.4	8.0
	12	35.6	51.0	42.2	48.5	30.7	9.4
	13	35.9	51.1	43.3	45.7	29.5	9.2
	14	38.1	51.1	44.5	48.6	31.4	8.5
	15	38.4	53.0	49.5	49.1	28.5	9.5
	16	38.9	52.4	43.9	46.2	30.9	10.2
	17	39.2	51.7	43.0	47.9	30.8	6.4
	18	40.7	53.1	47.5	48.8	30.8	10.5
	19	42.0	54.8	45.6	49.2	29.5	10.4
	20	42.1	55.7	49.3	50.7	34.8	6.9
	21	42.5	52.0	45.6	49.0	29.2	11.5
	22	42.7	52.9	44.9	48.9	28.4	10.5
	23	43.0	54.7	46.1	48.7	30.0	9.6
	24	43.4	53.0	44.8	48.7	30.6	9.7
	25	44.0	51.8	46.8	47.9	31.3	10.7
	26	44.1	54.3	44.1	51.4	34.5	8.3
	27	44.9	55.0	44.9	47.5	31.5	8.7
	28	45.6	53.5	46.9	48.3	29.3	11.0
	29	45.7	55.2	46.0	51.3	30.9	10.5
	30	46.4	54.3	46.1	53.0	34.7	10.7
	31	46.8	57.1	47.1	50.7	30.8	12.0
	32	46.9	53.4	48.0	52.5	30.4	11.8
	33	47.0	56.0	47.1	51.9	32.4	11.2
	34	47.2	55.6	45.8	50.7	27.6	11.5
	35	47.4	56.4	47.4	51.6	34.3	10.5
	36	48.2	53.8	46.9	50.5	33.1	12.1
	37	48.8	56.2	48.1	46.5	29.4	13.2
	38	49.2	56.2	47.2	53.1	32.5	8.7
	39	50.1	56.9	48.1	48.7	31.5	12.1
	40	52.0	60.8	51.9	55.5	32.3	14.7
	41	52.3	56.5	48.5	52.1	32.4	13.3
	42	52.8	54.6	48.6	51.3	32.5	14.7
	43	52.9	57.5	48.7	52.2	30.4	12.9
	44	53.3	55.4	47.5	33.2	33.8	15.0
	45	54.6	56.8	49.0	52.0	34.4	12.6
	46	56.4	58.1	50.1	55.4	35.6	14.9
	47	58.7	59.9	50.5	57.8	35.6	15.3
	48	59.9	58.0	51.2	55.5	55.1	15.0
	Mean ± SD	42.7±8.9	53.1±4.2	45.4±3.7	48.6±4.6	31.1±4.5	10.2±2.7
Large n=46	49	60.1	61.4	50.2	55.7	35.8	19.0
	50	61.3	58.5	50.7	53.5	35.2	16.5
	51	61.3	58.5	50.7	53.5	35.2	16.5
	52	64.5	64.1	52.4	56.4	34.2	16.8
	53	64.7	61.8	54.6	57.0	36.4	16.5
	54	66.5	62.8	52.0	55.1	34.2	16.1

	55	72.6	64.9	55.7	58.3	34.9	19.8
	56	72.8	64.4	54.9	56.2	34.3	17.3
	57	76.9	66.3	55.7	60.4	36.4	20.7
	58	79.9	65.1	58.5	57.8	36.5	22.0
	59	82.0	69.1	55.1	65.5	42.5	23.2
	60	82.0	69.1	55.1	65.5	42.5	23.2
	61	82.2	66.3	55.4	57.6	34.1	16.8
	62	82.2	66.3	55.4	57.6	34.1	16.8
	63	86.5	67.4	58.5	66.0	40.8	18.8
	64	87.6	69.0	59.4	62.7	39.3	19.2
	65	87.8	67.5	56.9	63.0	40.2	19.9
	66	88.7	68.3	58.5	63.9	43.2	18.8
	67	90.9	70.6	57.9	64.1	41.8	20.1
	68	94.6	66.8	55.8	59.1	38.3	19.9
	69	94.6	66.8	55.8	59.1	38.3	19.9
	70	95.9	68.5	59.5	64.3	40.0	21.4
	71	96.1	68.9	57.8	64.6	40.2	18.9
	72	96.4	65.1	60.9	64.8	44.5	24.0
	73	104.4	73.2	61.8	66.5	41.7	21.9
	74	105.5	68.4	60.7	64.8	36.7	21.8
	75	112.3	76.0	63.2	67.1	41.7	21.9
	76	112.5	75.7	61.5	69.0	42.4	25.1
	77	113.2	74.9	62.1	67.8	42.8	28.3
	78	113.8	74.7	65.4	68.0	41.1	30.9
	79	113.8	75.0	65.7	68.3	44.5	24.0
	80	114.1	70.2	31.6	65.0	35.6	21.3
	81	116.5	78.5	61.9	70.4	42.0	28.8
	82	121.5	80.1	63.9	72.3	43.1	23.5
	83	124.2	78.0	66.5	69.2	44.4	28.2
	84	126.1	69.3	65.3	69.3	46.1	33.4
	85	128.3	77.5	68.1	63.0	49.6	37.6
	86	129.3	79.1	61.9	71.3	45.0	26.1
	87	132.1	77.0	68.4	70.5	46.8	32.3
	88	132.7	79.3	67.0	68.9	45.9	34.5
	89	133.0	76.6	67.5	71.5	46.8	32.3
	90	140.0	83.1	70.4	75.8	50.4	32.2
	91	140.0	86.8	68.4	78.2	53.2	31.4
	92	150.7	80.5	68.2	78.5	50.1	38.5
	93	156.3	83.5	75.4	75.0	42.3	43.5
	94	162.6	85.3	67.5	79.3	52.4	34.3
	Mean ± SD	102.4 ± 27.2	71.3±7.1	59.8±7.3	65.0±6.8	41.3±5.2	24.2±6.9

WAW=whole animal weight; WMW=whole muscle weight

Appendix 9: Proximate composition of *C. sulcata*

Sample ID.		Moi	Protein		Fat		Ash		Carbo	
		% FW	%FW	%DW	%FW	%DW	%FW	%DW	%FW	%DW
S n=9	**1**	77.13	17.64	77.12	1.10	4.47	2.17	9.50	1.96	8.91
	2	76.84	18.67	80.60	0.83	3.28	2.22	9.60	1.44	6.52
	3	77.61	17.48	78.09	1.17	4.84	2.08	9.30	1.66	7.77
	4	77.61	17.79	79.45	1.15	4.53	1.88	8.40	1.57	7.62
	5	80.00	16.29	81.48	0.83	3.89	1.78	8.90	1.09	5.74
	6	77.68	16.89	75.69	1.02	4.30	2.10	9.40	2.31	10.62
	7	79.49	16.31	79.51	0.74	3.29	1.91	9.30	1.55	7.90
	8	76.17	18.71	78.51	1.04	3.85	2.29	9.60	1.80	8.04
	9	77.11	18.06	78.87	0.79	3.18	2.13	9.30	1.92	8.65
	Mean (± SD)	77.74 (± 1.24)	17.54 (± 0.90)	78.81 (± 1.75)	0.96 a (± 0.16)	3.96 a (± 0.61)	2.06 a (± 0.17)	9.26 a (± 0.38)	1.70 (± 0.35)	7.97 (± 1.40)
L n=7	**10**	75.47	20.57	83.84	0.97	3.67	1.94	7.90	1.05	4.58
	11	78.24	17.23	79.16	0.67	3.08	1.83	8.40	2.04	9.36
	12	78.30	17.07	78.67	0.70	3.22	1.80	8.30	2.13	9.81
	13	77.11	18.11	79.09	0.65	2.83	1.92	8.40	2.22	9.68
	14	80.00	15.63	78.16	0.65	3.27	1.62	8.10	2.09	10.46
	15	78.30	16.72	77.04	0.71	2.97	1.93	8.90	2.34	11.09
	16	78.24	17.72	81.44	0.66	2.74	1.85	8.50	1.53	7.33
	Mean (± SD)	77.95 (± 1.98)	17.58 (± 1.54)	79.63 (±2.29)	0.72 b (± 0.12)	3.11 b (± 0.31)	1.84 b (± 0.11)	8.36 b (± 0.32)	1.91 (± 0.46)	8.90 (± 2.24)

S= small; L= large; Moi=moisure;
Values with the same letter are not significant different using the LSD (5%)
DW=dry weight; FW= fresh weight; SD=standard deviation

Appendix 10: Amino acids profile of *C .sulcata* (% fresh weight)

AA	Small Group (n=5)					
	1	2	3	4	5	Mean±SD
Asp	1.52	1.42	1.23	1.38	1.44	1.40±0.11
Glu	2.79	2.78	2.41	2.87	2.93	2.75±0.20
Ser	0.82	0.75	0.66	0.81	0.79	0.77±0.07
His*	0.25	0.27	0.25	0.27	0.3	0.27±0.02
Gly	2.2	1.85	1.58	2.21	1.93	1.95±0.26
Thr*	0.68	0.67	0.61	0.71	0.73	0.68±0.05
Arg	2.05	1.98	1.76	2.07	2.18	2.01±0.16
Ala	0.96	0.9	0.79	0.91	0.94	0.90±0.06
Tau	1.33	1.93	1.88	2.06	2.46	1.93±0.41
Tyr	0.46	0.46	0.4	0.46	0.49	0.45±0.03
Val*	0.51	0.5	0.45	0.48	0.53	0.50±0.03
Phe*	0.49	0.49	0.44	0.49	0.53	0.49±0.03
Ile*	0.54	0.53	0.47	0.49	0.54	0.51±0.03
Lys*	1.2	1.24	1.11	1.14	1.3	1.20±0.08
Leu*	1.15	1.13	1	1.08	1.17	1.11±0.07
Pro	1.09	0.98	0.84	1.13	1.09	1.03±0.12
Cys	ND	0.07	0.06	0.05	0.05	0.06±0.01
Met*	ND	0.09	0.06	0.07	0.06	0.07±0.02
Trp*	ND	ND	1.13	ND	1.41	1.27±0.20
TAA	18.03	18.03	17.12	18.68	20.88	18.55±1.42
TEAA	4.81	4.92	5.52	4.73	6.56	5.31±0.77
TEAA/ TAA	0.27	0.27	0.32	0.25	0.31	0.29±0.03

AA	Large Group (n=7)							
	6	7	8	9	10	11	12	Mean±SD
Asp	1.57	1.32	1.28	1.03	1.31	1.42	1.57	1.36±0.19
Glu	3.29	2.76	2.61	2.13	2.67	2.86	3.13	2.78±0.38
Ser	0.84	0.8	0.73	0.57	0.76	0.78	0.81	0.76±0.09
His*	0.32	0.25	0.24	0.21	0.25	0.3	0.31	0.27±0.04
Gly	1.94	2.28	1.98	1.41	2.02	1.76	1.81	1.89±0.27
Thr*	0.79	0.69	0.65	0.53	0.68	0.71	0.73	0.68±0.08
Arg	2.39	2.04	1.96	1.57	1.93	2.08	2.06	2.00±0.24
Ala	1.02	0.88	0.82	0.67	0.84	0.9	0.95	0.87±0.11
Tau	2.36	1.89	1.68	1.49	1.78	1.9	1.8	1.84±0.27
Tyr	0.53	0.44	0.43	0.35	0.44	0.45	0.47	0.44±0.05
Val*	0.58	0.47	0.45	0.38	0.47	0.51	0.54	0.49±0.06
Phe*	0.57	0.47	0.44	0.37	0.45	0.49	0.49	0.47±0.06
Ile*	0.6	0.47	0.47	0.39	0.47	0.53	0.57	0.50±0.07
Lys*	1.46	1.05	1.04	0.88	1.03	1.22	1.19	1.13±0.18
Leu*	2.62	1.18	1.02	0.85	1.01	1.13	1.16	1.28±0.60
Pro	1.05	1.17	1.02	0.74	1.03	0.84	0.82	0.95±0.15
Cys	0.05	0.07	0.07	0.05	0.04	0.04	0.04	0.05±0.01
Met*	0.05	0.08	0.08	0.06	0.06	0.06	0.03	0.06±0.02
Trp*	1.35	1.26	1.18	1.00	1.01	1.01	0.53	1.05±0.27
TAA	23.36	19.56	18.16	14.67	18.26	19.00	19.02	18.86±2.56
TEAA	8.33	5.92	5.57	4.67	5.43	5.96	5.55	5.92±1.15
TEAA/ TAA	0.36	0.30	0.31	0.32	0.30	0.31	0.29	0.31±0.02

AA= Amino acid; TAA= total amino acids, TEAA= total essential amino acids;
TEAA/TAA= total essential amino acids /total amino acids; *= essential amino acid;
ND=not detected

Appendix 11: Amino acids profile of *C. sulcata* (% dry weight)

AA	Small Group (n=5)					
	1	2	3	4	5	Mean±SD
Asp	0.98	1.09	1.14	1.02	1.06	1.06±0.06
Glu	2.68	2.67	2.81	2.49	2.80	2.69±0.13
Ser	2.03	2.01	2.03	1.85	1.91	1.97±0.08
His*	1.94	1.97	2.03	1.81	1.92	1.93±0.08
Gly	2.13	2.11	2.07	1.94	1.94	2.04±0.10
Thr*	4.75	4.98	5.00	4.58	4.51	4.76±0.22
Arg	4.55	4.51	4.51	4.10	4.28	4.39±0.19
Ala	ND	0.37	0.22	0.24	0.27	0.28±0.06
Tau	ND	ND	5.43	4.63	0.00	3.35±2.93
Tyr	6.02	5.67	5.55	5.04	5.46	5.55±0.35
Val*	11.05	11.11	11.28	9.89	11.34	10.93±0.60
Phe*	3.25	2.98	3.04	2.70	3.22	3.04±0.22
Ile*	8.72	7.40	7.43	6.47	8.72	7.75±0.97
Lys*	8.12	7.92	8.39	7.23	8.20	7.97±0.45
Leu*	3.78	3.59	3.61	3.26	3.60	3.57±0.19
Pro	5.27	7.73	9.48	7.74	8.14	7.67±1.52
Cys	1.83	1.84	1.89	1.64	1.81	1.80±0.10
Met*	4.32	3.91	4.19	3.45	4.46	4.07±0.40
Trp*	ND	0.27	0.20	0.24	0.19	0.23±0.04
TAA	71.41	72.11	80.29	70.34	73.81	73.59±3.95
TEAA	19.06	19.70	25.24	22.67	18.69	21.07±2.81
TAAA/ TAA	0.27	0.27	0.31	0.32	0.25	0.29±0.03

AA	Large Group (n=7)							
	6	7	8	9	10	11	12	Mean±SD
Asp	1.27	0.99	0.95	0.83	1.24	1.04	1.25	1.08±0.17
Glu	3.11	2.69	2.59	2.15	2.91	2.84	2.90	2.74±0.31
Ser	2.26	1.84	1.80	1.54	2.09	1.96	2.16	1.95±0.24
His*	2.22	1.84	1.75	1.48	2.00	1.90	1.94	1.88±0.23
Gly	2.36	1.86	1.84	1.58	2.19	1.98	2.28	2.01±0.28
Thr*	5.71	4.14	4.10	3.56	5.02	4.34	4.74	4.52±0.71
Arg	10.26	4.63	4.04	3.43	4.64	4.23	4.60	5.12±2.31
Ala	0.19	0.32	0.30	0.26	0.24	0.23	0.12	0.24±0.07
Tau	5.28	4.94	4.66	4.04	4.13	4.24	2.12	4.20±1.02
Tyr	6.15	5.18	5.04	4.18	5.81	5.49	6.27	5.45±0.73
Val*	12.88	10.85	10.32	8.61	11.73	11.18	12.47	11.15±1.43
Phe*	3.29	3.14	2.89	2.30	3.19	3.20	3.24	3.03±0.35
Ile*	7.61	8.95	7.84	5.70	7.23	8.49	7.20	7.58±1.05
Lys*	9.38	8.01	7.76	6.33	8.55	8.11	8.19	8.05±0.92
Leu*	4.02	3.46	3.26	2.71	3.70	3.54	3.78	3.50±0.42
Pro	9.27	7.44	6.63	6.00	7.81	7.47	7.17	7.40±1.02
Cys	2.06	1.73	1.68	1.40	1.86	1.83	1.89	1.78±0.21
Met*	4.10	4.59	4.03	3.00	3.46	4.34	3.28	3.83±0.59
Trp*	0.18	0.28	0.29	0.19	0.17	0.17	0.16	0.21±0.05
TAA	91.59	76.88	71.79	59.29	77.97	76.57	75.76	75.69±9.53
TEAA	32.66	23.25	22.03	18.86	24.46	22.76	22.10	23.73±4.29
TEAA/ TAA	0.36	0.30	0.31	0.32	0.31	0.30	0.29	0.31±0.12

AA= Amino acid; TAA= total amino acids, TEAA= total essential amino acids;
TEAA/TAA= total essential amino acids /total amino acids; *= essential amino acid;
ND=not detected

Appendix 12: Fatty acid profile of *C. sulcata* (% fatty acids)

FA	Small Group								Large Group				
	1	2	3	4	5	6	7	Mean±SD	8#	9	10	11	Mean±SD
14:0	2.50	2.73	2.43	2.47	1.69	1.90	1.90	2.23±0.39	2.53	1.48	2.40	1.91	2.08±0.48
15:0	1.68	1.84	2.03	2.66	1.98	1.78	1.45	1.92±0.38	1.76	1.05	1.89	1.78	1.62±0.38
16:0	27.02	29.46	25.95	27.94	25.56	24.39	24.24	26.37±1.90a	22.44	12.79	21.76	21.73	19.68±4.60b
17:0	3.19	3.48	0.44	0.67	0.62	0.50	0.41	1.33±1.38	0.45	ND	0.60	0.52	0.52±0.08
18:0	7.02	7.67	5.74	7.11	7.50	6.16	8.28	7.07±0.88	7.34	4.20	8.13	7.15	6.71±1.72
20:0	1.98	ND	2.08	2.18	2.05	ND	ND	2.07±0.09a	ND	ND	ND	0.94	0.94±*b
22:0	0.94	ND	0.79	0.21	0.92	0.20	ND	0.61±0.38	0.41	ND	0.28	0.31	0.33±0.07
24:0	ND	ND	ND	ND	ND	ND	ND	*	0.80	ND	0.42	0.65	0.62±0.19
14:1	0.25	ND	0.22	0.27	0.27	0.36	ND	0.27±0.05a	0.17	ND	0.17	0.21	0.18±0.02b
15:1	0.52	ND	0.58	0.67	0.62	0.54	0.43	0.56±0.08	0.41	ND	0.51	0.59	0.50±0.09
16:1	0.29	0.94	0.23	0.28	0.30	0.26	0.23	0.36±0.26	0.30	1.12	0.32	0.22	0.49±0.42
17:1	0.36	1.79	0.86	0.31	0.93	0.17	2.14	0.94±0.76	0.09	ND	0.21	0.10	0.14±0.07
18:1	6.77	7.34	2.57	5.49	4.98	0.28	5.49	4.70±2.47	6.58	3.67	5.35	5.46	5.27±1.2
18:1	2.87	3.12	0.37	2.76	3.11	ND	2.23	2.41±1.05	1.71	ND	1.76	1.51	1.66±0.14
20:1	0.23	1.94	0.41	0.15	ND	1.75	1.76	1.04±0.86	1.17	ND	1.07	0.42	0.89±0.40
22:1	3.31	0.99	3.07	0.89	3.28	0.30	0.88	1.82±1.33	0.94	1.86	0.64	0.77	1.05±0.55
24:1 n9	0.81	ND	ND	0.36	ND	0.58	ND	0.58±0.18	ND	ND	ND	ND	*
14:2	0.26	ND	0.22	0.24	ND	0.23	0.21	0.23±0.02	0.26	ND	0.22	0.27	0.25±0.02
15:2	0.58	ND	0.43	0.42	0.51	0.39	0.44	0.46±0.07	0.48	ND	0.40	0.45	0.44±0.04
18:2	2.67	2.91	2.22	2.09	2.01	1.90	2.18	2.28±0.37	2.37	1.06	2.08	1.79	1.83±0.56
20:2	0.43	ND	0.41	0.37	0.47	0.34	0.54	0.43±0.07	0.40	ND	0.51	0.42	0.45±0.06
22:2	0.84	ND	0.70	0.20	0.96	0.25	0.91	0.64±0.31	0.37	ND	0.15	0.19	0.24±0.12
18:3 n3	0.60	1.23	0.38	0.27	0.29	0.31	ND	0.51±0.37	1.28	1.10	1.29	0.37	1.01±0.43
18:3 n6	ND	0.00	1.63	1.78	1.44	1.14	1.23	1.44±0.27a	0.15	ND	0.13	0.16	0.15±0.02b
20:3 n3	0.34	ND	0.21	0.18	0.33	0.22	0.34	0.27±0.08	0.23	ND	0.17	0.29	0.23±0.06
22:3	0.98	1.01	3.02	3.54	3.33	2.02	2.37	2.32±1.05	3.66	1.75	3.75	3.94	3.27±1.02
18:4 n3	ND	ND	ND	ND	ND	ND	ND	*	ND	ND	ND	ND	*
20:4 n6	9.07	9.74	12.89	11.86	12.70	7.85	10.25	10.62±1.77	10.88	5.23	12.17	11.52	9.95±3.19
22:4 n6	ND	ND	ND	ND	ND	0.25	ND	0.25±0.60*	0.20	ND	0.18	0.40	0.26±0.12
20:5 n3	5.26	5.73	5.29	3.85	5.17	3.61	7.15	5.15±1.19	5.88	2.72	6.21	5.16	4.99±1.58
22:5 n6	8.87	9.60	9.86	6.42	9.31	5.69	12.77	8.93±2.34	9.97	4.89	10.89	8.46	8.55±2.64
22:6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unkn	10.37	8.48	14.98	14.38	9.68	36.61	12.17	15.24±9.72	16.78	3.84	16.32	22.18	14.78±7.76

ND=not detected; *=mean value from less than 2 samples, or not available
Unkn=unknown; #=experimental error contained in this sample

Appendix 13: Vitamin E concentration of *C. sulcata*

Group	**Sample ID**	**Vitamin E Concentration** (mg/100g FW)
Small (n=6)	1	1.93
	2	2.09
	3	2.25
	4	1.95
	5	2.16
	6	2.56
	Mean±SD	2.16±0.23
Large (n=9)	7	2.01
	8	3.07
	9	2.88
	10	3.00
	11	2.65
	12	5.37
	13	4.23
	14	6.26
	15	3.91
	Mean±SD	3.71±1.38

FW= fresh weight

Appendix 14: Cholesterol concentration of *C. sulcata*

Group	**Sample ID**	**Cholesterol concentration** (mg/g FW)
Small (n=7)	1	1.44
	2	1.46
	3	1.26
	4	1.31
	5	1.28
	6	1.23
	7	1.29
	Mean ± SD	1.33 ± 0.09
Large (n=9)	8	1.33
	9	1.31
	10	1.34
	11	1.28
	12	1.34
	13	1.30
	14	1.33
	15	1.34
	16	1.32
	Mean ± SD	1.32 ± 0.02

FW= fresh weight