UNIVERSITY OF CAPE COAST

NUTRITIONAL QUALITY OF THE MANGROVE OYSTER, *CRASSOSTREA TULIPA*, IN GHANA AND HYDROGRAPHIC CONDITIONS OF THEIR HABITATS

FELIX EDUFIA AGBLEMANYO

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BY

FELIX EDUFIA AGBLEMANYO

Thesis submitted to the Department of Fisheries and Aquatic Sciences of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Master of Philosophy (M.Phil.) degree in Fisheries Science

NOVEMBER, 2021

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:..... Date:.....

Name:.....FELIX EDUFIA AGBLEMANYO.....

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:..... Date:.....

Name: .....PROF. EDWARD A. OBODAI.....

Co-Supervisor's Signature: ..... Date:.....

Name:.....PROF. MICHAEL B. ADINORTEY.....

ABSTRACT

Seafood contributes significantly to food security. The present study sought to provide information on the size, meat yield, proximate nutritional composition, minerals, amino acids, fatty acids and some antioxidant properties of *C. tulipa* (oyster) from three water bodies in the Greater Accra, Central and Western regions of Ghana. To maximize yield and maintain good physiological conditions of the oysters, spatiotemporal variations as well as effects of environmental conditions on the meat yield, proximate, minerals and amino acid composition of the oysters were also investigated. Oysters from "Densu" estuary were the biggest in size while those from "Narkwa" lagoon were the smallest. The nutritional quality assessment qualifies *C. tulipa* as a highly nutritious and a potential functional food. There were no significant differences in the proximate and amino acid contents of *C. tulipa* from the three water bodies ($P > 0.05$). *C. tulipa* from "Whin" estuary had significantly higher Fe and Zn contents, whereas those from Narkwa lagoon had significantly higher Ca and Na contents ($P < 0.05$). The proximate, mineral and amino acid compositions of *C. tulipa* from the various water bodies varied significantly during the months of study ($P < 0.05$). *C. tulipa* had higher concentrations of essential amino acids and had no limiting amino acids. Mineral concentrations of the oysters were all within recommended limits. Environmental factors, including temperature, pH, chlorophyll-a, precipitation and salinity, had significant effects on the meat yield, moisture, ash, sodium, zinc, iron and amino acid contents of *C. tulipa*. *C. tulipa* contained phenolic compounds and showed antioxidant properties. *C. tulipa* oil contained high amounts of polyunsaturated fatty acids, particularly eicosapentaenoic and docosahexaenoic acids (EPA and DHA).

KEYWORDS

Proximate composition

Minerals

Amino acids

Fatty acids

Antioxidant properties

Environmental factors

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DEDICATION

To the late Professor Emeritus Kobina Yankson.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

FAO	Food and Agriculture Organisation
MOFAD	Ministry of Fisheries and Aquaculture Development
SDG	Sustainable Development Goal
DPPH	2,2-diphenyl-1-picrylhydrazyl
K	Potassium
Na	Sodium
Ca	Calcium
Mg	Magnessium
P	Phosphorus
Zn	Zinc
Cu	Copper
Fe	Iron
AOAC	Association of Official Analytical Chemists
NMFS	National Marine Fisheries Service
WHO	World Health Organization
UNU	United Nations University
IITA	International Institute of Tropical Agriculture
LC	Liquid chromatography

UV	Ultraviolet
His	Histidine
Arg	Arginine
Ser	Serine
Gly	Glycine
Asp	Aspartic acid
Thr	Threonine
Cys	Cystine
Pro	Proline
Ala	Alanine
Lys	Lysine
Tyr	Tyrosine
Met	Methionine
Val	Valine
Iso	Isoleucine
Leu	Leucine
Phe	Phenylalanine
MESTI	Ministry of Environment, Science, Technology and Innovation

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 Background

The fisheries industry is a major source of reliable and affordable protein around the world, with shellfish playing a significant part. In 2017, molluscs and crustaceans accounted for 27.9 % of the world's aquaculture production of aquatic animals with 17.4 and 8.4 million tonnes respectively (FAO, 2019). The per-year capital by export for molluscs and crustaceans in 2017 was estimated at 55 billion USD (FAO, 2019). Aside from their economic worth, shellfishes are highly nutritious. Shellfish has a higher protein content than finfish and is a decent source of digestible protein (Venugopal & Gopakumar, 2017).

Shellfishes are rich in polyunsaturated omega-3 and omega-6 fatty acids, which have several health benefits, including reducing cardiovascular diseases and promoting child development and cognitive performance (Lombardo & Chicco, 2006). The health and nutritional benefits of shellfishes have led to their increasing demand by consumers and consequently, shellfishes are being used as a supplementary source of protein to reduce the high demand on the depleting stocks of finfish. In Ghana, shellfishes could also be used to decrease the high prevalence of malnutrition in the country.

Shellfish culture is exponentially increasing in the developed nations but there is currently no commercial shellfish farming in Ghana (Amenyogbe et al., 2018). Shellfisheries production in the country is thus low, with a total catch of around 95.45 metric tonnes excluding cephalopods in 2015 (MOFAD, 2016; Atindana et al., 2019). The lack of aquaculture or large-scale production of

shellfish has been attributed to insufficient research findings to ensure an efficient culture of these species, lack of private sector investment and poor nationwide acceptance or demand for shellfishes (Amenyogbe et al., 2018).

The West African mangrove oyster, *Crassostrea tulipa* (*C. tulipa*) is a bivalve of substantial economic value and can be found in several water bodies along the coast of West Africa. It is mostly used as a source of protein and a source of income for some coastal residents, and commercial exploitation is yet to begin. In Ghana, several researches have been undertaken to commence the commercial production of *C. tulipa* (Yankson, 1990; Yankson & Moyse, 1991; Yankson et al., 1994; Obodai et al., 2007; Sutton et al., 2012; Chuku et al., 2020; Osei et al., 2021). Nutritional profiling of food and food-based products is gaining much interest within the food processing industry due to the growing awareness and preference of consumers on the nutritional value of what they consume (Nielsen, 2017). Thus, identifying and publicizing the healthy components in *C. tulipa* may be a valuable marketing technique.

Environmental conditions resulting from climatic conditions and certain anthropogenic practices have been shown to influence the development and survival of fish, with shellfishes being the most vulnerable (Atindana et al., 2019). The key environmental factors governing oyster physiology and reproduction are physicochemical parameters and nutrient availability. Temperature, salinity, chlorophyll-a content, and other exogenous factors affect oyster metabolism as well as their nutritional composition (Brown & Hartwick, 1988; Liu et al., 2010; Yildiz et al., 2011; Chakraborty et al., 2016). Nutritional

quality and biochemical composition of fish have, therefore, been used as bio-indicators of water quality (Munyasya et al., 2015; Gonçalves et al., 2016).

Studies on the biochemical composition of oysters from two coastal water bodies in Ghana, Benya lagoon and Pra estuary, revealed significant differences among the moisture, protein, carbohydrate, calcium, iron and phosphorus contents in oysters from the different water bodies (Yankson et al., 1994). Environmental factors that influence these variations and can help improve the yield as well as maintain good physiological conditions of oysters are, thereby, needed in Ghana, where efforts are being made to begin the commercial exploitation of oysters. Understanding the impact of environmental conditions on the nutritional and biochemical compositions of oysters in Ghana will also contribute to the creation of policies and management strategies that will ensure the long-term stability of Ghana's coastal ecosystems as well as the country's food security.

As a result, this study aimed to provide a nutritional profile involving proximate, amino acid and mineral compositions of oysters from three water bodies in Ghana as well as to determine the effects of environmental factors (temperature, salinity, pH, chlorophyll-a, dissolved oxygen and precipitation) on the nutritional quality of the oysters. Nutraceutical properties (phenolic content, fatty acid composition, DPPH scavenging activity and total antioxidant capacity) of different extracts of *C. tulipa* were also studied.

1.2 Problem Statement and Justification

Fish is the most preferred source of animal protein in Ghana, with about 75 % of its annual production (400,000 tonnes) being consumed locally (FAO, 2016). The remaining 25 % serves as the second most important non-traditional export after horticultural products (FAO, 2016). However, the current annual fish production in Ghana forms only about 40% of the local demand, giving rise to a national fish deficit (FAO, 2016). This has resulted from over-exploitation leading to a rapid loss of traditional food fisheries in the subsistence fishing communities of Ghana (Aheto, Aduomih, et al., 2011; Asare et al., 2019). Thus, efforts at providing a supplementary source of fish to reduce pressure on capture fisheries are being made through aquaculture.

Shellfish are noted for their high protein content and other important nutritional compositions. They also have other considerable economic importance, and their commercial exploitation will contribute greatly to the sustainability of Ghana's fisheries and economy (Obodai & Yankson, 1999). Studies are still ongoing and much has also been done to facilitate the commercial exploitation of *Crassostrea tulipa* in Ghana. Thus, information regarding the biochemical composition (Yankson et al., 1994), *in vitro* fertilization and rearing (Yankson, 1990; Obodai et al., 2007), larvae production with cryopreserved spermatozoa (Yankson & Moyse, 1991), feeding ecology and filtration rates (Sutton et al., 2012), optimising spat culture (Obodai, 2000; Chuku et al., 2020), depuration effects on microbial content (Obodai et al., 2010), heavy metal composition (Otchere, 2003; Essumang et al., 2010) and much other important information on mangrove oysters in Ghana are available.

Efforts have also been made by some oyster fishers in Ghana to begin 'rearing' of the species in the Densu estuary, near Tsokomey in the Greater Accra Region (Chuku, 2019). Coastal dwellers are the major consumers of oysters in Ghana, and the oysters are consumed either steamed, spiced, fried, or smoked (Atindana et al., 2019).

Nutritional profiling of oysters has resulted in an increase in demand for oysters in countries like India (Asha et al., 2014) as most consumers now demand food products that are nutritious and of high quality (Nielsen, 2017). Information on the nutritional composition of raw ingredients also assists food manufacturers in producing foods that reflect the quality demands of consumers, meet the quality standards of the manufacturer and comply with government regulations (Nielsen, 2017).

Nutritional assessment studies on oysters from Benya Lagoon and Pra estuary in Ghana have thus been conducted (Yankson et al., 1994). However, such studies have not been conducted on oysters from other water bodies, such as Whin estuary, Narkwa lagoon, and Densu estuary where there are vibrant oyster fisheries and thriving oyster populations. Furthermore, a current evaluation of the nutritional and health benefits of *C. tulipa* is needed, since evidence from three decades ago could be imprecise due to climate changes in their environment.

Yankson et al. (1994) observed significant differences among nutritional constituents in oysters from two different water bodies in Ghana. These variations are influenced by environmental factors including temperature,

salinity and chlorophyll-a (Brown & Hartwick, 1988; Liu et al., 2010; Yildiz et al., 2011; Chakraborty et al., 2016; Bejaoui et al., 2020) and they may be used as quality improvement measures for oyster production if their effects on the nutritional composition of oysters in Ghana are known. In the wake of climate change and increasing anthropogenic factors affecting water bodies, assessing the impact of environmental factors on the nutritional content of oysters would also lead to the formulation of smart policies to ensure the survival of Ghana's coastal ecosystems.

1.3 Objectives

The study sought to provide a nutritional profile of oysters from three water bodies namely Densu estuary, Narkwa lagoon and Whin estuary in Ghana and to determine the effects of hydrographic conditions on the nutritional quality of the oysters.

The specific objectives were to:

1. Investigate the spatiotemporal variations in proximate (moisture, ash, fibre, protein, fat, carbohydrate and energy value), mineral (K, Na, Ca, Mg, P, Zn, Cu & Fe) and amino acid compositions of *C. tulipa* from the three coastal water bodies
2. Examine the possible effects of environmental conditions (Chlorophyll a, pH, temperature, dissolved oxygen, salinity and precipitation) of the three coastal water bodies on the nutritional composition of *C. tulipa*.

3. Estimate the phenolic content, fatty acid composition, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and total antioxidant capacity of different extracts of *C. tulipa* from the three coastal water bodies.

1.4 Significance of the study

The fishing industry constitutes an important sector of national economic development. Fish is one of the most highly traded food commodities globally, worth USD 145 billion per year, with developed countries responsible for nearly 60% of its overall value (FAO, 2019). In addition, fish has the potential to help meet the need for healthy, low environmental impact diets for the projected 10 billion people on earth in 2050.

The findings of this study will help in maximizing the yield and quality of oysters, lead to an increase in demand for oysters in the country and also contribute to the formulation of smart policies to ensure the protection of Ghana's coastal ecosystems. The study will also contribute new information to the already existing scientific information on oysters.

This study emphasizes the possibility of assisting in the management of poverty, hunger and malnutrition, as well as assisting in the response to climate change, all while contributing to the sustainable management of natural resources as outlined in the 2030 Agenda, especially SDG 14: "Conserve and sustainably use the oceans, seas and marine resources for sustainable development."

1.5 Hypotheses

1. H_1 There are spatial (among water bodies) and temporal variations in the nutritional composition of *C. tulipa.*

2. H_1 Hydrographic conditions influence the nutritional composition of *C. tulipa*.

1.6 Delimitations of the study

Only three coastal water bodies out of the several water bodies that support oyster culture in Ghana were selected for the study. The study looked at most of the important nutrients in oysters including proximate (moisture, ash, fibre, protein, fat, carbohydrate and energy value), mineral (K, Na, Ca, Mg, P, Zn, Cu & Fe), amino acid and fatty acid compositions as well as some antioxidant properties. However, some nutritional components, like vitamins, were not examined.

1.7 Limitations of the study

Field sampling was done monthly from November 2020 to June 2021. However, due to restrictions by local authorities at New Amanful in the Western Region of Ghana, sampling was not carried out in November 2020 at the Whin estuary.

1.8 Organisation of the study

This thesis comprises six chapters. Chapter one provides background information on the study; the problem statement and justification; the objectives of the study; the significance of the study; hypotheses; delimitations; and limitations. By reviewing relevant literature, chapter two describes oysters in

detail, their nutritional qualities, and how these nutritional qualities are affected by different environmental factors. The materials and procedures used in the research are described in chapter three. Chapter four presents the results obtained from the study. Chapter five discusses the results obtained in the study with reference to previous studies. The last chapter, chapter six, provides conclusions from the study and some recommendations on the way forward. List of references and appendices have also been provided.

CHAPTER TWO

LITERATURE REVIEW

By reviewing relevant literature, this chapter describes oysters in detail, their nutritional qualities, and how these nutritional qualities are affected by different environmental factors.

2.1 Oysters

Oysters comprise any member of the family Ostreidae (true oysters) or the family Aviculidae (pearl oysters) (Bayne, 2017). True oysters are often used as food, while pearl oysters are valued for their precious pearls. This write-up focuses on the true oysters, the genera *Crassostrea, Saccostrea and Ostrea*, of the family Ostreidae (Littlewood, 1994; Hautmann, 2001; Bayne, 2017). *Crassostrea* and *Saccostrea* belong to the subfamily Crassostreinae, while *Ostrea* belongs to the subfamily Ostreinae (*Table 1*) (Bayne, 2017).

Oysters are bivalves with just one adductor muscle (monomyarian), and in their postlarval stage, when they lose their foot, they are cemented to a substrate by their left valve. Oyster shells have rough surfaces, usually dirty gray in colour and their two valves differ in shape. The left valve that is attached to substrates is flat while the right is usually convex in shape and smaller. The inner surfaces of both valves are smooth and white. However, the abductor muscle scar of Crassostreinae is coloured while that of Ostreinae is not (Bayne, 2017). Oysters are distinguished from other bivalves by their highly irregular shell shape. Environmental constraints dictate their shell form, and they can grow on neighbouring objects, including other oysters (Bayne, 2017).

Table 1: *Taxonomic classification of oysters*

Taxonomic rank	Name
Phylum	Mollusca
Class	Bivalvia
Subclass	Pteriomorphia
Order	Ostreida
Superfamily	Ostreoidea
Family	Ostreidae
Subfamily	Ostreinaea
Tribe	Ostreini (Ostrea)
Family	Flemingostreidae
Subfamily	Crassostreinaea
Tribe	Striostreini (Saccostrea)
Tribe	Crassostreini (Crassostrea)

(Littlewood, 1994; Hautmann, 2001; Bayne, 2017)

Oysters are widely distributed in tropical and temperate waters but are absent in the polar oceans (*Figure 1*) (Gosling, 2008; Bayne, 2017). They are primarily coastal, inhabiting intertidal or shallow subtidal zones in estuaries, marshes and bays. They usually cling to sedimentary bottoms or aggregate on mangrove roots. The distribution, survival and growth of oysters are highly influenced by several hydrographic factors such as temperature, salinity, pH and availability of food. For example, Yankson (1990) reported that oysters in Ghana (*C. tulipa*) exhibit optimum fertilization and larval development at temperature and salinity ranges of 25 - 30 °C and 20 - 30 ppt, respectively.

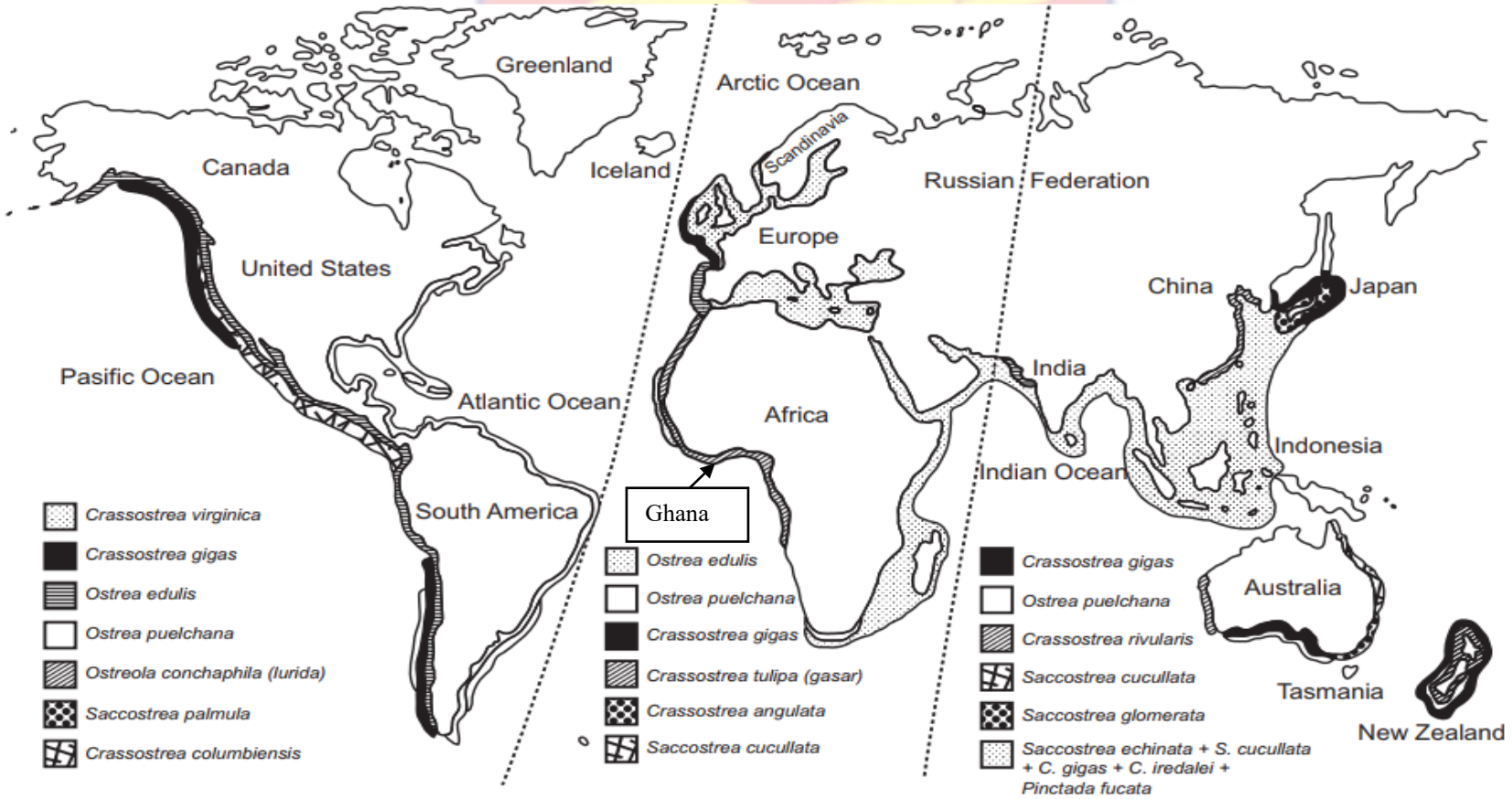

Figure 1: Map displaying the geographical distribution of major oyster species in the world. Adopted from Bayne, (2017)

Oysters are of great economic value. In the US alone, oyster landings generate about USD190 million annually (NMFS, 2015). In addition to their traditional market value, oysters as well as other bivalves provide broader ecosystem services such as carbon sequestration, nutrient remediation, coastal defense and provision of nursery grounds for fish (Olivier et al., 2020; Willer & Aldridge, 2020). Despite their numerous benefits, production of oysters and other bivalves is low in the tropics (Wijsman et al., 2019; Olivier et al., 2020; Willer & Aldridge, 2020). According to Willer and Aldridge (2020), the tropics have about 1,000,000 km^2 of underdeveloped coastlines which are ideal for productive bivalve farming, and in Africa, if only 1 % of these undeveloped coastlines are developed, 33.3 Mt of bivalve meat will be produced, which will feed about 195 million people.

2.2 Nutritional Profiling

Nutritional profiling refers to making known the nutrient content of a food and the contribution of a food to a healthy diet (Maillot et al., 2008). It is also defined as the judgement which is made about the relative "healthfulness" of a food as revealed by the nutritional composition of the food (Bussell, 2005). The nutritional profile of a food is not only legally required in many countries, but most consumers now demand food products that are safe and of high quality (Nielsen, 2017). The nutritional profile of raw ingredients also assists food manufacturers in producing foods that fulfil the quality demands of consumers, meet the quality standards of the manufacturer, and abide by government regulations (Nielsen, 2017).

Nutritional profiling has had a crucial role in the marketing of oysters to consumers. Promotion of oysters and other bivalve products in China as highly palatable and affordable protein sources boosted consumer demand and was pivotal to China's success in bivalve aquaculture (Mao et al., 2019; Willer & Aldridge, 2020). A similar observation of increased demand for oysters was also reported in India (Asha et al., 2014).

2.2.1 Proximate composition

According to Self (2005), the proximate composition of a food product refers to the sum of protein, crude fat, carbohydrate, crude fibre, moisture and ash expressed as a percentage composition of the food. The major component of oysters is moisture and it depicts their succulence, which is an important sensory characteristic. In Ghana, the moisture content of *C. tulipa* was reported to be within 80 – 83 % (Yankson et al., 1994; Osei, 2019). Similar results have been reported elsewhere (Cochet et al., 2015; Min et al., 2020), while lower moisture contents have also been reported (Ajana, 1980; Castro & de Mattio, 1987; Opeh, 2018). For example, Opeh (2018) reported moisture content of 54.01 % for *C. rhizophorea* in Nigeria.

Proteins, lipids and carbohydrates serve as sources of energy for most living organisms. They have other significant physiological roles, such as repairing worn-out tissues, growing muscles, and serving as precursors for metabolic pathways and cushions for vital organs. According to Yankson et al. (1994), the protein, fat and carbohydrate composition of oysters in Ghana compared favourably with those of other commercial bivalves. Yankson et al. (1994) reported protein, fat and

carbohydrate contents of 59.3 ± 2.2, 8.0 ± 0.6 and 20.0 ± 2.5 % for *C. tulipa* from Benya lagoon and 67.7 ± 2.2, 9.8 ± 1.3 and 8.1 ± 0.4 % for *C. tulipa* from Pra estuary, respectively. A recent study by Osei (2019) reported higher fat and carbohydrate contents, and a lower protein content for *C. tulipa* in Ghana as compared to those reported by Yankson et al. (1994). Silva et al. (2020) reported lower protein contents of 3.35 – 9.93 % and fat contents of 1.29 – 3.04 % for *C. gasar* in Brazil. Per wet weight bases, Asha et al. (2014) reported 9.41 ± 0.85, 3.25 ± 0.32 and 3.2 ± 0.13 % for protein, fat and carbohydrate content for *C. madrasensis*.

Dietary fibre content consists of complex polysaccharides which are usually nondigestible by humans. Due to the several important functions of dietary fibre, such as decreasing intestinal transit time, increasing the volume of faecal bulk, as well as decreasing cholesterol and glycaemic levels, their consumption has been linked to a reduction in the incidence of several types of diseases (Dhingra et al., 2012). Crude fibre content of a food denotes only about 50 % of the dietary fibre content in the food. This is because the determination of crude fibre content using acid and base digestion procedures results in the loss of soluble and some insoluble dietary fibre in the food sample. A crude fibre content of 1.98 % was reported for *C. tulipa* from the Densu estuary in Ghana (Osei, 2019). However, it was less than what had been reported for *C. gasar* by Johnnie et al. (2020).

Ash content signifies the mineral or inorganic elements of a food product. Oysters tend to have relatively high ash contents due to their ability to filter and bioaccumulate minerals from their environment (Bates et al., 2021). In Ghana, Osei

(2019) reported an ash content of 3.04 ± 0.13 % for *C. tulipa* from the Densu estuary, whereas Yankson et al. (1994) reported higher ash contents for *C. tulipa* from Benya lagoon (12.7 ± 1.0 %) and Pra estuary (14.4 ± 1.6 %). Lower ash contents (0.63 – 2.71 %) were also reported for oysters in India, China and Brazil (Asha et al., 2014; Min et al., 2020; Silva et al., 2020).

2.2.2 Mineral composition

Minerals have a wide range of functions and potentials in the body's metabolism and homeostasis, including bone formation, hormone production, and nerve impulse transmission (Gharibzahedi & Jafari, 2017; Wang et al., 2021). Minerals cannot be synthesized by living organisms and, based on body requirements, they are categorized into two groups, which are the macro or major minerals and micro or minor minerals. Major minerals are needed in relatively high amounts and the minor minerals are needed in very small quantities (McDowell, 2003). Major minerals include Ca, Na, S, P, Mg and Cl, whereas the minor minerals include Fe, Cu, Zn, Mn, Se, Co, Cr, I, Mo, etc. (McDowell, 2003).

Most of the major minerals, such as Ca, Mg, P, Na, K and Cl, are found in the electrolytes of body fluids and soft tissues where they play important roles such as maintaining acid-base balance and osmotic pressure (McDowell, 2003). Most micro-minerals, such as Fe, Cr, Mn and Zn are components of ribonucleic acid, which is used for protein synthesis (McDowell, 2003). Minerals have several other important health benefits such as hormone production, formation of red blood cells and boosting of the immune system (McDowell, 2003; Wang et al., 2021). Despite

their importance, minerals can be toxic at high concentrations and there are regulations regarding their consumption or toxicity levels (Capra, 2006).

Oysters are rich sources of minerals, and this is partly due to their rapid filter feeding, enabling them to accumulate more minerals (Wang et al., 2018; Bates et al., 2021). Yankson et al. (1994) reported higher concentrations of P, Ca and Fe for *C. tulipa* in Ghana than those in foods deemed to be good sources (dried skim milk, black pudding, and calf's brain). Oysters, according to Chakraborty et al. (2016) are a rich source of Ca, Zn and Fe. Bates et al. (2021) and Catry et al. (2021) reported higher concentrations of Zn and Cu in oysters as compared to other bivalve species collected from the same water bodies. Similar results were obtained for *C. tulipa* in Ghana when their trace element concentrations were compared to those of the bloody cockle (*Anadara senelis*) and brown mussel (*Perna perna*) by Otchere (2003). *C. tulipa* was found to have relatively high concentrations of Zn (43 – 278 mg/100g), Cu (1.7 – 7.4 mg/100g), Fe (28 – 70 mg/100g), and Mn (1.1 – 2.0 mg/100g) in three lagoons (Sakumo, Ningo, and Benya) in Ghana (Otchere, 2003). *C. tulipa* from Pra estuary in Ghana also had 2810 ± 583, 58.7 ± 8.9 and 1693 ± 226 mg/100 g for Ca, Fe and P, whereas those from Benya lagoon had 964 ± 274, 29.7 ± 4.9 and 923 ± 95 mg/100 g, respectively (Yankson et al., 1994). In contrast, Sowah (2019) reported lower concentrations of Zn and Cu in *C. tulipa* from Narkwa lagoon, and Densu and Whin estuaries in Ghana.

2.2.3 Amino acid composition

The quality of a food protein is primarily defined by its amino acid constituents and digestibility. Amino acids, in addition to serving their main

purpose as the building blocks of proteins, have other important physiological roles such as being carriers of oxygen, carbon dioxide, vitamins and enzymes (Chalamaiah et al., 2012). In marine bivalves, amino acids are major components of their intracellular fluids and are important in maintaining their osmotic pressure and balance (Silva & Wright, 1994; Berger & Kharazova, 1997; Ivanina et al., 2020). Based on nutritional requirements, amino acids are grouped into essential and nonessential amino acids. Essential amino acids are not synthesized by the human body and are required in diets. The body synthesizes nonessential amino acids, but they are also helpful in diets since they provide more than 50 % of the total nitrogen consumed (D'Mello, 2003). The quality of a food protein can be assessed by comparing its essential amino acid concentrations to those of a reference protein (egg protein) or the amino acid requirements of an organism, such as the amino acid reference pattern for adults or children by FAO/WHO/UNU (2007). When essential amino acid concentrations do not meet their required amounts, protein quality is negatively affected. These amino acids hinder protein synthesis and are termed as limiting amino acids.

Shellfish have high amounts of digestible proteins which are of very high quality (Venugopal & Gopakumar, 2017). Oysters as well as other shellfish species have been reported to have chemical scores of 100 % and above (Venugopal & Gopakumar, 2017). This signifies that they do not have limiting amino acids. Chen et al. (2012) also reported chemical scores greater than 100 % for *C. rivularis* in China. In contrast, methionine has been reported as a limiting amino acid in oysters by several studies (Qin et al., 2018; Jiang et al., 2019; Qin et al., 2021). This can be

related to the 10 % and 50 % loss of methionine and cysteine, respectively, when protein is hydrolysed directly without pre-treatment with nitrogen gas, an antioxidant or performic acid (Spindler et al., 1984). However, other amino acids, such as valine and leucine were reported as limiting in *C. madrasensis* in India (Asha et al., 2014).

In Ghana, *C. tulipa* has been reported to have higher protein content (Yankson et al., 1994; Osei, 2019). However, no records were found on their amino acid constituents.

2.3 Variability patterns in the nutritional quality of oysters

Biochemical constituents such as amino acids, protein, fat, carbohydrate and minerals which make up the nutritional quality of oysters are influenced by energy requirements and physiological states of the oysters. According to Silva et al. (2020), Nutrient availability and physicochemical parameters are the key environmental factors influencing oyster physiology and reproduction. Several studies have, therefore, been conducted to determine how physiological states such as sex and gametogenic cycle, and environmental factors such as climatic seasons and physicochemical parameters affect the nutritional quality of oysters (Yankson et al., 1994; Yildiz et al., 2011; Chakraborty et al., 2016; Qin et al., 2018; Silva et al., 2020; Qin et al., 2021). Understanding how the changes in nutritional quality occur can help in improving the yield and quality of oysters.

2.3.1 Reproductive cycle and nutritional quality

Ren et al. (2003) studied variations in the reproductive cycle and the nutritional contents of pacific oysters in New Zealand and found that the

biochemical composition of the oysters followed cycles of somatic growth and reproduction. Yildiz et al. (2011) and Qin et al. (2018) also reported similar observations where protein and fat contents of oysters increased during gonad development but decreased after spawning. Other studies reported higher protein and lower glycogen and carbohydrate contents during gametogenesis, which they related to the conversion of glycogen to proteins during gametogenesis (Ruiz et al., 1992; Dridi et al., 2007; Qin et al., 2018). Total amino acid content, especially essential amino acids, has been shown to increase with gonad formation and maturation in *C. hongkongensis* (Qin et al., 2021). Changes in the soft tissue weight during reproduction cycles also influence the mineral composition of oysters and other bivalves (Otchere, 2003). Variations in polyunsaturated fatty acids of *C. hongkongensis* in relation to their reproductive cycle have been reported by Qin et al. (2021). Due to the sharp decrease in biochemical constituents of oysters right after spawning, Yildiz et al. (2011) reported those periods as not suitable for oyster harvesting. According to Qin et al. (2021), the best period to harvest *C. hongkongensis* is during their inactive gonad stages.

2.3.2 Climatic seasons and nutritional quality

Yankson et al. (1994) studied seasonal changes in the nutritional composition of *C. tulipa* in Ghana. The study showed that *C. tulipa* from different water bodies in Ghana had higher condition indices during the rainy season; however, their nutrient components did not vary with respect to the climatic seasons. Also, the stability of physicochemical parameters of a lagoon as compared to an estuary resulted in *C. tulipa* from the Benya lagoon having less variability in

their nutritional components. Silva et al. (2020) reported high proteins, fats, carbohydrates and omega-3 fatty acids in *C. gasar* during the rainy seasons in Brazil. Otchere (2003), studied seasonal variations in the trace minerals in *C. tulipa* and other bivalves and found that Zn and Fe increased, whereas Cu decreased during the wet season in Ghana. Seasonal variations in the free amino acid contents of *C. gigas* have also been reported (Qin et al., 2021). According to Silva et al. (2020), the increased nutritional constituents during the rainy seasons were due to the increase in food availability. Yildiz et al. (2011) also reported winter as not a suitable period for harvesting flat oysters (*Ostrea edulis*) in Turkey.

2.3.3 Physicochemical factors and nutritional quality

Oysters tend to close their valves under unfavourable conditions, which affect their metabolic rates as well as their biochemical constituents (Lombardi et al., 2013; Porter & Breitburg, 2016). Environmental factors such as pH, temperature, salinity, chlorophyll-a and precipitation have been reported by several studies to influence the nutritional quality of oysters (Yildiz et al., 2011; Chakraborty et al., 2016; Lemasson et al., 2019; Ivanina et al., 2020; Silva et al., 2020; Bates et al., 2021; Mosca et al., 2021).

An increase in temperature, pH and a decrease in precipitation led to a decrease in the moisture content and an increase in ash content of *C. gasar* (Silva et al., 2020). The changes in the ash and moisture contents reflect coping mechanisms for environmental stress by oysters. According to Haider et al. (2020) and Ivanina et al. (2020), amino acids and minerals such as Na, Mg and K, which

make up the ash contents of food products, are involved in the maintenance of acid-base balance and osmotic pressure of oysters.

Ocean acidification and warming conditions led to significant reductions in the protein, fat and carbohydrate contents of *Magallana gigas* (Lemasson et al., 2019). These changes in energy reserves indicate physiological stress and might jeopardize the organism's survival in the long term (Lemasson et al., 2019). According to Pogoda et al. (2013) and Lemasson et al. (2019), oysters have species-specific responses to changes in environmental conditions. Other oyster species showed stability in their energy reserves and other different responses to changes in their environmental conditions (Lemasson et al., 2019; Silva et al., 2020). Anacleto et al. (2014) and Lemasson et al. (2019) reported that some bivalves may possess adaptive coping strategies which prevent significant alterations in their energy reserves due to changes in environmental conditions.

Exposure to high salinities, anoxia, turbidity or drilling effluents resulted in significant fluctuations in the free amino acid composition of *C. virginica* (Powell et al., 1982). Hosoi et al. (2003) reported a remarkable increase and decrease in free amino acids of *C. gigas* when exposed to an abrupt increase and decrease in salinity, respectively. Enzymes (glutamate dehydrogenase and glutamate-oxaloacetate transaminase) involved in synthetic pathways of some amino acids were reported to increase in *C. gigas* when exposed to high salinities (Wickes & Morgan II, 1976) and could account for some of the observations made by Powell et al. (1982) and Hosoi et al. (2003). Oysters have also been reported to take up amino acids from their environment (Rice & Stephens, 1987).

According to Chakraborty et al. (2016), the fatty acid composition of bivalves is closely related to the foods they consume. Previous studies have found positive relationships between chlorophyll-a concentrations and the fatty acid composition of oysters (Dridi et al., 2007; Chakraborty et al., 2016). High chlorophyll-a concentrations indicate a wide range of microalgae species and abundance (Dridi et al., 2007). Ratios of polyunsaturated fatty acids (Docosahexaenoic acid: Ecosapentaenoic acid) have been used to predict ingested foods by oysters, with ratios > 1 indicating a diet dominated by dinoflagellates and ratios < 1 indicating a diet dominated by diatoms (Budge & Parrish, 1998).

Oysters are easily disturbed by changes in environmental conditions (Rice & Stephens, 1987). Studies on the influence of environmental conditions on the biochemical constituents of oysters are important because they help in maximizing yield as well as maintaining good physiological conditions of the oysters. In Ghana, the effects of seasonal variations on the biochemical constituents of *C. tulipa* have been studied (Yankson et al., 1994; Otchere, 2003). However, information on the influence of physicochemical factors and food availability on their biochemical constituents is limited.

CHAPTER THREE

MATERIALS AND METHODS

The materials and procedures used in the research are described in this chapter. Detailed descriptions of the study locations and methods for field sampling, laboratory analysis, and data analysis used for this study have been provided.

3.1 Study sites

Three coastal water bodies namely Densu estuary, Narkwa lagoon, and Whin estuary found in the Greater Accra, Central, and Western Regions of Ghana, respectively, were used in the present study. These study sites were selected due to the presence of thriving populations of *C. tulipa* and research works undertaken in these sites to facilitate the sustainable exploitation of *C. tulipa* (Obodai & Yankson, 2002; Janha et al., 2017; Asare et al., 2019; Sowah, 2019; Chuku et al., 2020; Osei et al., 2021).

3.1.1 Densu estuary

The Densu estuary is found between 5°34′07″ N, 0°16′43″ W and 5°30′21″ N, 0°20′02″ W in the Greater Accra Region of Ghana. Some neigbouring communities of the Densu estuary are Tsokomey, Tetegu, and Faana of the Ga South Municipal District. The estuary is part of a 120 km long river basin with a catchment area of 2564 km^2 (Carboo et al., 1999). Densu estuary has a vibrant oyster fishery where the oyster fishers are mainly females who are usually Gas or Ewes (Osei et al., 2020). There is an association for oyster fishers at Densu estuary

known as Densu Oyster Pickers Association (DOPA) which ensures the sustainable exploitation of their oyster resources.

3.1.2 Narkwa lagoon

The Narkwa lagoon is located in the Central Region of Ghana specifically between 5°12′17″ N, 0°56′22″ W and 5°12′32″ N, 0°54′41″ W. Narkwa, Ekumpoano, and Atwa of the Ekumfi district are some of the neighbouring communities of the lagoon. Narkwa lagoon is an open lagoon with a surface area of ~1.2 km^2 which mainly serves as a landing site and fishing ground for the surrounding communities (Asare et al., 2019). Oyster picking is the second most popular livelihood activity at Narkwa and the pickers are dominated by females. Oysters are harvested all year round with periods of high harvest from August to March and low harvest from April to August. The prices of oysters from the Narkwa lagoon are relatively low making the pickers harvest more to increase their income (Asare et al., 2019).

3.1.3 Whin estuary

The Whin estuary is found in the Western Region of Ghana and lies between 4°52′52″ N, 1°46′47″ W and 4°52′30″ N, 1°46′04″ W. Some neighbouring towns of the Whin estuary are New Amanful, Adakope, and Apremdo of the Ahanta West district. The Whin estuary is funnel-shaped which allows a large freshwater-seawater interchange (Aheto, Mensah, et al., 2011). Wednesday is a no-fishing day in the Whin estuary. The fishing activity in the Whin estuary involves mainly the use of cast net, set net, and hand fishing to catch fishes like *Periophthalmus barbarus and Sarotherodon melanotheron.* Oyster picking is also done at the Whin

estuary and the pickers are dominated by women. The oysters harvested are usually spiced, fried, smoked, and packaged for sale (Atindana et al., 2019).

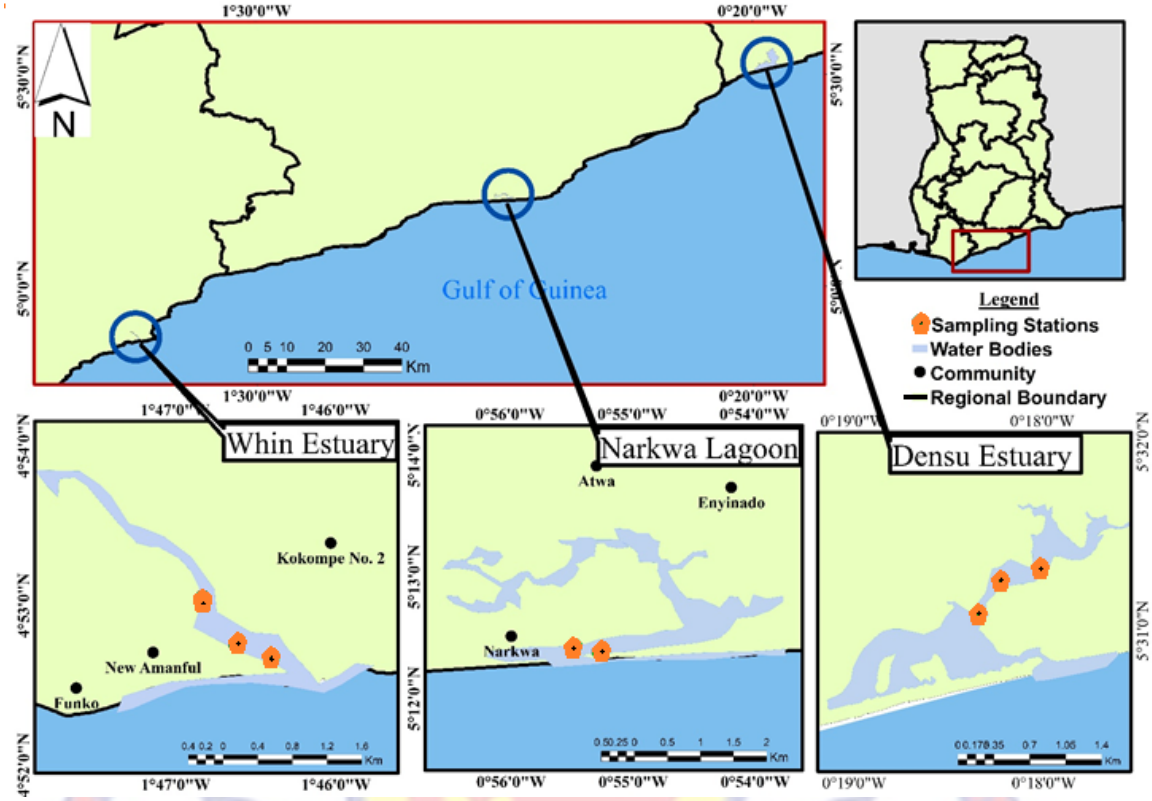

Figure 2: Maps of the study sites (Densu estuary, Narkwa lagoon and Whin estuary).

3.2 Experimental design

Matured oysters, oysters with shell height > 40 mm (Yankson, 1996), were collected monthly (Nov 2020 – Jun 2021) from 2 - 3 stations where they were found in the various water bodies. Oysters were obtained from 3 stations in the Whin and Densu estuaries, and 2 stations in the Narkwa lagoon (*Figure 2*). At the different sampling locations, physicochemical parameters of the water, namely salinity, temperature, dissolved oxygen, and pH were measured *in-situ*. Water samples for chlorophyll-a analysis were collected into high-density opaque plastic bottles. To

prevent contamination of the water before *in-situ* measurements of the physicochemical parameters, water samples were taken after the *in-situ* measurements were made and before oyster samples were collected. Oyster samples from the different stations were pooled during each sampling month for each water body before the nutritional quality assessment was conducted.

3.3 Field sampling

Sampling was done monthly from November 2020 to June 2021, however, due to restrictions by local authorities, sampling was not carried out in November 2020 at Whin estuary. At each sampling, physicochemical parameters of the water: salinity, temperature, dissolved oxygen, and pH, were measured *in-situ* at the various sampling locations using a multiparametric water quality checker (Eutech PCD650). Total monthly precipitation data were obtained from an online climate database (Tutiempo.net, 2021) (Appendix A).

High-density 1 L opaque plastic bottles were labeled using permanent markers and the labels consisted of the sample ID, date and time of sampling, and the sampling station. The bottles were then rinsed thrice before water samples were taken. Water samples were taken by immersing a Van Dorn water sampler 30 cm below the water surface in the water body. Water collected by the water sampler was poured into large containers and then sub-sampled into the bottles which were immediately stored on ice in an ice chest.

Oyster samples were obtained by handpicking from the sandy-mud substratum of the Densu estuary and Narkwa lagoon. With the help of a cutlass, oyster samples were collected from the stilt roots of red mangroves spanning along

the banks of the Whin estuary. Approximately 20 oysters were picked from each sampling station and were kept in plastic bags, labeled, and stored on ice. All samples taken were transported to the Fisheries and Coastal Research laboratory, University of Cape Coast for chlorophyll-a analysis of water samples, and cleaning, sorting out, and freezing of oyster samples for further analyses.

3.4 Chlorophyll-a analysis

Chlorophyll-a concentration of each water sample was determined using the spectrophotometric method as described by Aminot and Rey (2000). Using a glass fibre filter paper (Whatman Grade 934-AH), 500 mL of water were filtered under gravity. The filter paper containing the filtration residue was then folded, rolled, and placed into a 50 mL falcon tube. A total volume of 10 mL of 90 % acetone were added to each tube and the mixture was kept overnight at 4 ºC. Each mixture was then centrifuged (using Eppendorf ® Centrifuge 5430) at 500 x g for 10 min. The supernatant from each centrifuge tube was carefully transferred into glass cuvettes and absorbance of each mixture was measured at 750, 664, 647, and 630 nm (A_{750}, A_{664}, A_{647}, and A_{630}, respectively) against a 90% acetone blank. The concentration of chlorophyll-a in each sample was estimated using equation (1) (Jeffrey & Humphrey, 1975).

$$Chlorophyll - a = \frac{(11.85(A_{664} - A_{750}) - 1.54(A_{647} - A_{750}) - 0.08(A_{630} - A_{750})) \times Ve}{L \times Vf} \quad (1)$$

Where;

L = Cuvette light-path (cm).

Ve = Volume of 90 % acetone (mL).

Vf = Volume of water filtered (L).

Chlorophyll-a concentrations are in unit mg m^{-3}.

3.5 Morphometrics and meat yield of oysters

The shell width, shell length, and shell height measurements of the oysters were taken after they were thoroughly cleaned using a scrubbing brush and water. The shell width was taken by measuring the depth of the maximum longitudinal axis, the shell length by measuring the maximum lateral axis, and the shell height by measuring the maximum distance between the anterior and posterior axis (*Figure 3*). With the help of calipers, all distance measurements were obtained to the nearest 0.1 mm. The oysters were then weighed, shucked and meat weights were taken. The meats were stored at -20 °C for further analysis. Percentage meat yield of the oysters was determined using the equation of Freeman (1974):

$$\%\ Meat\ Yield = \frac{Meat\ wet\ weight}{Total\ weight} \times 100 \quad (2)$$

Figure 3: Shell width (SW), shell height (SH) and shell length (SL) measurement of *C. tulipa.*

3.5 Nutritional quality analysis

The oyster meat samples for each month from each water body were divided into three portions. Two portions were oven-dried at 50 ºC for 72 h and then powdered for proximate nutritional composition analysis, mineral composition analysis, and antioxidant properties determination while the remaining portion of the meat was minced for amino acid and fatty acid composition analyses. Meats for amino and fatty acids analyses were not oven-dried to prevent the oxidation of the fatty acid and amino acid constituents of the meat.

3.5.1 Proximate nutritional composition

Proximate nutritional composition which involved the moisture, ash, fibre, protein, fat and carbohydrate content of the oyster meats were determined as per standard protocols of the Association of Official Analytical Chemists (AOAC, 2005).

Moisture content

Porcelain-crucibles were cleaned, dried in an oven (Dry-Line® 56 Prime) at 105 ºC and then weighed. Approximately, 12 g of fresh oyster meat were weighed into each crucible and each crucible dried in an oven at 105 ºC. After 48 h of drying, each sample was cooled and then weighed. The moisture content of each oyster meat sample was determined using the equation:

$$\%\, moisture = \frac{weight\ loss}{mass\ of\ sample} \times 100 \qquad (3)$$

Ash content

Into cleaned, dried, and weighed crucibles, 2 g of powdered oyster meat were weighed and preheated in an oven at 105 ºC for 1 h. The samples were then incinerated in a muffle furnace at 550 ºC overnight. The ashed samples were cooled and weighed. Ash content of each oyster meat sample was calculated using the equation:

$$\% \, ash = \frac{mass \, of \, ash}{mass \, of \, sample} \times 100 \quad (4)$$

Fibre content

Powdered oyster meat (1 g) was weighed into a 500 mL beaker to which 100 mL of 1.25 % H_2SO_4 was added and boiled for 30 min in a fume hood. The solution was filtered using a Whatman Grade 591 filter paper and the residue was transferred into a 500 mL beaker where 100 mL of 1.25 % NaOH were added. The mixture was boiled in a fume hood and then filtered using a Whatman Grade 591 filter paper. The residue was placed into a crucible, dried in an oven at 105 ºC for 3 h, cooled in a desiccator, weighed, and then incinerated at 550 ºC in a muffle furnace. After incineration, the samples were weighed and the fibre content of each oyster meat sample was calculated as;

$$\% \, fibre = \frac{mass \, of \, residue - mass \, of \, ash}{mass \, of \, sample} \times 100 \quad (5)$$

Fat content

The Soxhlet extraction method was used for estimating the fat content of the oysters. Powdered oyster meat samples (10 g) were weighed into labeled

thimbles and covered with cotton wool. Each sample was placed into a Soxhlet unit. Fat extraction was carried out by refluxing 100 mL of petroleum ether through the samples at 40 ºC for 4 h. Oils extracted were collected into a weighed extraction flask. Residual ether in the extracted oil was evaporated by placing extraction flask with oil into an oven at 105 ºC for 1 h. The flask was cooled in a desiccator and weighed. The fat content of the oyster meat was determined using the equation:

$$\% \, fat = \frac{mass \, of \, oil}{mass \, of \, sample} \times 100 \quad (6)$$

Protein content

The protein content of each oyster meat sample was determined using the Kjeldahl method which involves digestion, distillation, and titration processes. Firstly, 0.2 g of powdered oyster meat was digested by mixing with 4.4 mL of digestion solution (175 mL of H_2O_2, 0.21 g of Se, 7 g of Li_2SO_4, and 210 mL of H_2SO_4) in a digestion flask and then heated at 360 ºC for 2 h inside a fume hood. Also, a blank was made by digesting the above reagents without any sample in them. Each digest was transferred into an Elenmeyer flask and topped up with distilled water to a volume of 50 mL. An aliquot (10 mL) of each digest was added to 10 mL of 45 %, NaOH solution in a Kjeldahl distilling unit and distilled. The distillate was collected into 10 mL of 4 % boric acid solution containing 3 drops of mixed indicators (1.5:1, bromocresol green, and methyl red in 2 L of 90 % ethanol). The distillate (50 mL) was titrated with 0.1 N HCl until the solution changed from green to wine red. The titre values obtained were used to calculate the protein content of the oyster meat using the equation:

$$\%\ Nitrogen = \frac{(\text{Sample titre value} - \text{Blank titre value}) \times 0.1 \times 0.01401}{sample\ weight \times 10} \times 100 \quad (7)$$

$$\%\ Protein = \%\ Nitrogen \times 6.25 \quad (8)$$

Carbohydrate content

The carbohydrate composition of the oyster meat was determined by difference using the equation:

$$\%\ Carbohydrate = 100\ \% - (\%\ ash + \%\ fibre + \%\ protein + \%\ fat) \quad (9)$$

% moisture was not included in the equation (9) because % ash, fibre, protein, and fat were determined per dry weight basis as well as the % carbohydrate was also determined per dry weight basis.

Energy value

The energy value of the oysters was determined using the equation of (Crisan & Sands, 1978):

$$Energy\ value\ (kcal/100g) = (4\ x\ \%protein) + (9\ x\ \%fat) + (4\ x\ \%\ carbohydrate) \quad (10)$$

3.5.2 Mineral composition

Major minerals: sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and minor minerals; iron (Fe), copper (Cu), and zinc (Zn) compositions of the oyster meat were determined. Before the mineral analysis, the powdered samples were digested to destroy their organic matter constituents. The digestion procedure is the same as that of the protein content determination.

Phosphorus content

Phosphorus content was determined using the ascorbic acid method as described by IITA (1982), with slight modifications. The method requires the use of two reagents: reagent A (12:10 w/v ammonium molybdate: distilled water + 0.2908:100 w/v potassium antimony tartrate: distilled water + 1 L of 2.5 M H_2SO_4, volume made up to 2 L with distilled water) and reagent B (ascorbic acid) to form a colour forming reagent. The colour-forming reagent was prepared by dissolving 1.56 g of reagent B to every 200 mL of reagent A.

An aliquot of digested samples (2 mL) was transferred into a volumetric flask and 10 mL of distilled water were added after which 4 mL of the colour forming reagent were added. The mixture was allowed to stand for 15 min after which absorbance was taken with a spectrophotometer at 882 nm. The procedure was repeated by replacing the sample with 2 mL of Phosphorus (0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0μg per mL of digested blank). The absorbance and concentrations of the standards were used to extrapolate the Phosphorus content of each sample.

Calcium and magnesium content

The calcium and magnesium contents were determined using the ethylene diamine tetra-acetic acid (EDTA) method as described by Page and Keeney (1982). Briefly, 10 mL of each digested sample was diluted to 150 mL with distilled water, 15 mL of pH 10 buffer solution (16.9 g of $AlCl_3$ dissolved in 143 mL of NH_4OH and then diluted to 250 mL with distilled water) and 1 mL each of KCN, $HONH_2 \cdot HCl$, $C_6FeK_4N_6$ and $C_6H_{15}NO_3$. Each final solution was titrated against 0.005 M EDTA after adding five drops of erichrome Black T. Calcium was

determined by repeating the above procedure without adding 15 mL of pH 10 buffer solution (16.9 g of $AlCl_3$ dissolved in 143 mL of NH_4OH and then diluted to 250 mL with distilled water). The Ca and Mg contents were calculated using the equations:

$$\% \text{ Ca} = \frac{0.005 \text{ x } 40.08 \text{ x } T_{Ca}}{\text{Mass of sample}} \times 100 \quad (11)$$

$$\% \text{ Mg} = \frac{[(0.005 \text{ x } T_{Ca+Mg}) - (0.005 \text{ x } T_{Ca})] \times 24.31}{\text{Mass of ample}} \times 100 \quad (12)$$

Where, T_{Ca} = titre value for calcium titration, $T_{Ca + Mg}$ = titre value for calcium and magnesium titration, 0.005 = conc of EDTA, 40.08 = Mr(Ca) and 24.31 = Mr(Mg).

Iron, copper and zinc content

The concentrations of the microminerals Fe, Cu and Zn were determined using the flame atomic absorption spectrometer as described by Motsara and Roy (2008). Samples and standard solutions of Fe, Cu and Zn (1, 2, 3, 4 and 5 μg/mL) were aspirated one by one at wavelengths of 213.9, 324.8 and 248.3 nm, respectively, and absorbance readings were recorded. A standard curve was generated using the absorbance and concentrations of the standards and was used to extrapolate the micromineral content of each sample solution.

Consumption estimates of minerals.

Adequate daily intake of K and upper levels of daily intake of Na, P, Mg, Ca, Fe, Cu and Zn as defined by Australia/New Zealand (Capra, 2006) were compared to the mean of mineral concentrations per wet weight of *C. tulipa* from the different water bodies if 109 g of *C. tulipa* is consumed daily (the daily

consumption rate of oysters in Ghana as reported by Essumang et al. (2018)). No single regulatory agency had the defined adequate daily intake or upper levels of daily intake for all the minerals estimated in this study, except for Food Standards Australia New Zealand (FSANZ).

3.5.3 Amino acid composition

The Pico-Tag technique, as described by Zheng et al. (2015), was used to estimate the amino acid content of oyster meat. Briefly, 50 mg of minced oyster meat were hydrolysed by adding 2 mL of 6 N HCl containing 0.1 % phenol and heated at 110 ºC for 24 h in corked 15 mL glass tubes filled with nitrogen. The digested samples were centrifuged for 15 min at 2600 x g to force any acid that may have condensed on the side of the tube down to the bottom of the tube and filtered through a 1.5 µm pore size glass fibre filter paper before derivatization. Amino acids in hydrolysed samples and amino acid standards were derivatized by mixing 10 µL of each hydrolysed sample or standards with 100 µL of redrying reagent (methanol: water: triethylamine, 2:1:1 v/v/v) and dried completely at 60 ºC under nitrogen gas, after which 20 µL of phenyl isothiocyanate (PITC) or Edman's reagent (methanol: water: triethylamine: PITC, 7:1:1:1 v/v/v/v) was added. Each mixture was kept in the dark for 30 min and then dried completely at 60 ºC under nitrogen gas. The derivatives were diluted with 1 mL of sample diluent (900 µL of 0.05 % formic acid and 100 µL of 60 % acetonitrile) before analyzing with a Shimadzu Prominence Ultrafast liquid chromatographic (UFLC) system equipped with two LC-20AD pumps.

A Luna® 3 µm C18(2) 100 Å, LC column (150 x 4.6 mm) was used and was maintained at 40 °C in a column oven. The derivatized amino acids were detected with a UV detector SPD 20AX at a wavelength of 256 nm. The mobile phase was made up of (A) 0.1 % formic acid and (B) acetonitrile. Gradient elution of 0 – 4 min (20 % B), 4 – 31 min (20 – 70 % B), 31 – 33 min (70 % B), 33 – 33.01 min (20 % B) was used with a flow rate and an injection volume of 0.8 mL/min and 5 µL, respectively. All reagents were prepared with Milli-Q ultrapure water. The concentrations of the amino acids were determined using the external standard technique and they were identified by comparing their retention times to their corresponding standards. The amino acid concentrations were reported as g/100 g ww of oyster meat. The calibration linearity (regression equation and R^2), precision (% RSD), limit of detection (LOD), limit of quantitation (LOQ) and retention times of the amino acids have been presented in Appendices B - D.

Protein quality estimation

The following dietary parameters were calculated based on the amino acid profile of the oyster meats:

Chemical score of oyster meat was determined using the FAO/WHO/UNU (2007) comparison pattern, which is the least amino acid score. The amino acid score (AAS) was calculated as the ratio of a gram of amino acid in dry weight protein to the amount of the same amino acid in a reference diet multiplied by 100. The FAO/WHO/UNU (2007) amino acid reference pattern for adults was used as the reference diet.

Essential amino acid index (EAAI) was determined according to Oser (1959). The EAAI was calculated by multiplying the ratio of the amount of each essential amino acid in the oyster meat to the amount of the same amino acid in the reference protein by 100. Log 10 of each value was determined and their mean was calculated. Finally, antilog of the mean was used to derive the EAAI.

Biological value (BV) of oyster meat was determined using the equation of Oser (1959):

$$BV = 1.09 \times EAAI - 11.7 \quad (13)$$

3.6 Determination of medicinal properties

Medicinal properties which involved the phenolic content, DPPH scavenging activity, total antioxidant capacity, and fatty acid composition of the oyster meats were determined on pooled monthly samples for each water body.

3.6.1 Solvent extraction

Bioactive compounds were extracted by dissolving 5 g of powdered oyster meat in absolute methanol and kept for 24 h at room temperature. Each mixture was heated in a water bath at 50 ^{o}C while shaking. Each resultant mixture was filtered through a Whatman grade 591 filter paper and the filtrate was collected. The extraction procedure was repeated by replacing absolute methanol with 70 % ethanol, 70 % methanol and absolute hexane. Filtrates for absolute methanol and absolute hexane were dried in a desiccator whereas those of 70 % ethanol, 70 % methanol were dried at 50 ^{o}C using a water bath. Yield of extract was determined by finding the ratio of the mass of the extract to the mass of the sample and multiplied by 100. The yield of the extract was used to determine the solvent which

was more suitable for the extraction. The extracts were stored in a desiccator and used for the phenolic content, DPPH scavenging activity and total antioxidant capacity determinations.

3.6.2 Phenolic content estimation

The total phenolic content of the oyster meat extracts was determined using the Folin–Ciocalteu method (Singleton & Rossi, 1965). Briefly, 100 μL of dissolved extract were mixed with 750 μL of 10% v/v Folin–Ciocalteu reagent followed by 750 μL of 7.5%, w/v Na_2CO_3. Absorbance readings were taken against a blank with a Jenway 7315 ® spectrophotometer after incubating the mixture in the dark for 90 min at room temperature. Gallic acid was used as standard and the concentrations were repressented as mg of gallic acid equivalent (GAE) per gram of extract using the equation;

$$Concentration\ of\ extract = C_s \times \frac{V_e}{M_e} \quad (14)$$

Where;

C_s = Concentration extrapolated from standard curve

V_e = Volume of extract

M_e = Mass of extract

3.6.3 DPPH scavenging activity

DPPH radical scavenging activity was assessed as described by Kimura et al. (2002). Extracts or standard (ascorbic acid) of different concentrations (0.008-0.032 mg/mL) were incubated with 2 mL of 47.5 µg/mL DPPH solution in the dark

for 30 min after shaking vigorously. Absorbance readings of the mixture and a control (mixture with extract replaced with extraction solvent) were taken at 517 nm. The % DPPH inhibition by the extracts or standards were determined using the equation:

$$\%\ inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (15)$$

Where, $A_{control}$ is the absorbance measured for the control and A_{sample} is the absorbance for the extract or standard with DPPH mixture.

3.6.4 Total antioxidant capacity

Total antioxidant capacity of the extracts was determined using the phosphomolybdate assay as described by Garrat (1964). Briefly, 3 mL of reagent solution (0.6 M H_2SO_4, 0.004 M $(NH_4)6Mo_7O_{24}$ and 0.028 M Na_2PO_4) was added to 1 mL of dissolved extract and the mixture was heated at 90 °C for 90 min. The mixtures were allowed to cooled at room temperature and their absorbance readings were taken at 695 nm using a Jenway 7315 ® spectrophotometer against a blank. Ascorbic acid was used as standard and the concentrations were represented as mg of ascorbic acid equivalent (AAE) per gram of extract.

3.6.5 Fatty acid composition

Fat was extracted from minced oyster meat using Folch's method (Folch et al., 1957) with some modifications. Briefly, 1 g of sample was homogenized for 30 sec with 7 ml of methanol in a beaker, after which 14 mL of chloroform were added and then homogenized again. The homogenate was filtered through Whatman no. 40 filter paper into a 15 mL glass tube. The beaker was rinsed again with an

additional 12 ml of 2:1 (v/v) chloroform/methanol, homogenized for another 5 sec to rinse the probe, filtered, and filtrate added to the homogenized sample. The mixture was agitated for 10 min after adding 8 mL of 0.88 % NaCl. The mixture was centrifuged for 5 min at 1000 x g to allow the mixture to become biphasic. The upper, aqueous layer was removed using a Pasteur's pipette. The chloroform layer remaining in the tube was evaporated using a rotary evaporator at 40 ºC to obtain extracted fat.

The extracted fat was derivatized into fatty acyl methyl esters (FAME) before identification and quantification. Each sample was dissolved in 4 mL of hexane and 2 mL of 0.5 N sodium methoxide in methanol was added. The tubes were capped tightly, heated in a water bath for 10 min at 50 ºC and 0.1 ml of glacial acetic acid followed by 5 ml of saturated NaCl solution were added. The tubes were agitated for 10 min, centrifuged at 1000 x g for 10 min and the upper organic layer was transferred into clean, labeled tubes for GC-MS analysis.

A "Perkin Elmer GC Clarus 580 gas chromatograph interfaced with a Perkin Elmer (Clarus SQ 8 S) mass spectrometer equipped with ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane, DB-5) fused capillary column (30×0.25 µm ID×0.25 µm DF)" was used for the GC analysis. The initial temperature of the oven was 80 °C, held for 2 mins and then ramped up by 15 °C/min to 150°C which was finally ramped up by 3 °C/min to 250 °C and maintained for 4 min. The carrier gas was Helium (99.999 %), the flow rate was 1.6 mL/min, and an injection volume 1 µL of sample was used for the GC-MS detection. The transfer temperature and ion-source temperature were held at 280 and 220 °C, respectively. Mass spectra were

taken at 70 eV; a scan-interval of 0.5 s and fragments from 45 to 4500 Da. The solvent delay was from 0 to 3 min, and the total GC/MS running time was 34.5 min. Using mass spectra libraries in the databases of the National Institute of Standard and Technology (NIST) and Wiley, mass spectra obtained were identified and matches ≥750 were regarded as acceptable (Appendix H). Relative abundance (% area) of the fatty acid methyl esters (FAMEs) were determined using the peak area (Appendix G) of each selected fatty acid methyl ester expressed as a percentage of the sum of areas of all selected FAMEs.

Index of atherogenic (AI) were measured using the equation of Ulbricht and Southgate (1991) to describe the atherogenic ability of the oyster meat:

$$AI = \frac{12{:}0 + 4 \times 14{:}0 + 16{:}0}{MUFA + n{-}3\ PUFA + n{-}6\ PUFA} \quad (16)$$

3.6 Statistical analysis

Homogeneity of variance of the dataset was determined using Levene's test and non-parametric analyses were used when variances were heterogeneous. One-way Analysis of Variance (ANOVA) was used to determine the monthly variations within the nutritional constituents of *C. tulipa.* The Kruskal-Wallis test was used to determine spatial variations in morphometrics, meat yield, and nutritional composition of *C. tulipa*, as well as the physicochemical parameters of the water bodies, and when significant differences were found, pairwise comparisons were made using the Mann-Whitney/Wilcoxon rank-sum test.

Principal component analysis was conducted using correlation matrices to determine whether the nutritional constituents of the oysters or the physicochemical

parameters of the water bodies were correlated and whether there were patterns associated with the location of oysters. Biplots were then created to visualize the location of oysters and the magnitude of variable importance in ordination space.

Multivariate comparisons were also conducted using the permutational multivariate analysis of variance (PERMANOVA) based on Euclidean distances and a permutation = 999 to determine whether there were differences in the overall physicochemical parameters of water bodies or the morphological or nutritional properties of the oysters from the different water bodies.

Multiple linear regression models were used to determine the influence of the environmental factors on the nutritional composition of the oysters. Statistical analyses were conducted in R version 4.1.1 and SPSS version 25 and statistical significance was established at $\alpha = 0.05$.

CHAPTER FOUR

RESULTS

This chapter presents the results obtained for the study. The results are represented with graphs and tables where necessary and were analysed statistically as described in chapter three.

4.1 Physicochemical Parameters of the Water Bodies

Monthly trends of temperature, pH, salinity, dissolved oxygen and chlorophyll-a content of Densu estuary, Narkwa lagoon and Whin estuary for the period of study are shown in *Figure 4.*

4.1.1 Temperature

The temperature ranges were 28.88 ± 0.61 – 32.55 ± 0.27 °C for Densu estuary, 27.43 ± 0.39 – 31.87 ± 0.12 °C for Narkwa lagoon and 30.12 ± 0.56 – 32.17 ± 1.62 °C for Whin estuary (*Figure 4A)*. The highest temperatures were recorded in January, April and March 2021 for Densu estuary, Narkwa lagoon and Whin estuary, while their lowest temperatures were recorded in May, January and June 2021, respectively. The three water bodies showed different monthly trends for temperature and there were significant differences (Kruskal-Wallis, $P < 0.05$) in temperature among the three water bodies for all the months studied. In general, the mean temperatures of Densu estuary (31.31 ± 1.34 °C) and Whin estuary (31.50 ± 1.16 °C) were similar and were significantly higher (Wilcoxon, $P < 0.05$) than that of Narkwa lagoon (29.51 ± 1.42 °C).

4.1.2 pH

The three water bodies showed a similar trend in pH where higher pH values (pH > 7.2) were recorded from February to May 2021 *(Figure 4B)*. The pH ranges were 7.19 ± 0.01 – 8.25 ± 0.19 for Densu estuary, 7.05 ± 0.07 – 7.99 ± 0.07 for Narkwa lagoon and 7.19 ± 0.05 – 8.04 ± 0.12 for Whin estuary, respectively. The highest pH values were recorded in March 2021 for all the water bodies, while the lowest pH values were obtained in December 2020 for Densu estuary, November 2020 for Narkwa lagoon and January 2021 for Whin estuary. In general, pH values for the water bodies were similar, however, the pH of Narkwa lagoon was significantly lower than that of Densu estuary (Wilcoxon, $P < 0.05$) *(Figure 4B)*.

4.1.3 Salinity

The monthly mean of salinity for Narkwa lagoon was higher than those of Densu and Whin estuaries during the period of study, except for December 2020 and April 2021 where the salinity of Narkwa lagoon was either equal to or lower than the salinities of the estuaries *(Figure 4C)*. Salinity ranges were 9.66 ± 3.01 – 25.69 ± 2.04 ppt for Densu estuary, 18.45 ± 2.27 – 29.39 ± 0.26 ppt for Narkwa lagoon and 11.48 ± 1.76 – 22.12 ± 1.77 ppt for Whin estuary. The highest salinities were recorded in April 2021 for Densu estuary and in May 2021 for Narkwa lagoon and Whin estuary, whereas the lowest salinities were observed in November 2020 for Densu estuary and in December 2020 for Narkwa lagoon and Whin estuary. In general, the mean salinities for Densu (17.41 ± 5.24 ppt) and Whin (16.24 ± 5.66 ppt) estuaries were similar, however, they were significantly lower than the salinity of Narkwa lagoon (24.12 ± 4.76 ppt) (Wilcoxon, $P < 0.05$).

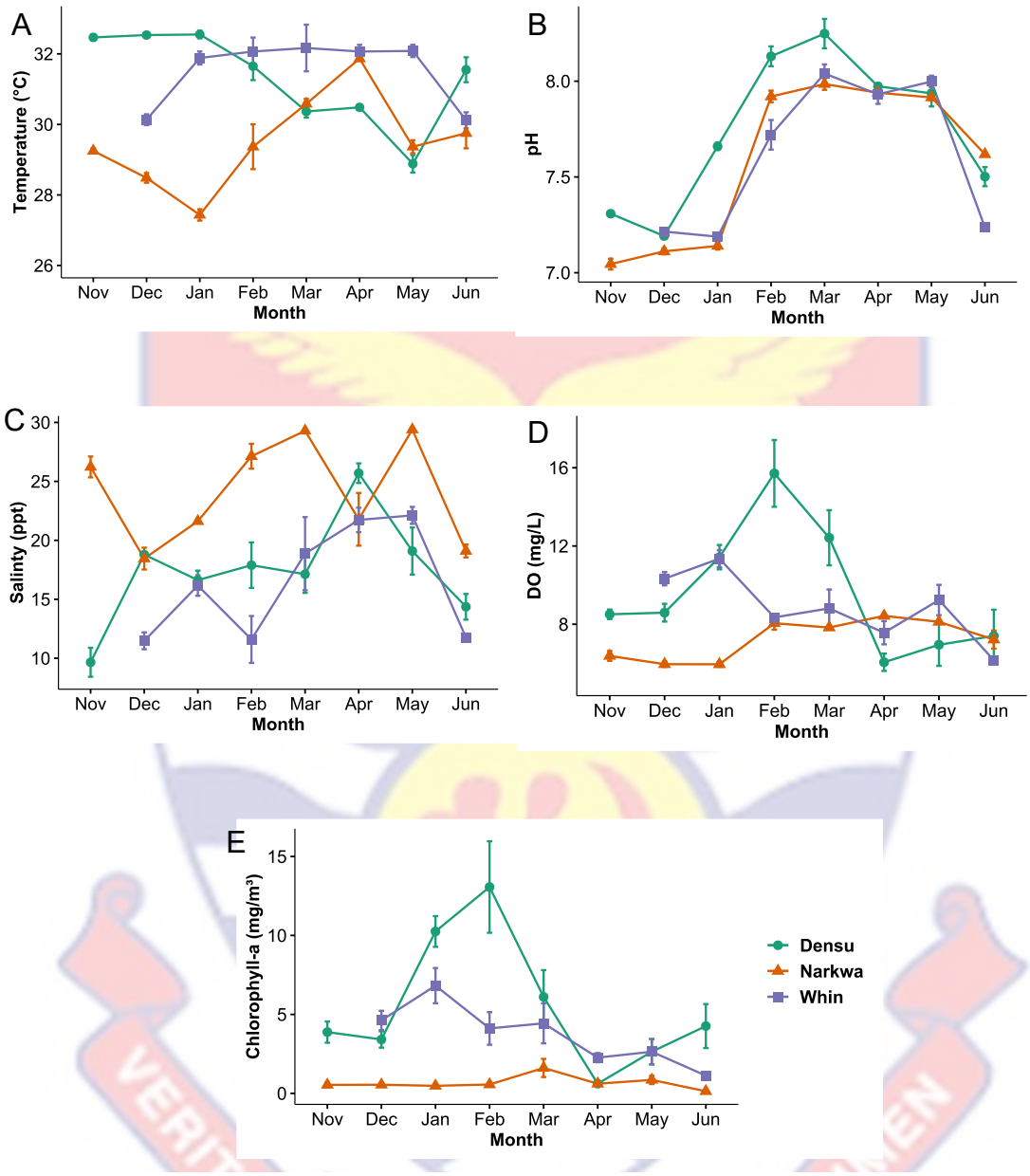

Figure 4: Physicochemical parameters **A**. Temperature, **B.** pH, **C.** Salinity, **D.** Dissolved Oxygen (DO) and **E.** Chlorophyll-a of Densu estuary, Narkwa lagoon and Whin estuary from November 2020 to June 2021. (Error bars = standard errors of the means of six readings)

4.1.4 Dissolved Oxygen (DO)

The three water bodies showed different monthly trends in dissolved oxygen concentrations during the period of study *(Figure 4D)*. Densu estuary recorded higher concentrations of dissolved oxygen from January to March 2021. Whin estuary also recorded higher concentrations of dissolved oxygen in December 2020 and January 2021, whereas in Narkwa lagoon, dissolved oxygen concentrations were higher after January 2021. The ranges for dissolved oxygen concentrations were 6.06 ± 1.08 – 15.71 ± 4.18 mg/L for Densu estuary, 5.95 ± 0.31 – 8.43 ± 0.07 mg/L for Narkwa lagoon and 6.16 ± 0.27 – 11.35 ± 1.06 mg/L for Whin estuary. The overall mean of dissolved oxygen contents for Densu (9.63 ± 3.89 mg/L) and Whin (8.83 ± 2.05 mg/L) estuaries were similar and significantly higher (Wilcoxon, $P < 0.05$) than that of Narkwa lagoon (7.24 ± 1.09 mg/L).

4.1.5 Chlorophyll-a

Chlorophyll-a concentration ranges were 0.61 ± 0.17 – 13.07 ± 7.10 mg/m^3 for Densu estuary, 0.13 ± 0.09 – 1.61±1.41 mg/m^3 for Narkwa lagoon and 1.13 ± 0.48 – 6.82 ± 2.73 mg/m^3 for Whin estuary *(Figure 4E)*. The monthly means of chlorophyll-a trends for the three water bodies were similar to those of their dissolved oxygen concentrations. The highest chlorophyll-a concentration was recorded in February 2021 for Densu estuary, March 2021 for Narkwa lagoon and January 2021 for Whin estuary, whereas their lowest were recorded in April 2021 for Densu estuary and June 2021 for Narkwa lagoon and Whin estuary. In general, the mean of chlorophyll-a concentration for Densu (5.53 ± 5.03 mg/m^3) and Whin

(3.72 ± 2.61 mg/m^3) estuaries were similar and were significantly higher (Wilcoxon, $P < 0.05$) than that of Narkwa lagoon (0.67 ± 0.68 mg/m^3).

4.1.6 Comparison of physicochemical parameters of the three water bodies

Principal component analysis relating physicochemical parameters to their water bodies showed that principal component 1 (Dim1) and principal component 2 (Dim2) accounted for 49.7 and 26 % of variance in the physicochemical parameters, respectively. Temperature, chlorophyll-a and dissolve oxygen were positively correlated and had strong positive contributions to principal component 1 while in principal component 2, there were strong negative contributions of salinity and pH. Densu estuary had the largest variability in physicochemical characteristics during the period of study. Narkwa lagoon was associated with higher salinities, whereas Densu and Whin estuaries were associated with higher dissolved oxygen, temperature and chlorophyll-a *(Figure 5)*.

A multivariate comparison of the three water bodies based on their physicochemical parameters using permutational multivariate analysis of variance (PERMANOVA) revealed that Densu and Whin estuaries were statistically similar and were significantly different from the Narkwa lagoon ($F = 27.3$, $R^2 = 0.288$ $P = 0.001$).

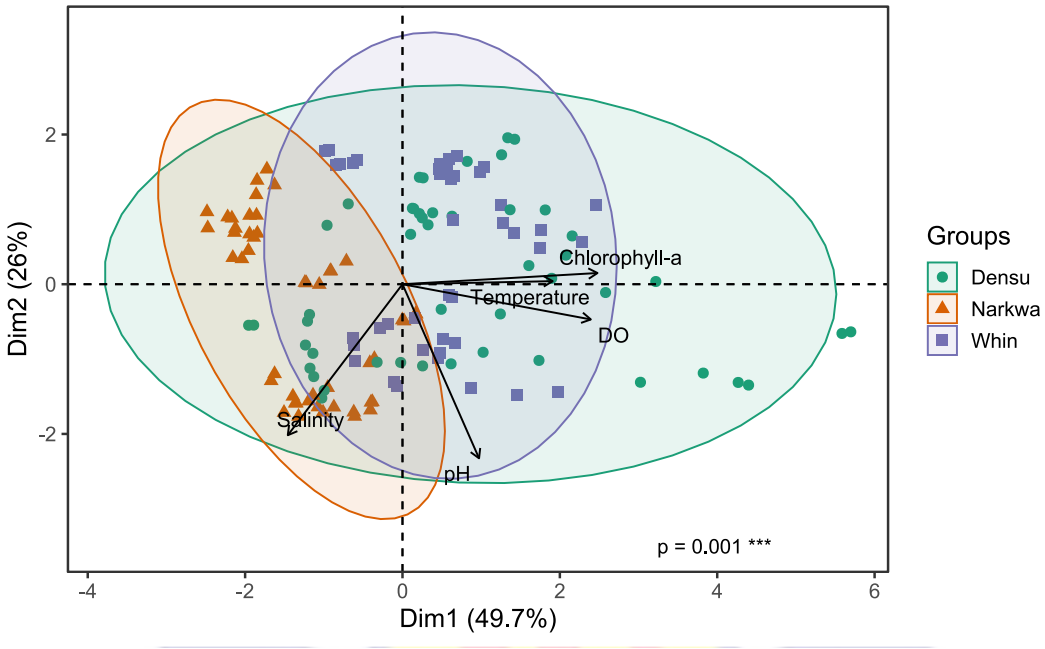

Figure 5: Physicochemical parameters ordered by principal component analysis according to Location (Densu estuary, Narkwa lagoon and Whin estuary). P value represents statistical comparisons between the three water bodies using PERMANOVA (*** = $P \leq 0.001$)

4.2 Morphometric Characteristics of Oysters

4.2.1 Shell height, shell length and shell width

Shell height ranges of *C. tulipa* used for the study were 64.5 – 164 mm for Densu estuary, 41 – 107 mm for Narkwa lagoon and 54 – 136 mm for Whin estuary. Shell length ranges were 33 – 88 mm for Densu estuary, 22.5 – 74 mm for Narkwa lagoon and 31 – 88 mm for Whin estuary, while those of their shell widths were 16 – 55 mm, 10 – 47 mm and 15 – 53 mm, respectively (*Figure 6*).

The shell sizes of *C. tulipa* from Densu estuary were significantly greater than those of Narkwa lagoon and Whin estuary (PERMANOVA, $P < 0.05$). The shell sizes of *C. tulipa* from Whin estuary were also significantly bigger than those of Narkwa lagoon (PERMANOVA, $P<0.05$).

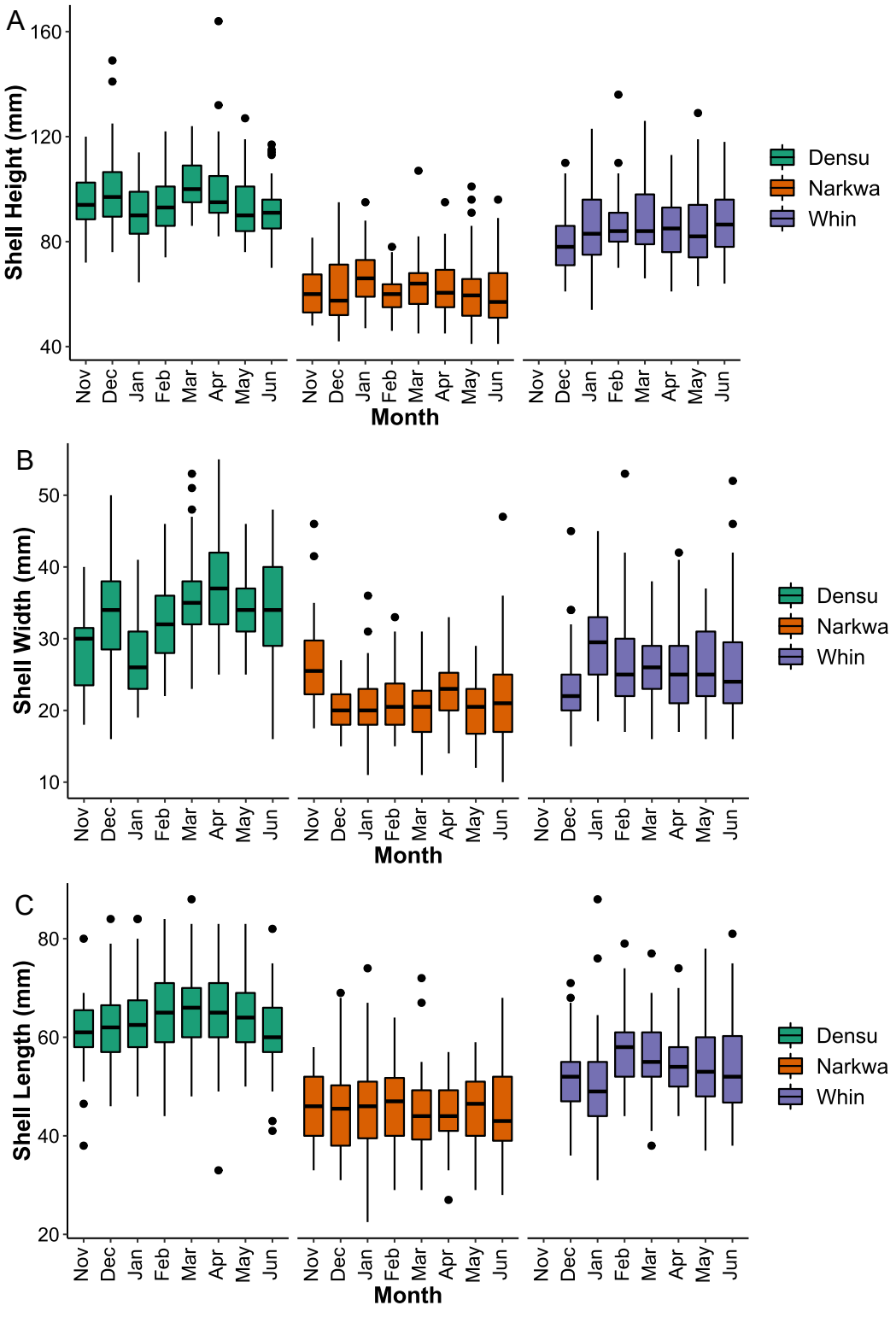

Figure 6: Morphometric characteristics; **A.** Shell height **B.** shell width and **C.** shell length of *C. tulipa* sampled monthly (November 2020 to June 2021) from Densu estuary, Narkwa lagoon and Whin estuary.

4.2.2 Meat yield

Monthly mean meat yields for oysters from Densu estuary and Narkwa lagoon showed a similar trend, with the exception of December 2020 and January 2021 where meat yield for oysters from Narkwa lagoon increased, while those of Densu estuary decreased. The meat yield for oysters from Whin estuary decreased gradually from December 2020 (13.93 ± 3.39 %) to May 2021 (9.55 ± 2.26 %) after which it increased in June 2021 (10.68 ± 2.76 %). The highest meat yields for oysters were recorded in November 2020 for Densu estuary (15.91 ± 3.13 %), January 2021 for Narkwa lagoon (16.90 ± 3.27 %) and December 2020 (13.93 ± 3.39 %) for Whin estuary, whereas the lowest were recorded in April 2021 for both Densu estuary (9.28 ± 1.93 %) and Narkwa lagoon (12.31 ± 2.20 %), and in May 2021 for Whin estuary (9.55 ± 2.26 %) (*Figure 7*).

In general, meat yield for oysters from Densu (11.69 ± 2.93 %) and Whin (12.01 ± 3.17 %) estuaries were similar; however, each was significantly lower than that of oysters from Narkwa lagoon (14.14 ± 2.99 %) (Wilcoxon, $P < 0.05$). The means of meat weight were 10.38 ± 3.26 g, 3.66 ± 1.70 g and 5.84 ± 2.42 g for oysters from Densu estuary, Narkwa lagoon and Whin estuary, respectively. Also, the means of their total weights were 91.42 ± 27.33 g, 26.32 ± 12.13 g and 50.18 ± 22.21 g for Densu estuary, Narkwa lagoon and Whin estuary, respectively.

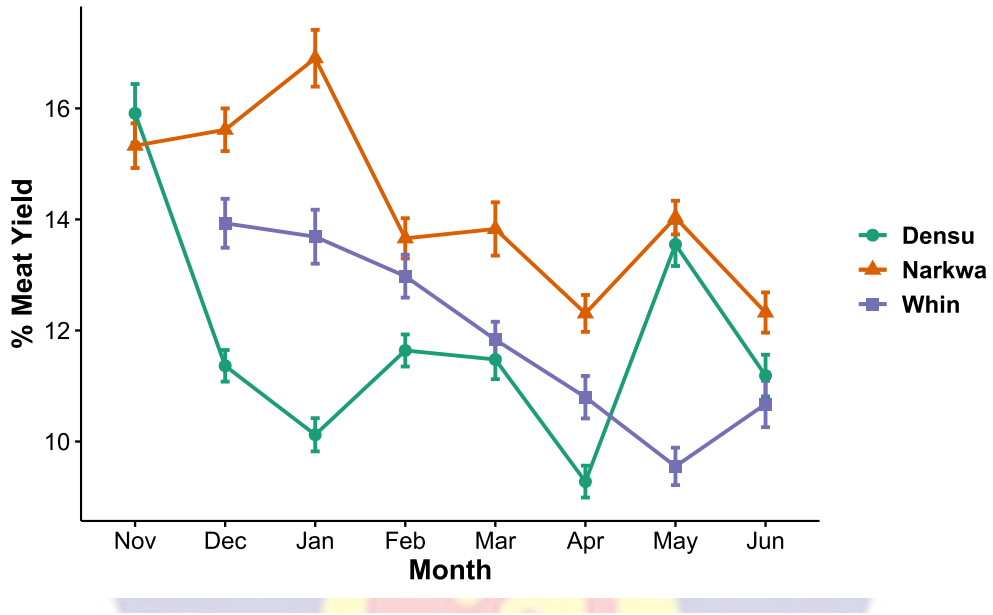

Figure 7: Meat yield for *C. tulipa* sampled monthly (November 2020 to June 2021) from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = Standard errors of the means of 35 – 50 readings)

4.3 Proximate Nutritional Composition of Oysters

4.3.1 Moisture content

Means of moisture content were 85.23 ± 2.29 %, 85.76 ± 1.91 % and 84.47 ± 1.76 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively (*Figure 8A*). The moisture contents of *C. tulipa* from Densu estuary and Narkwa lagoon were relatively high from December 2020 to March 2021. *C. tulipa* from Whin estuary recorded the highest moisture content in December 2020 which decreased thereafter. Highest moisture contents were recorded in December 2020 for Densu (88.20 ± 0.45 %) and Whin (88.23 ± 0.28 %) estuaries, and in January 2021 for Narkwa lagoon (88.30 ± 0.81 %). The lowest moisture contents were obtained in June, April and May 2021 for Densu estuary (81.46 ± 0.34 %), Narkwa lagoon (82.62 ± 0.38 %) and Whin estuary (82.66 ± 0.27 %), respectively. There were significant monthly variations in moisture content of *C. tulipa* from the various water bodies (ANOVA, $P < 0.05$). In general, there were no significant differences in the moisture content of *C. tulipa* from the different water bodies (Kruskal-Wallis, $P>0.05$), however, significant variations existed in November 2020, March 2021 and May 2021 (Kruskal-Wallis, $P<0.05$).

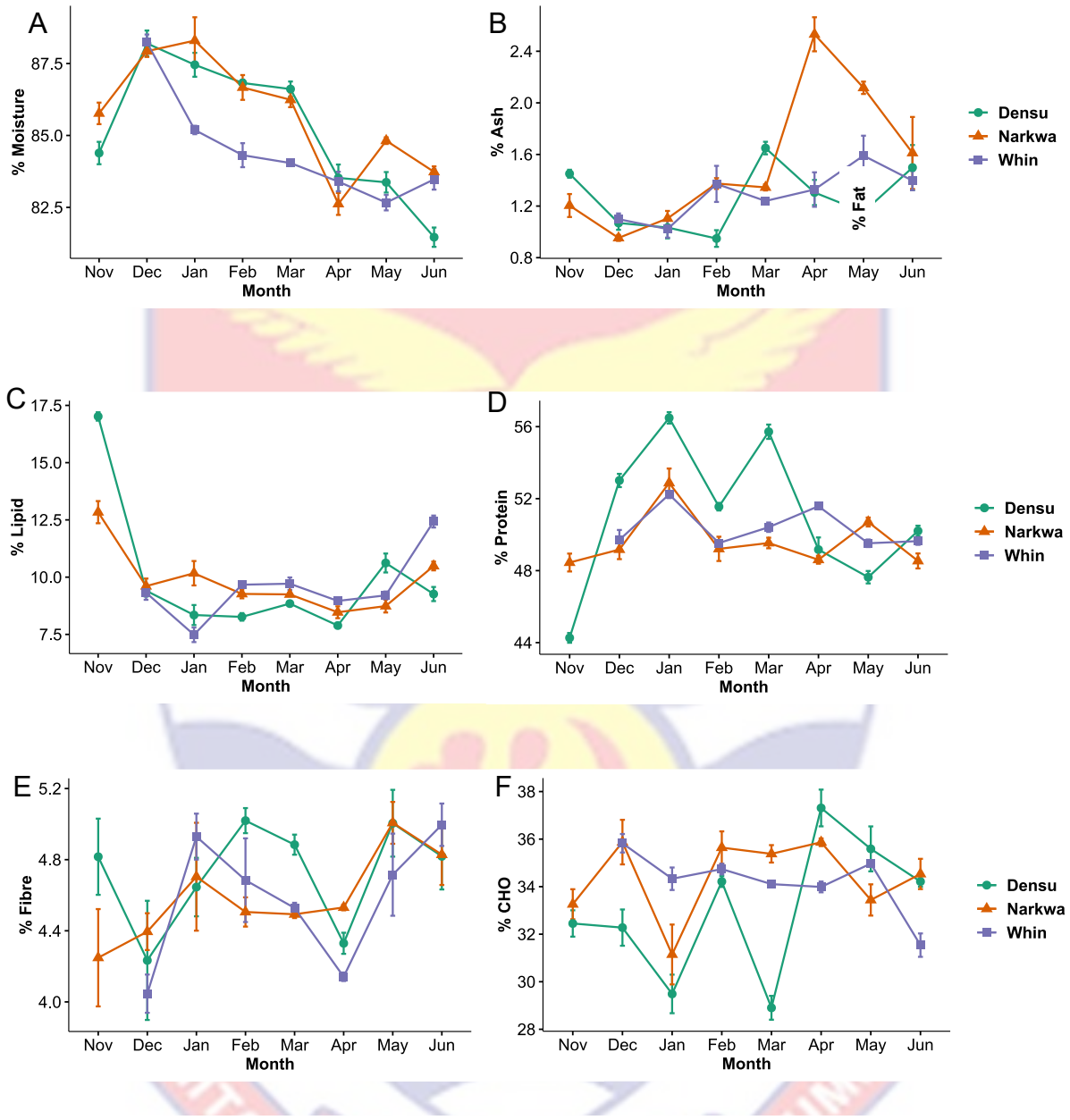

Figure 8: Proximate nutritional composition; **A.** moisture **B.** ash **C.** fat **D.** protein **E.** fibre and **F.** carbohydrate (CHO) contents per dry weight of *C. tulipa* sampled monthly (November 2020 to June 2021) from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = standard deviations of the means of triplicate

4.3.2 Ash content

Ash content ranges were 0.95 ± 0.06 – 1.65 ± 0.05 %, 0.95 ± 0.02 – 2.53 ± 0.13 % and 1.02 ± 0.07 – 1.59 ± 0.15 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. There were significant monthly variations in ash content of *C. tulipa* from the various water bodies (ANOVA, $P < 0.05$). The highest ash contents were recorded in March, April and May 2021 for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, whereas their lowest were recorded in February 2021, December 2020 and January 2021, respectively (*Figure 8B*). The mean ash contents were 1.26 ± 0.25 %, 1.53 ± 0.52 % and 1.29 ± 0.20 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. In general, there were no significant differences in the ash contents of *C. tulipa* from the different water bodies (Kruskal-Wallis, $P > 0.05$), however, significant variations were observed in November 2020, March 2021 and May 2021 (Kruskal-Wallis, $P<0.05$).

4.3.3 Protein content

There were significant monthly variations in the mean protein contents of *C. tulipa* from the various water bodies (ANOVA, $P < 0.05$) with ranges of 44.26 ± 0.27 – 56.49 ± 0.32 %, 48.45 ± 0.50 – 52.87 ± 0.80 % and 49.52 ± 0.21 – 52.23 ± 0.20 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. Highest protein contents were recorded in January 2021 for *C. tulipa* from all the study water bodies, whereas, the lowest were recorded in November 2020 for *C. tulipa* from Densu estuary and Narkwa lagoon, and in May 2021 for *C. tulipa* from Whin estuary (*Figure 8D*). Means of protein content were 51.01 ± 3.93

%, 49.64 ± 1.49 % and 50.38 ± 1.07 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. In general, there were no significant differences in the protein content of *C. tulipa* from the different water bodies (Kruskal-Wallis, $P > 0.05$), however, significant variations were observed in November 2020, March 2021, April 2021, May 2021 and June 2021 (Kruskal-Wallis, $P < 0.05$).

4.3.4 Fat content

There were significant monthly variations in the fat content of *C. tulipa* from the various water bodies (ANOVA, $P < 0.05$) with ranges of 7.89 ± 0.08 – 17.02 ± 0.18 %, 8.47 ± 0.26 – 12.84 ± 0.48 % and 7.48 ± 0.32 – 12.43 ± 0.26 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. Highest fat contents were recorded in November 2020 for *C. tulipa* from Densu estuary and Narkwa lagoon, and in June 2021 for those of Whin estuary. Lowest fat contents were also recorded in April 2021 for *C. tulipa* from Densu estuary and Narkwa lagoon, and in January 2021 for those of Whin estuary.

In general, there were no significant differences in the fat content of *C. tulipa* from the different waterbodies (Kruskal-Wallis, $P > 0.05$), however, significant variations were observed among *C. tulipa* from the three water bodies in all the months of study except for December 2020 (Kruskal-Wallis, $P<0.05$). Means of fat content were 9.96 ± 2.85 %, 9.85 ± 1.35 % and 9.54 ± 1.42 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively (*Figure 8C*).

4.3.5 Fibre content

Fibre content ranges were 4.23 ± 0.34 – 5.02 ± 0.07 %, 4.25 ± 0.27 – 5.01 ± 0.12 % and 4.05 ± 0.11 – 5.00 ± 0.12 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. Highest fibre contents were recorded in February, May and June 2021 for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. The lowest fibre contents were recorded in December 2020 for *C. tulipa* from Densu and Whin estuaries, and in November 2020 for *C. tulipa* from Narkwa lagoon. There were significant monthly variations in the fibre content of *C. tulipa* from the various water bodies (ANOVA, $P < 0.05$). In general, there were no significant differences in the fibre content of *C. tulipa* from the different water bodies (Kruskal-Wallis, $P > 0.05$), however, significant variations were observed in November 2020, March 2021 and April 2021 (Kruskal-Wallis, $P < 0.05$) (*Figure 8E*).

4.3.6 Carbohydrate content

Mean carbohydrate contents were 33.06 ± 2.81 %, 34.39 ± 1.71 % and 34.21 ± 1.29 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively (*Figure 4.7B*). There were significant monthly variations in the carbohydrate content of *C. tulipa* from the various water bodies (ANOVA, $P < 0.05$) with ranges of 28.90 ± 0.50 – 37.31 ± 0.77 %, 31.15 ± 1.26 – 35.87 ± 0.16 % and 31.54 ± 0.49 – 35.82 ± 0.39 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. Highest carbohydrate contents were recorded in April 2021 for *C. tulipa* from Densu estuary, in December 2020 for *C. tulipa* from Whin estuary, and in April 2021 and December 2020 for *C. tulipa* from Narkwa

lagoon. The lowest carbohydrate contents were observed in March, January and June 2021 for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively (*Figure 8F*).

In general, there were no significant differences in the carbohydrate content of *C. tulipa* from the different water bodies (Kruskal-Wallis, $P > 0.05$), however, significant variations were observed from January 2021 to April 2021 (Kruskal-Wallis, $P < 0.05$).

4.3.7 Energy Value

Energy values were in the ranges of 416.90 ± 0.58 – 460.02 ± 1.13 kcal/100g, 414.08 ± 1.79 – 442.38 ± 2.18 kcal/100g and 413.61 ± 1.71 – 436.57 ± 1.17 kcal/100g for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively (*Figure 11*). Highest energy values were recorded in November 2020 for *C. tulipa* from Densu estuary and Narkwa lagoon, and in June 2021 for *C. tulipa* from Whin estuary, whereas the lowest energy values were recorded in April 2021 for *C. tulipa* from Densu estuary and Narkwa lagoon, and in December 2020 for *C. tulipa* from Whin estuary. In general, there were no significant differences in the energy value of *C. tulipa* from the different water bodies (Kruskal-Wallis, $P > 0.05$); however, on a month by month basis, significant variations were observed in all the months of study except for December 2020 and February 2021 (Kruskal-Wallis, $P<0.05$) (*Figure 9*).

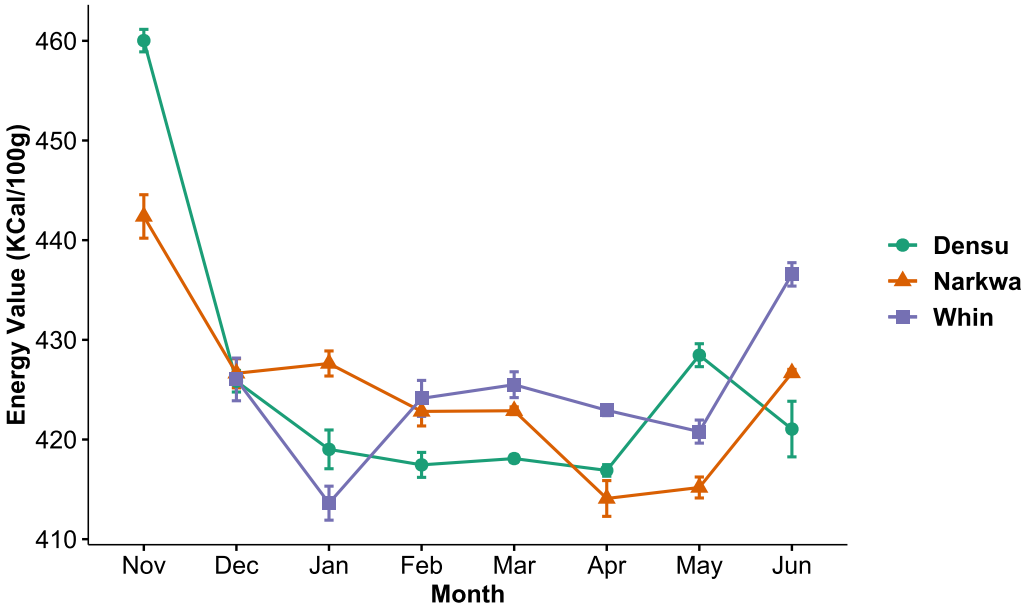

Figure 9: Energy values per dry weight of *C. tulipa* sampled monthly (November 2020 to June 2021) from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = standard deviations of the means of triplicate determinations)

4.1.6 Comparison of *C. tulipa* from different water bodies based on their proximate nutritional properties.

Principal component analysis relating proximate nutritional properties of *C. tulipa* to their water bodies showed that principal component 1 (Dim1) and principal component 2 (Dim2) accounted for 35.9 and 27.6 % of variance in the proximate nutritional compositions, respectively (*Figure 12*). There were positive correlations between the fat content and energy value, and between ash and carbohydrate contents, while a negative correlation was observed between moisture, the ash and carbohydrate contents of *C. tulipa* (*Figure 12*). In principal component 1, there were strong positive contributions of fat and energy value, and a strong negative contribution of proteins while in principal component 2, there was a strong negative contribution of moisture and strong positive contributions of ash and carbohydrate. *C. tulipa* from Densu estuary had the largest variability in proximate nutritional properties during the period of study, followed by Narkwa lagoon and then Whin estuary (*Figure 12*). *C. tulipa* from Narkwa lagoon was associated with high ash and carbohydrate contents, whereas Densu estuary was associated with high protein, fat and energy contents. However, the proximate nutritional properties of *C. tulipa* from the three water bodies were not significantly different (PERMANOVA; $F = 0.62$ $R^2 = 0.018$, $P = 0.622$).

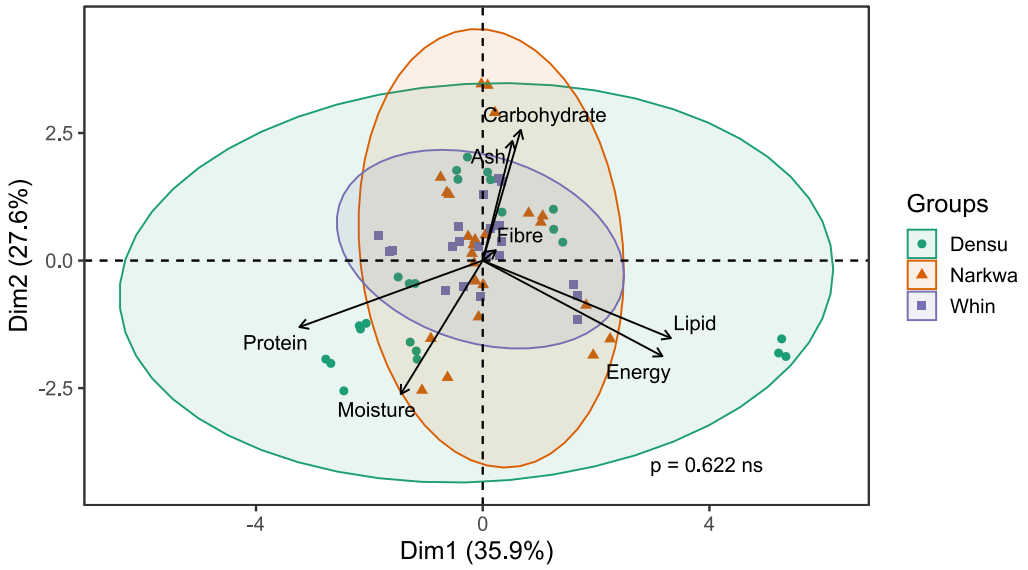

Figure 10: Proximate nutritional composition of *C. tulipa* ordered by principal component analysis according to their location (Densu estuary, Narkwa lagoon and Whin estuary). P value represents statistical comparisons in *C. tulipa* from the three water bodies using PERMANOVA (*ns* = $P > 0.05$)

4.4 Mineral Composition of Oysters

Potassium, sodium, phosphorus, magnesium, calcium, zinc, copper and iron contents of *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary were determined and are illustrated in *Figure 11 and 12*. Potassium, sodium phosphorus, magnesium and calcium have been grouped as macro-minerals (*Figure 11*) and zinc, copper and iron have been grouped as micro-minerals (*Figure 12*).

4.4.1 Macro-mineral contents

On average, calcium concentration (20.53 ± 3.46 mg/g) was the highest of the macro-minerals determined in *C. tulipa* from the various water bodies followed by phosphorus (9.66 ± 0.89 mg/g) and then potassium (3.56 ± 0.51 mg/g). Magnesium concentration (1.27 ± 0.20 mg/g) was the least of the macro-mineral determined after sodium concentration (2.98 ± 0.56 mg/g). *C. tulipa* from Whin estuary recorded the highest potassium (3.59 ± 0.49 mg/g) and phosphorus (9.92 ± 0.91 mg/g) concentrations, whereas *C. tulipa* from Narkwa lagoon recorded the highest calcium (23.05 ± 2.11 mg/g) and sodium (3.12 ± 0.54 mg/g) concentrations. The highest magnesium concentration was obtained by *C. tulipa* from both Whin estuary (1.32 ± 0.19 mg/g) and Narkwa lagoon (1.32 ± 0.22 mg/g). *C. tulipa* from Densu estuary obtained the lowest potassium, phosphorus, calcium and magnesium concentrations, whereas those from Whin estuary recorded the lowest sodium concentration (*Figure 11*).

In general, phosphorus and potassium concentrations were not significantly different (Kruskal-Wallis, $P>0.05$) for *C. tulipa* from the different water bodies;

however, there were significant differences (Kruskal-Wallis, $P<0.05$) in calcium, sodium and magnesium concentrations of *C. tulipa* from the different water bodies.

4.4.2 Micro-mineral contents

On average, zinc concentration was the highest of the micro-minerals determined in *C. tulipa* from the various water bodies followed by iron and then copper. *C. tulipa* from Whin estuary recorded the highest concentrations of all the micro-minerals determined. *C. tulipa* from Narkwa lagoon recorded the lowest concentrations of zinc and copper whereas those from Densu estuary recorded the lowest concentration of iron (*Figure 12*). In general, the concentrations of copper in *C. tulipa* from the various water bodies were not significantly different (Kruskal-Wallis, $P>0.05$). However, there were significant differences in the iron and zinc concentrations of from the different water bodies (Kruskal-Wallis, $P<0.05$).

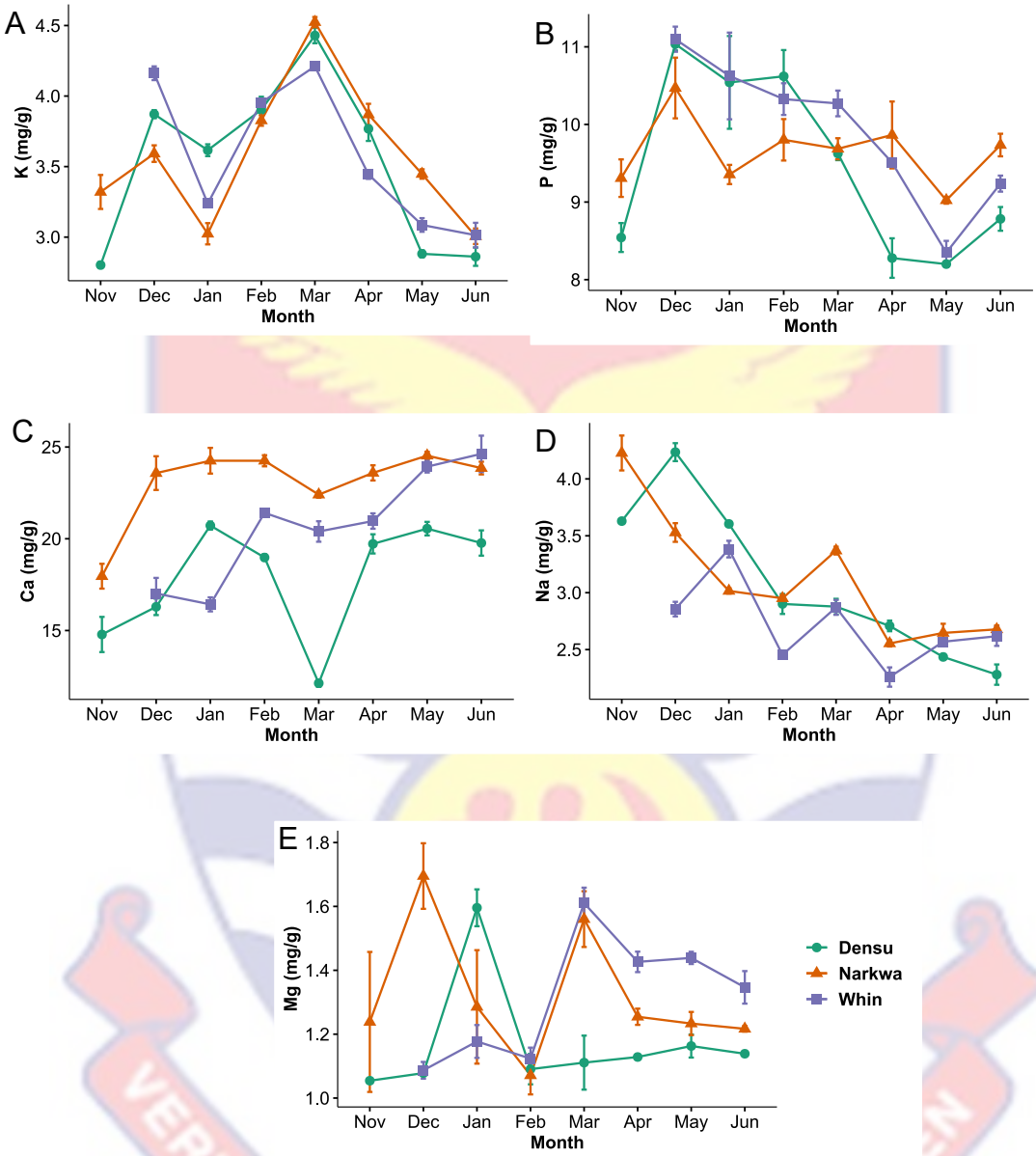

Figure 11: Macro-minerals; **A**. potassium (K) **B.** phosphorus (P) **C.** sodium (Na) **D.** calcium (Ca) and **E.** magnesium (mg) per dry weight of *C. tulipa* sampled monthly (November 2020 to June 2021) from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = standard deviations of the means of triplicate determinations)

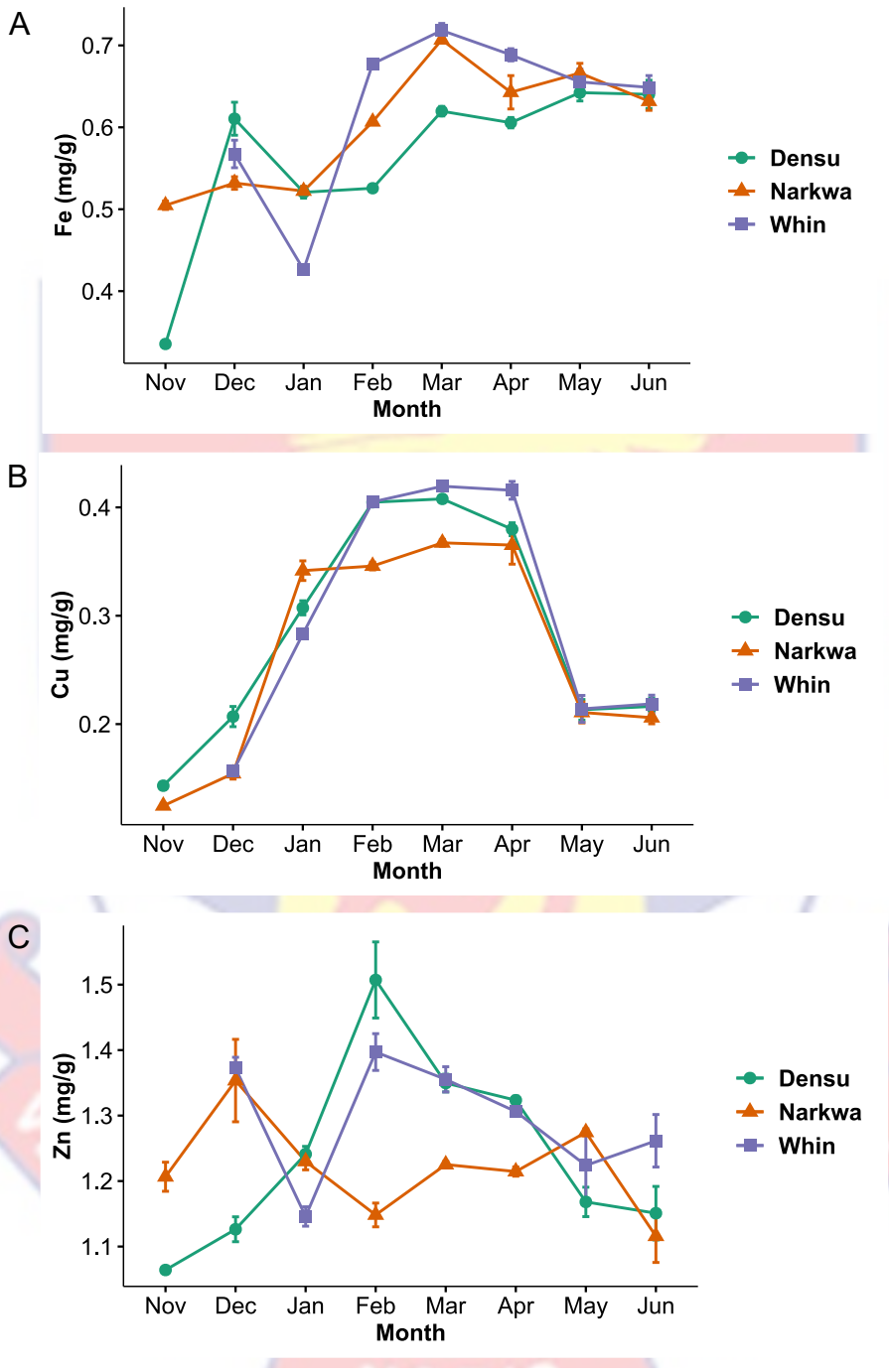

Figure 12: Micro-minerals; **A**. iron (Fe) **B.** copper (Cu) and **C.** zinc (Zn) per dry weight of *C. tulipa* sampled monthly (November 2020 to June 2021) from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = standard deviations of the means of triplicate determinations)

4.4.3 Comparison of *C. tulipa* from different water bodies based on their mineral contents.

Principal component analysis relating mineral concentrations of *C. tulipa* to their water bodies showed that principal component 1 (Dim1) and principal component 2 (Dim2) accounted for 33.2 and 26.1 % of variance in the mineral compositions, respectively (*Figure 13*). There were strong positive contributions of iron, copper, zinc and potassium to Dim1 while in Dim2, there were strong negative contributions of sodium, phosphorus and potassium and a strong positive contribution of calcium. *C. tulipa* from Densu estuary had the largest variability in mineral compositions during the period of study, followed by Whin estuary and then Narkwa lagoon (*Figure 13*). Multivariate analysis revealed that *C. tulipa* from the three water bodies were significantly different based on their mineral contents (PERMANOVA; $F = 11.521$, $R^2 = 0.259$, $P = 0.001$).

4.4.3 Consumption estimates of minerals

The mean mineral concentrations that would be consumed if 109 g wet weight (ww) of oyster is eaten daily were all below the adequate intake or the upper level of daily intake as defined for adults in Australia/New Zealand. Copper and zinc concentrations were the closest to their respective daily limits (*Table 2*).

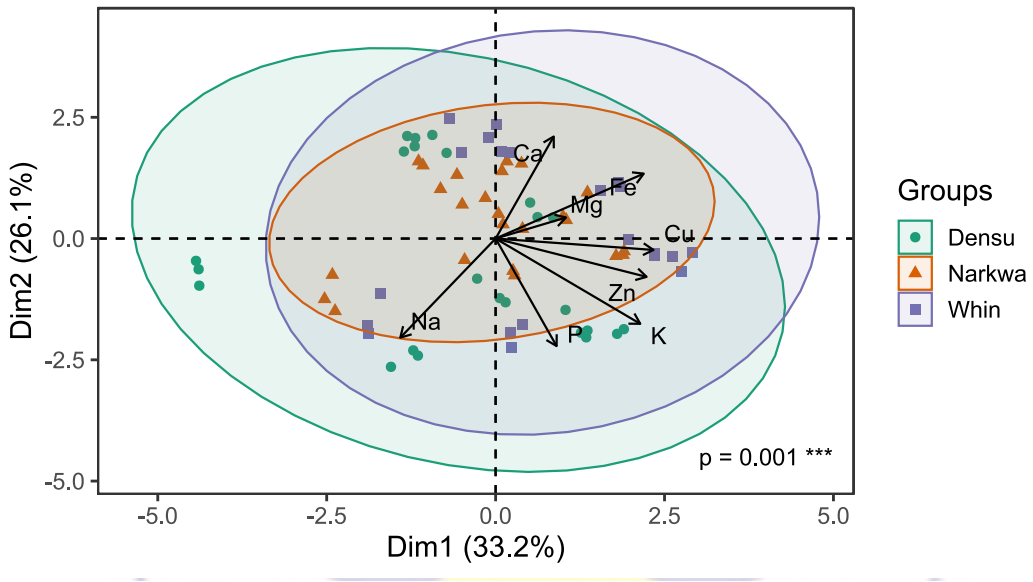

Figure 13: Mineral composition of *C. tulipa* ordered by principal component analysis according to their location (Densu estuary, Narkwa lagoon and Whin estuary). P value represents statistical comparisons of *C. tulipa* from the three water bodies using PERMANOVA (*** = $P \leq 0.001$)

Table 2: *Comparison of the mean mineral composition of C. tulipa to the maximum recommended levels of each mineral.*

Mineral	Mean intake if 109 g ww is consumed per day			**Upper level of daily intake**
	Densu	Narkwa	Whin	
K mg/day	56.27	55.51	60.72	**3800***
Na mg/day	49.35	48.45	45.97	**2300**
P mg/day	151.26	149.87	167.86	**4000**
Mg mg/day	18.72	20.48	22.27	**350**
Ca mg/day	285.91	357.81	350.10	**2500**
Fe mg/day	9.00	9.34	10.60	**45**
Cu mg/day	4.56	4.10	5.11	**10**
Zn mg/day	19.86	18.95	21.91	**40**

* = adequate intake was used instead of the upper level of daily intake because the upper level was not available. Regulated values are for adults and are defined by Australia/New Zealand. Upper level of daily intake is defined as the highest average daily nutrient intake likely to pose no adverse effects.

4.5 Amino acid profile

4.5.1 Amino acid profile of *C. tulipa* from Densu estuary

Total amino acid (TAA) contents of *C. tulipa* from Densu estuary ranged from 10.61 ± 0.3 to 14.95 ± 0.9 g/100g ww (*Table 3*). Total amino acid concentrations were relatively low in January and May 2021 and were relatively high in March, April and June 2021. On average, essential amino acids (His, Arg, Thr, Lys, Met, Val, Iso, Leu and Phe) formed 78 % of the total amino acids with Lys (2.32 ± 0.40 g/100g ww) being the most abundant and Met (0.21 ± 0.03 g/100g ww) being the least abundant essential amino acid. The monthly mean total non-essential amino acids followed a similar trend as those of total essential amino acids; however, the lowest mean of total non-essential amino acids was observed in May 2021, whereas that of the essential amino acids was observed in Jan 2021. Also, the highest mean of total non-essential amino acid was observed in June 2021, whereas that of the essential amino acids was recorded in March 2021. On average, Ser (0.03 ± 0.01 g/100 g ww) was the least abundant non-essential amino acid and Pro (1.07 ± 0.16 g/100 g ww) was the most abundant non-essential amino acid. Delicious amino acids (Gly, Asp & Ala) varied from 0.48 ± 0.03 g/100 g ww to 0.74 ± 0.13 g/100 g ww with the highest being recorded in March 2021 and the lowest in May 2021. The mean essential/non-essential amino acids ratio (EAA/NEAA) was 3.55 ± 0.22 g/100 g ww (*Table 3*).

Table 3: *Monthly mean (± SD) of amino acid composition (g/100g ww) of C. tulipa sampled (from November 2020 – June 2021) from the Densu estuary.*

Amino acid	Month							
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
His	0.22±0.00	0.21±0.01	0.20±0.02	0.20±0.01	0.25±0.01	0.26±0.01	0.20±0.01	0.26±0.01
Arg	1.12±0.04	1.12±0.00	1.05±0.10	1.16±0.11	1.49±0.09	1.33±0.08	0.94±0.02	1.33±0.01
Ser	0.02±0.02	0.02±0.00	0.03±0.00	0.02±0.00	0.03±0.00	0.04±0.01	0.02±0.00	0.05±0.02
Gly	0.21±0.00	0.25±0.03	0.18±0.02	0.24±0.01	0.29±0.00	0.27±0.05	0.18±0.01	0.28±0.01
Asp	0.13±0.00	0.15±0.03	0.21±0.10	0.14±0.02	0.17±0.03	0.17±0.03	0.11±0.01	0.13±0.01
Thr	1.29±0.01	1.28±0.01	0.92±0.07	1.09±0.00	1.50±0.16	1.30±0.08	1.04±0.02	1.29±0.02
Cys	0.20±0.03	0.27±0.01	0.25±0.03	0.28±0.01	0.10±0.00	0.27±0.01	0.20±0.00	0.36±0.03
Pro	0.95±0.01	1.04±0.07	0.90±0.08	1.01±0.00	1.27±0.09	1.21±0.16	0.92±0.12	1.22±0.02
Ala	0.19±0.00	0.20±0.00	0.20±0.03	0.20±0.00	0.28±0.04	0.26±0.04	0.18±0.01	0.25±0.01
Lys	2.01±0.08	2.12±0.02	1.92±0.11	2.26±0.18	2.86±0.18	2.76±0.21	1.91±0.10	2.76±0.07
Tyr	0.72±0.04	0.74±0.01	0.71±0.08	0.77±0.04	0.95±0.05	0.91±0.02	0.68±0.02	0.90±0.02
Met	0.20±0.01	0.19±0.01	0.18±0.03	0.21±0.01	0.25±0.01	0.22±0.01	0.18±0.01	0.27±0.01
Val	0.74±0.05	0.72±0.09	0.64±0.03	0.69±0.04	0.99±0.04	0.92±0.02	0.68±0.04	0.82±0.00
Iso	0.57±0.01	0.58±0.02	0.52±0.05	0.56±0.03	0.72±0.06	0.66±0.03	0.50±0.02	0.67±0.01
Leu	1.14±0.02	1.16±0.03	0.85±0.17	1.20±0.11	1.45±0.14	1.21±0.03	1.02±0.04	1.35±0.07
Phe	2.04±0.16	2.04±0.04	1.86±0.07	1.97±0.31	2.35±0.09	2.10±0.02	1.83±0.08	2.14±0.03
TAA	11.76±0.4	12.09±0.1	10.62±0.6	12.02±0.9	14.95±0.9	13.89±0.6	10.61±0.3	14.08±0.0
EAA	9.33±0.25	9.42±0.11	8.13±0.32	9.34±0.80	11.84±0.6	10.75±0.4	8.31±0.09	10.88±0.1
NEAA	2.44±0.10	2.67±0.01	2.49±0.30	2.67±0.08	3.10±0.27	3.14±0.25	2.30±0.16	3.20±0.07
DAA	0.54±0.00	0.60±0.06	0.59±0.11	0.58±0.03	0.74±0.13	0.70±0.06	0.48±0.03	0.66±0.01
EAA/TAA	0.79±0.00	0.78±0.00	0.77±0.02	0.78±0.01	0.79±0.01	0.77±0.01	0.78±0.01	0.77±0.01
EAA/NEAA	3.83±0.05	3.53±0.03	3.29±0.28	3.49±0.20	3.82±0.14	3.43±0.15	3.62±0.22	3.40±0.10

His = Histidine, Arg = Arginine, Ser = Serine, Gly = Glycine, Asp = Aspartic acid, Thr = Threonine, Cys = Cystiene, Pro = Proline, Ala = Alanine, Lys = Lysine, Tyr = Tyrosine, Met = Methionine, Val = Valine, Iso = Isoleucine, Leu = Leucine, Phe =Phenylalanine. TAA = Total amino acids, EAA = Total essential amino acids, NEAA = Total non-essential amino acids, DAA = Total delicious amino acids (Gly, Asp & Ala). Values are means ± SD of duplicate determinations.

4.5.2 Amino acid profile of *C. tulipa* from Narkwa lagoon

Total amino acid contents of *C. tulipa* from Narkwa lagoon ranged from 8.92 ± 0.7 to 15.18 ± 0.9g/100 g ww (*Table 4*). Total amino acid concentrations increased gradually from November 2020 to April 2021 after which the concentrations plateaued. On average, essential amino acids formed 77 % of the total amino acids with Lys (2.31 ± 0.52 g/100 g ww) being the most abundant and Met (0.21 ± 0.04 g/100 g ww) being the least abundant essential amino acid. The monthly mean of total non-essential amino acids and essential amino acids followed a similar trend as that of total amino acids. Ser (0.03 ± 0.01 g/100 g ww) was the least abundant non-essential amino acid and Pro (1.04 ± 0.24 g/100 g ww) was the most abundant non-essential amino acid. Delicious amino acids (Gly, Asp & Ala) also varied from 0.45 ± 0.02 g/100 g ww to 0.78 ± 0.16 g/100 g ww with the highest being observed in March 2021 and the lowest in November 2020. The mean essential/non-essential amino acids ratio was 3.44 ± 0.19 g/100 g ww.

Table 4: *Monthly mean (± SD) of amino acid composition (g/100g ww) of C. tulipa sampled (from November 2020 – June 2021) from the Narkwa lagoon.*

Amino acid	Month							
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
His	0.16±0.01	0.18±0.02	0.19±0.01	0.22±0.01	0.24±0.01	0.29±0.02	0.25±0.00	0.26±0.02
Arg	0.90±0.07	1.01±0.00	0.85±0.04	1.15±0.00	1.31±0.02	1.48±0.08	1.50±0.00	1.50±0.03
Ser	0.02±0.01	0.02±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.05±0.01	0.03±0.01	0.04±0.02
Gly	0.16±0.00	0.17±0.00	0.17±0.02	0.23±0.01	0.38±0.15	0.30±0.01	0.25±0.02	0.29±0.03
Asp	0.14±0.01	0.16±0.05	0.14±0.03	0.13±0.01	0.17±0.02	0.17±0.01	0.15±0.01	0.15±0.02
Thr	0.67±0.05	0.81±0.01	0.70±0.01	0.97±0.04	1.15±0.06	1.37±0.02	1.12±0.01	1.21±0.07
Cys	0.23±0.03	0.26±0.03	0.24±0.01	0.33±0.01	0.30±0.04	0.35±0.07	0.18±0.02	0.30±0.01
Pro	0.73±0.05	0.82±0.03	0.75±0.03	1.03±0.07	1.21±0.14	1.23±0.13	1.24±0.10	1.30±0.09
Ala	0.16±0.01	0.18±0.00	0.15±0.00	0.19±0.01	0.23±0.01	0.29±0.02	0.25±0.01	0.26±0.02
Lys	1.63±0.12	1.82±0.07	1.65±0.01	2.32±0.00	2.65±0.10	2.79±0.23	2.69±0.07	2.92±0.18
Tyr	0.62±0.04	0.72±0.04	0.66±0.06	0.77±0.01	0.89±0.02	1.00±0.08	0.97±0.05	0.96±0.05
Met	0.17±0.00	0.16±0.02	0.15±0.00	0.21±0.00	0.23±0.02	0.23±0.00	0.26±0.01	0.27±0.02
Val	0.52±0.01	0.62±0.05	0.56±0.02	0.66±0.01	0.76±0.03	0.87±0.05	0.93±0.02	0.87±0.03
Iso	0.43±0.05	0.47±0.03	0.51±0.04	0.56±0.01	0.64±0.04	0.76±0.05	0.71±0.02	0.70±0.05
Leu	0.86±0.03	0.93±0.07	0.97±0.01	1.20±0.02	1.32±0.11	1.48±0.17	1.50±0.07	1.42±0.18
Phe	1.53±0.25	1.61±0.49	2.05±0.25	2.15±0.23	2.16±0.00	2.50±0.04	2.33±0.05	2.26±0.12
TAA	8.92±0.7	9.96±0.6	9.78±0.4	12.16±0.0	13.65±0.4	15.18±0.9	14.33±0.3	14.69±0.9
EAA	6.88±0.58	7.63±0.60	7.64±0.37	9.44±0.15	10.45±0.4	11.78±0.6	11.27±0.1	11.39±0.7
NEAA	2.05±0.13	2.33±0.03	2.14±0.03	2.72±0.11	3.21±0.03	3.39±0.28	3.06±0.22	3.29±0.20
DAA	0.45±0.02	0.51±0.05	0.45±0.02	0.56±0.03	0.78±0.16	0.76±0.00	0.65±0.05	0.69±0.07
EAA/TAA	0.77±0.00	0.77±0.02	0.78±0.01	0.78±0.01	0.76±0.01	0.78±0.01	0.79±0.01	0.78±0.00
EAA/NEAA	3.35±0.07	3.27±0.31	3.57±0.12	3.47±0.20	3.26±0.09	3.48±0.11	3.69±0.23	3.46±0.00

His = Histidine, Arg = Arginine, Ser = Serine, Gly = Glycine, Asp = Aspartic acid, Thr = Threonine, Cys = Cystiene, Pro = Proline, Ala = Alanine, Lys = Lysine, Tyr = Tyrosine, Met = Methionine, Val = Valine, Iso = Isoleucine, Leu = Leucine, Phe =Phenylalanine. TAA = Total amino acids, EAA = Total essential amino acids, NEAA = Total non-essential amino acids, DAA = Total delicious amino acids (Gly, Asp & Ala). Values are means ± SD of duplicate determinations.

4.5.3 Amino acid profile of *C. tulipa* from Whin estuary

Total amino acid contents of *C. tulipa* from Whin estuary ranged from 10.44 ± 0.37to 16.15 ± 0.82 g/100 g ww (*Table 5*). Total amino acid concentrations peaked twice in January and May 2021; however, the highest total amino acid concentration was recorded in January 2021, whereas the lowest was recorded in December 2020. On average, essential amino acids formed 78 % of the total amino acids with Lys (2.56 ± 0.44 g/100 g ww) being the most abundant and Met (0.23 ± 0.05 g/100 g ww) being the least abundant essential amino acid. The monthly mean of total non-essential amino acids and essential amino acids followed a similar trend as that of total amino acids. Ser (0.04 ± 0.01 g/100 g ww) was the least abundant non-essential amino acid and Pro (1.17 ± 0.23 g/100 g ww) was the most abundant non-essential amino acid. Delicious amino acids (Gly, Asp & Ala) also varied from 0.50 ± 0.01 g/100g ww to 0.72 ± 0.05 g/100g ww with the lowest being recorded in December 2020 and the highest in May 2021. The mean essential/non-essential amino acids ratio was 3.48 ± 0.15 g/100g ww.

Table 5: *Monthly mean (± SD) of amino acid composition (g/100g ww) of C. tulipa sampled (from November 2020 – June 2021) from the Whin estuary.*

Amino acid	Month							
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
His		0.19±0.01	0.20±0.02	0.27±0.03	0.22±0.01	0.25±0.01	0.29±0.00	0.24±0.00
Arg		1.02±0.03	1.15±0.10	1.61±0.09	1.35±0.03	1.37±0.06	1.57±0.03	1.19±0.03
Ser		0.03±0.00	0.03±0.01	0.03±0.01	0.04±0.01	0.04±0.01	0.04±0.00	0.05±0.03
Gly		0.19±0.01	0.21±0.03	0.27±0.02	0.23±0.01	0.30±0.00	0.30±0.03	0.26±0.01
Asp		0.13±0.00	0.13±0.01	0.16±0.03	0.18±0.05	0.15±0.01	0.17±0.02	0.12±0.01
Thr		1.00±0.05	1.14±0.07	1.40±0.08	1.31±0.03	1.40±0.02	1.31±0.02	1.18±0.06
Cys		0.26±0.02	0.24±0.02	0.41±0.05	0.33±0.00	0.25±0.00	0.40±0.02	0.33±0.00
Pro		0.85±0.02	0.91±0.03	1.43±0.15	1.12±0.08	1.28±0.01	1.44±0.01	1.14±0.02
Ala		0.18±0.00	0.20±0.03	0.24±0.02	0.21±0.00	0.25±0.00	0.26±0.00	0.23±0.00
Lys		1.86±0.06	2.13±0.08	3.08±0.16	2.56±0.06	2.76±0.05	3.04±0.03	2.46±0.02
Tyr		0.68±0.01	0.77±0.08	1.05±0.07	0.91±0.06	0.93±0.00	1.10±0.01	0.82±0.03
Met		0.17±0.00	0.19±0.01	0.28±0.01	0.22±0.02	0.25±0.01	0.30±0.02	0.23±0.01
Val		0.57±0.02	0.72±0.13	0.89±0.05	0.84±0.12	0.86±0.02	0.94±0.01	0.74±0.04
Iso		0.49±0.01	0.58±0.07	0.78±0.06	0.66±0.04	0.69±0.01	0.80±0.01	0.61±0.01
Leu		1.04±0.01	1.15±0.09	1.67±0.09	1.31±0.00	1.39±0.03	1.53±0.17	1.17±0.06
Phe		1.80±0.17	1.98±0.16	2.56±0.09	1.97±0.36	2.38±0.24	2.50±0.08	2.02±0.10
TAA		10.44±0.37	11.73±0.50	16.15±0.82	13.47±0.25	14.56±0.38	15.99±0.03	12.80±0.25
EAA		8.13±0.31	9.24±0.39	12.55±0.47	10.46±0.27	11.37±0.40	12.28±0.04	9.84±0.22
NEAA		2.31±0.06	2.49±0.11	3.60±0.35	3.02±0.03	3.19±0.02	3.71±0.01	2.96±0.03
DAA		0.50±0.01	0.55±0.07	0.68±0.07	0.62±0.04	0.70±0.01	0.72±0.05	0.62±0.00
EAA/TAA		0.78±0.00	0.79±0.00	0.78±0.01	0.78±0.01	0.78±0.01	0.77±0.00	0.77±0.00
EAA/NEAA		3.52±0.04	3.71±0.01	3.49±0.21	3.47±0.12	3.56±0.15	3.31±0.02	3.33±0.04

His = Histidine, Arg = Arginine, Ser = Serine, Gly = Glycine, Asp = Aspartic acid, Thr = Threonine, Cys = Cystiene, Pro = Proline, Ala = Alanine, Lys = Lysine, Tyr = Tyrosine, Met = Methionine, Val = Valine, Iso = Isoleucine, Leu = Leucine, Phe =Phenylalanine. TAA = Total amino acids, EAA = Total essential amino acids, NEAA = Total non-essential amino acids, DAA = Total delicious amino acids (Gly, Asp & Ala). Values are means ± SD of duplicate determinations.

4.5.4 Comparison of amino acid profiles of *C. tulipa* from the three water bodies.

In general, the amino acid profiles of *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary were similar. There were no significant differences between the total amino acid, total essential amino acid, total non-essential amino and delicious amino acid concentrations of *C. tulipa* from the three water bodies (Kruskal-Wallis, $P>0.05$). Principal component analysis relating amino acid concentrations of *C. tulipa* to their water bodies showed that principal component 1 (Dim1) and principal component 2 (Dim2) accounted for 71.7 and 7.1 % of variance in the amino acid compositions, respectively (*Figure 14*). Alanine, proline, tyrosine and all the essential amino acids were positively correlated and they contributed positively to principal component 1 while in principal component 2, there were strong positive contributions of cysteine and serine, which were also positively correlated. *C. tulipa* from Narkwa lagoon had the largest variability in amino acids that contributed to Dim1, followed by Whin estuary, whereas *C. tulipa* from Densu estuary had the largest variability in amino acids that contributed to Dim2 during the period of study. Multivariate comparison revealed that the amino acid compositions of *C. tulipa* from the three water bodies were not significantly different (PERMANOVA; $F = 1.76$, $R^2 = 0.076$ $P = 0.171$).

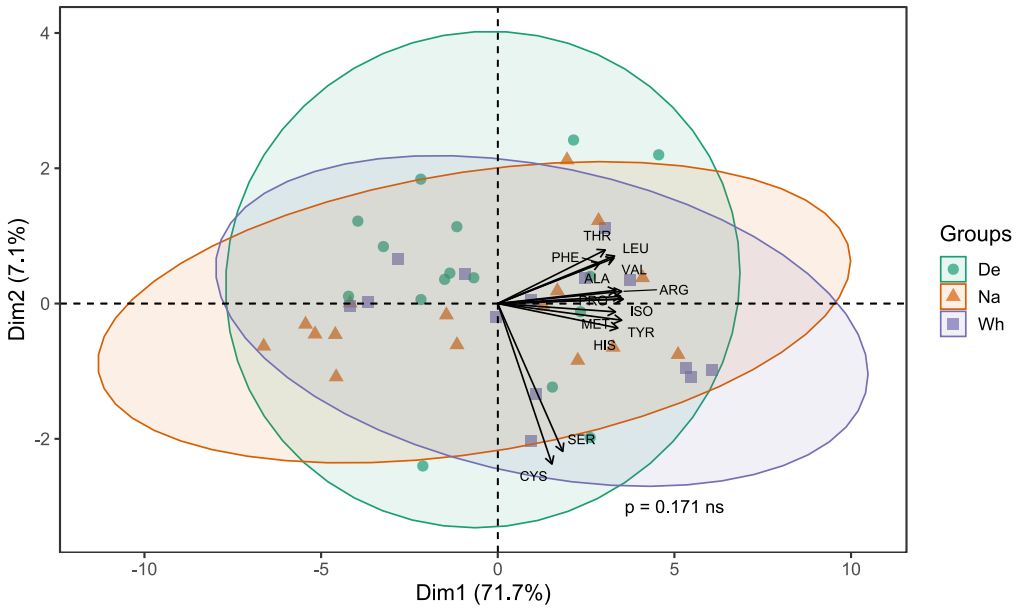

Figure 14: Amino acid composition of *C. tulipa* ordered by principal component analysis according to their location (Densu estuary = De, Narkwa lagoon = Na and Whin estuary = Wh). Only variables with strong contributions to the principal components were shown on the figure. *P*-value represents statistical comparisons between amino acids of *C. tulipa* from the three water bodies using PERMANOVA (*ns = not significant*)

4.5.5 Protein quality of *C. tulipa* based on their amino acids profile

Essential amino acid concentrations of *C. tulipa* were all above the recommended amino acid pattern for adults as defined by FAO/WHO/UNU. Amino acid score (AAS) ranges were 101.6 – 506.3 %, 104.9 – 535.6 % and 101.3 – 519.9 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. Histidine recorded the least AAS, whereas Phenylalanine + Tyrosine recorded the highest AAS. There was no limiting amino acid and the chemical scores were 101.6, 104.9 and 101.3 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. The essential amino acid index (EAAI) values were 117.6, 119.3 and 121.5 %, whereas the biological values (BV) were 116.5, 118.4 and 120.7 % for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. *C. tulipa* from Narkwa lagoon recorded the highest chemical score, whereas those from Whin estuary recorded the highest EAAI and BV.

Table 6: *Mean of protein quality scores for C. tulipa from Densu estuary, Narkwa lagoon and Whin estuary.*

Amino acid	FAO/WHO pattern mg/g protein	AAS (%)		
		Densu	Narkwa	Whin
His	15	101.6	104.9	101.3
Ile	30	134.3	140.2	141.4
Leu	59	134.6	143.9	144.5
Lys	45	349.6	360.1	365.8
Met + Cys	22	140.5	153.7	161.5
Phe + Tyr	38	506.3	535.6	519.9
Thr	23	357.4	305.4	350.0
Val	39	134.2	130.2	131.2
Chemical score (%)	-	101.6	104.9	101.3
EAAI (%)	-	117.6	119.3	121.5
BV (%)	-	116.5	118.4	120.7

AAS = Amino acid score, EAAI = essential amino acid index & BV = Biological value. $\%\ AAS = \frac{amino\ concentration\ in\ dw\ of\ Oyster}{reference\ amino\ acid\ concentration} \times 100$

4.6 Effect of environmental factors of water bodies on the nutritional properties of *C. tulipa.*

Multiple regression analyses showing the effects of environmental factors of the various water bodies on the nutritional properties of *C. tulipa* are shown in *Tables 4.6, 4.7 & 4.8*. pH, temperature, precipitation, salinity and chlorophyll-a were the environmental factors used as predictors in the regression analyses. Dissolved oxygen was excluded to prevent multicollinearity because it was highly correlated with chlorophyll-a ($p < 0.0005$). Precipitation data are shown in Appendix A.

4.6.1 Relationship of environmental variables with meat yield and proximate nutritional composition.

There were significant relationships of the environmental factors with meat yield ($F_{(5,17)} = 3.249$, $p = 0.031$, $R^2 = 0.489$), moisture ($F_{(5,17)} = 4.003$, $p = 0.041$, $R^2 = 0.541$) and ash ($F_{(5,17)} = 2.997$, $p = 0.040$, $R^2 = 0.469$). Temperature was the most significant predictor of meat yield ($p = 0.033$) and there was a negative relationship between temperature and the meat yield of *C. tulipa*. The most significant predictors of moisture and ash contents of *C. tulipa* were pH and chlorophyll-a ($p < 0.05$). pH and chlorophyll-a contributed negatively and positively to moisture, while on the other hand, showed opposite contributions to the ash content of *C. tulipa,* respectively. The results also showed a significant positive relationship of chlorophyll-a with the protein content of *C. tulipa;* however, the multiple regression model was not significant ($F_{(5,17)} = 1.774$, $p = 0.172$, $R^2 = 0.343$) (*Table 7*). Non-significant models were also recorded for the fat ($F_{(5,17)} = 1.615$, $p = 0.210$, $R^2 =$

0.332), fibre ($F_{(5,17)} = 2.033$, $p = 0.125$, $R^2 = 0.374$), carbohydrate ($F_{(5,17)} = 0.728$, $p = 0.612$, $R^2 = 0.176$) and energy ($F_{(5,17)} = 1.815$, $p = 0.163$, $R^2 = 0.348$) content of *C. tulipa.*

4.6.2 Relationship of environmental variables with mineral composition.

There were significant relationships of the environmental factors with sodium ($F_{(5,17)} = 5.260$, $p = 0.004$, $R^2 = 0.607$), iron ($F_{(5,17)} = 3.535$, $p = 0.023$, $R^2 = 0.510$) and zinc ($F_{(5,17)} = 3.095$, $p = 0.036$, $R^2 = 0.476$) contents of *C. tulipa*. pH ($p < 0.0005$) and salinity ($p = 0.009$) were the most significant predictors of sodium and they showed a negative and positive relationship with the sodium content of *C. tulipa*, respectively. pH also showed significant positive relationships with the iron and zinc contents of *C. tulipa* ($p < 0.005$) (*Table 8*). Non-significant models were recorded for phosphorus ($F_{(5,17)} = 1.564$, $p = 0.223$, $R^2 = 0.315$), potassium ($F_{(5,17)} = 1.051$, $p = 0.421$, $R^2 = 0.236$), calcium ($F_{(5,17)} = 1.830$, $p = 0.160$, $R^2 = 0.350$), magnesium ($F_{(5,17)} = 0.210$, $p = 0.953$, $R^2 = 0.058$) and copper ($F_{(5,17)} = 1.588$, $p = 0.217$, $R^2 = 0.318$).

Table 7: *Partial coefficients of multiple regression showing the relationship of environmental factors with the meat yield and proximate composition of C. tulipa.*

Nutritional Property	Term	Coef	SE Coef	T-value	P-value
Meat yield	(Constant)	50.225	10.390	4.834	0.000
	pH	-2.236	1.134	-1.971	0.065
	Temperature	-0.701	0.302	-2.320	**0.033***
	Precipitation	0.007	0.010	0.763	0.456
	Salinity	0.036	0.089	0.401	0.693
	Chlorophyll-a	0.062	0.143	0.434	0.669
Moisture	(Constant)	120.218	9.786	12.284	0.000
	pH	-2.755	1.069	-2.579	**0.020***
	Temperature	-0.549	0.285	-1.928	0.071
	Precipitation	-0.013	0.009	-1.403	0.178
	Salinity	0.104	0.084	1.237	0.233
	Chlorophyll-a	0.412	0.135	3.051	**0.007***
Ash	(Constant)	-4.063	1.901	-2.138	0.047
	pH	0.483	0.208	2.329	**0.032***
	Temperature	0.066	0.055	1.185	0.252
	Precipitation	0.001	0.002	0.722	0.480
	Salinity	-0.005	0.016	-0.280	0.783
	Chlorophyll-a	-0.073	0.026	-2.786	**0.013***
Protein	(Constant)	56.799	14.759	3.848	0.001
	pH	-0.702	1.611	-0.436	0.669
	Temperature	-0.167	0.429	-0.388	0.703
	Precipitation	-0.007	0.014	-0.550	0.590
	Salinity	0.131	0.126	1.037	0.314
	Chlorophyll-a	0.533	0.203	2.622	**0.018***

Models with at least a significant predictor were shown. * signifies p-value < 0.05.

Table 8: *Partial coefficients of multiple regression showing the relationship of environmental factors with the mineral proximate composition of C. tulipa.*

Nutritional Property	Term	Coef	SE Coef	T-value	P-value
Sodium (Na)	(Constant)	8.823	2.472	3.569	0.002
	pH	-1.341	0.270	-4.970	**0.000***
	Temperature	0.099	0.072	1.373	0.188
	Precipitation	-0.002	0.002	-0.738	0.471
	Salinity	0.063	0.021	2.965	**0.009***
	Chlorophyll-a	0.070	0.034	2.046	0.057
Iron (Fe)	(Constant)	-0.710	0.455	-1.561	0.137
	pH	0.159	0.050	3.211	**0.005***
	Temperature	0.004	0.013	0.313	0.758
	Precipitation	0.000	0.000	0.766	0.454
	Salinity	0.000	0.004	-0.124	0.903
	Chlorophyll-a	-0.013	0.006	-2.099	0.051
Copper (Cu)	(Constant)	-1.092	0.514	-2.125	0.049
	pH	0.159	0.056	2.843	**0.011***
	Temperature	0.004	0.015	0.250	0.805
	Precipitation	6.1E-05	0.000	0.129	0.899
	Salinity	0.001	0.004	0.268	0.792
	Chlorophyll-a	0.005	0.007	0.644	0.528

Models with at least a significant predictor were shown. * Signifies p-value < 0.05.

4.6.3 Relationship of environmental variables with amino acid composition.

Total amino acids (TAA), essential amino acids (EAA) and non-essential amino acids (NEAA) were used for the multiple regression models instead of the individual amino acids because most of the amino acids were correlated in the principal component analysis (*Figure 14*). There were significant relationships of the environmental factors with TAA ($F_{(5,17)} = 14.336$, $p < 0.0005$, $R^2 = 0.808$), EAA ($F_{(5,17)} = 13.975$, $p < 0.0005$, $R^2 = 0.804$) and NEAA ($F_{(5,17)} = 12.298$, $p = < 0.0005$, $R^2 = 0.783$). pH, temperature, precipitation and chlorophyll-a were all significant predictors of TAA, EAA and NEAA ($p < 0.05$). Temperature, pH and precipitation showed positive relationships, whereas chlorophyll-a showed a negative relationship the TAA, EAA and NEAA of *C. tulipa* (*Table 9*).

Table 9: *Partial coefficients of multiple regression showing the relationship of environmental factors with the amino acid composition of C. tulipa.*

Nutritional Property	Term	Coef	SE Coef	T-value	P-value
EAA	(Constant)	-27.953	5.052	-5.533	0.000
	pH	3.200	0.552	5.800	**0.000***
	Temperature	0.489	0.147	3.327	**0.004***
	Precipitation	0.012	0.005	2.517	**0.022***
	Salinity	-0.060	0.043	-1.377	0.186
	Chlorophyll-a	-0.268	0.070	-3.845	**0.001***
NEAA	(Constant)	-8.115	1.543	-5.260	0.000
	pH	0.842	0.168	4.997	**0.000***
	Temperature	0.157	0.045	3.502	**0.003***
	Precipitation	0.004	0.001	2.514	**0.022***
	Salinity	-0.009	0.013	-0.679	0.506
	Chlorophyll-a	-0.079	0.021	-3.714	**0.002***
TAA	(Constant)	-36.073	6.414	-5.624	0.000
	pH	4.049	0.700	5.781	**0.000***
	Temperature	0.645	0.187	3.455	**0.003***
	Precipitation	0.015	0.006	2.587	**0.019***
	Salinity	-0.069	0.055	-1.254	0.227
	Chlorophyll-a	-0.347	0.088	-3.918	**0.001***

* signifies p value < 0.05. EAA = total essential amino acids, NEAA = total non-essential amino acids and TAA = Total amino acids

4.7 Medicinal properties of *C. tulipa*

Phenolic contents, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities and total antioxidant capacities of different solvent (70 % ethanol, 70 % methanol, absolute methanol and absolute hexane) extracts of *C. tulipa* from the different water bodies are shown in *Figure 16, 17 and 18.* The percentage yields of the various extracts have been shown in *Figure 15*. Fatty acid profile of extracted oils from *C. tulipa* are also shown in *Table 10.*

4.7.1 Percentage yield of extracts

For *C. tulipa* from the three water bodies, absolute methanol (28.18 % - 33.86 %) yielded the highest amount of extract, followed by 70 % ethanol (25.35 % - 30.00 %) and then 70 % methanol (16.01 % - 25.73 %) with absolute hexane (2.57 % - 4.37 %) recording the least yield. *C. tulipa* from Densu estuary recorded the highest yield when methanol and 70 % methanol were used for the extraction, whereas *C. tulipa* from Narkwa lagoon and Whin estuary recorded the highest yield for 70 % ethanol and hexane, respectively (*Figure 15*).

4.7.2 Phenolic content of extracts

Using gallic acid as standard, the phenolic contents of the different extracts of *C. tulipa* were determined from the regression equation, $y = 0.9996x - 0.0269$ with an $R^2 = 0.995$ (Appendix E). The phenolic contents were expressed as gallic acid equivalents (GAE) per gram of extract. In general, phenolic content was highest for 70 % ethanol extracts as compared to those of the other extracts of *C. tulipa* from the various water bodies. The second highest phenolic content was recorded for 70 % methanol, followed by methanol and the lowest was recorded for

hexane extracts. Ranges of phenolic contents were 40.55 ± 1.82 – 10.64 ± 0.31 mg GAE/g, 31.45 ± 0.65 – 8.55 ± 0.18 mg GAE/g and 61.05 ± 0.31 – 10.32 ± 0.07 mg GAE/g for *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary, respectively. With the exception of the 70 % ethanol extract where *C. tulipa* from Whin estuary recorded the highest phenolic content, *C. tulipa* from Densu estuary recorded the highest phenolic content for all the extracts (*Figure 16*).

4.7.3 DPPH scavenging activity of extracts

All the extracts showed DPPH scavenging activities which were concentration-dependent ($P < 0.05$) and are shown in *Figure 17*. Overall, 70 % ethanol showed the highest DPPH scavenging activity. In all the extracts, *C. tulipa* from Whin estuary showed the highest DPPH scavenging activity. For the 70 % and absolute methanol extracts, *C. tulipa* from Densu estuary recorded the lowest DPPH scavenging activity, whereas for 70 % ethanol and hexane, *C. tulipa* from Narkwa lagoon showed the lowest DPPH scavenging activity. The DPPH scavenging activity of the extracts were all lower than that of the standard (Ascorbic acid), however, DPPH scavenging activity of the 70 % ethanolic extract of *C. tulipa* from Whin estuary was closer to that of the standard.

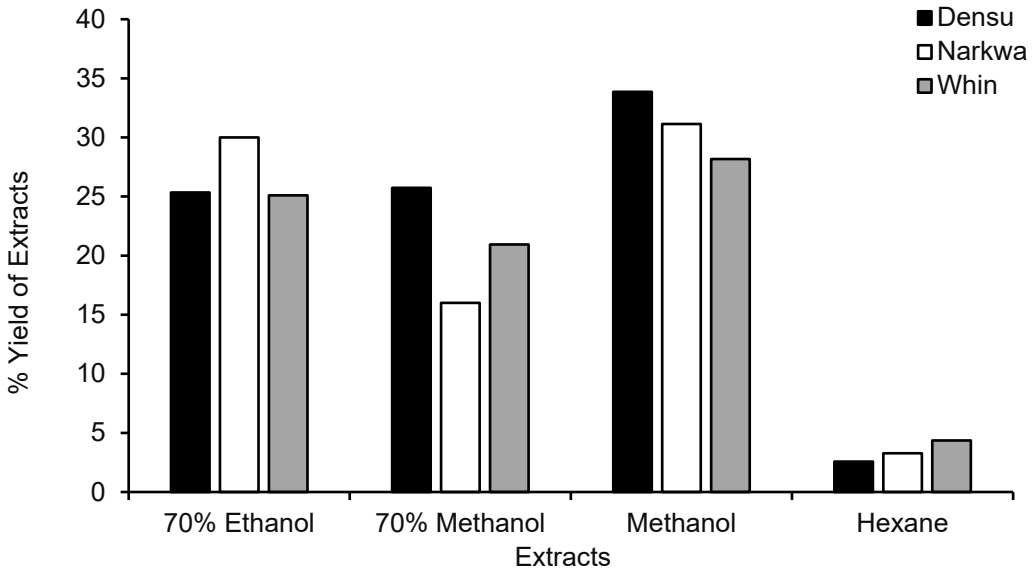

Figure 15: Percentage yield of different solvent extracts of *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary.

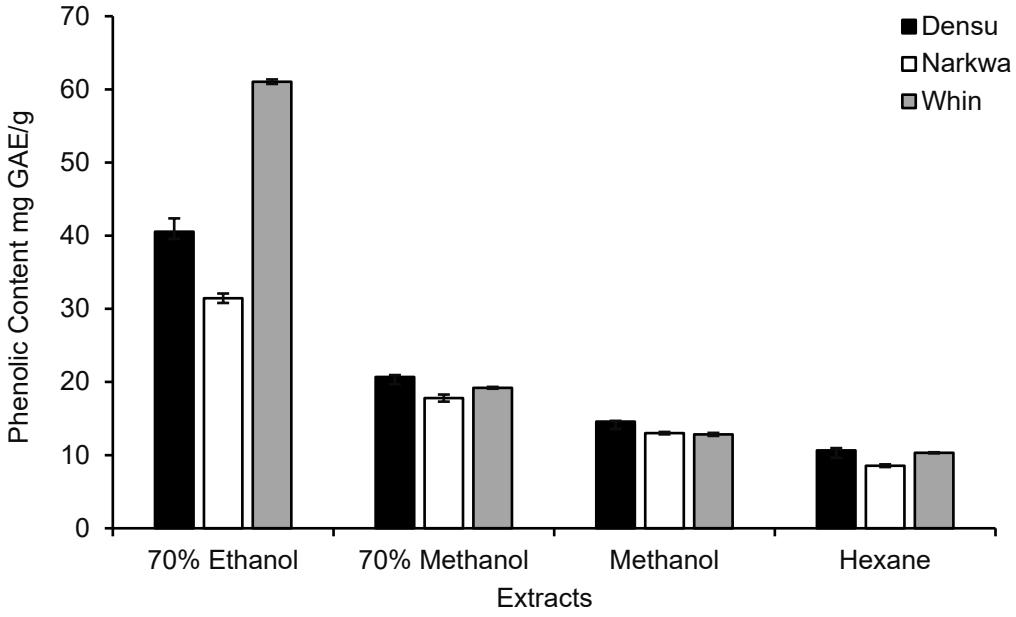

Figure 16: Phenolic content of different solvent extracts of *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = standard deviations of the means of duplicate determinations)

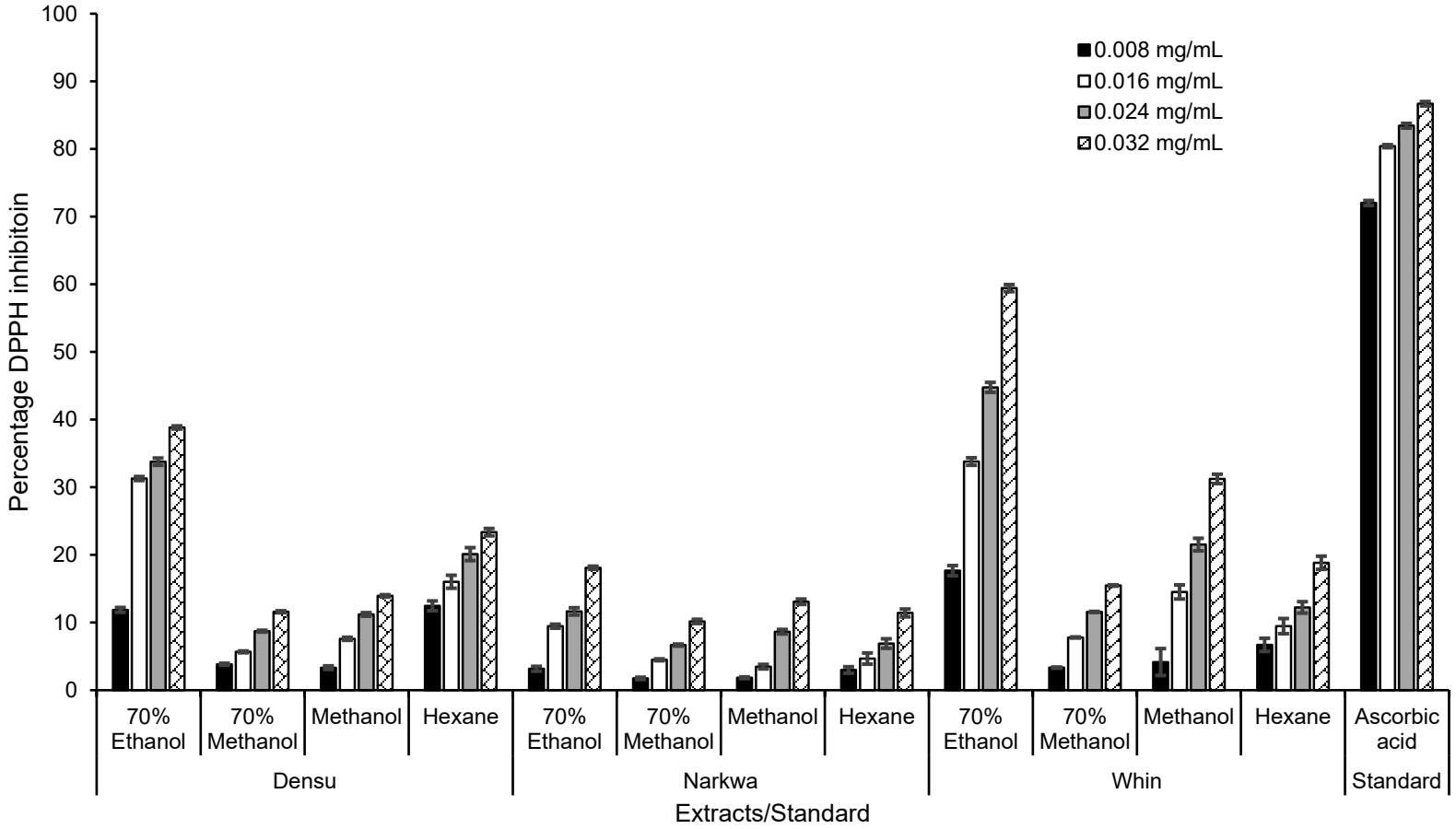

Figure 17: Percentage DPPH inhibition of different solvent extracts of *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = standard deviations of the means of triplicate determinations)

4.7.4 Total Antioxidant Capacity

Using ascorbic acid as standard, the total antioxidant capacity of the different extracts of *C. tulipa* were determined from the regression equation, y = 7.895x - 0.0471 with an R^2 = 0.99 (Appendix F). The total antioxidant capacities of the extracts are expressed as ascorbic acid equivalents (AAE) per gram of extract. *C. tulipa* from different water bodies showed different trends in the total antioxidant capacity of their extracts as follows; hexane > 70 % ethanol > methanol > 70 % methanol for *C. tulipa* from Densu estuary, methanol > hexane > 70 % methanol > 70 % ethanol for *C. tulipa* from Densu estuary and hexane > 70 % ethanol > 70 % methanol > methanol for *C. tulipa* from Whin estuary, respectively. Overall, the hexane extract of *C. tulipa* from Whin estuary recorded the highest total antioxidant capacity (354.16 ± 11.05 mg AAE/g), whereas the 70 % methanol extract of *C. tulipa* from Densu estuary recorded the lowest (64.85 ± 5.83 mg AAE/g) (*Figure 18*).

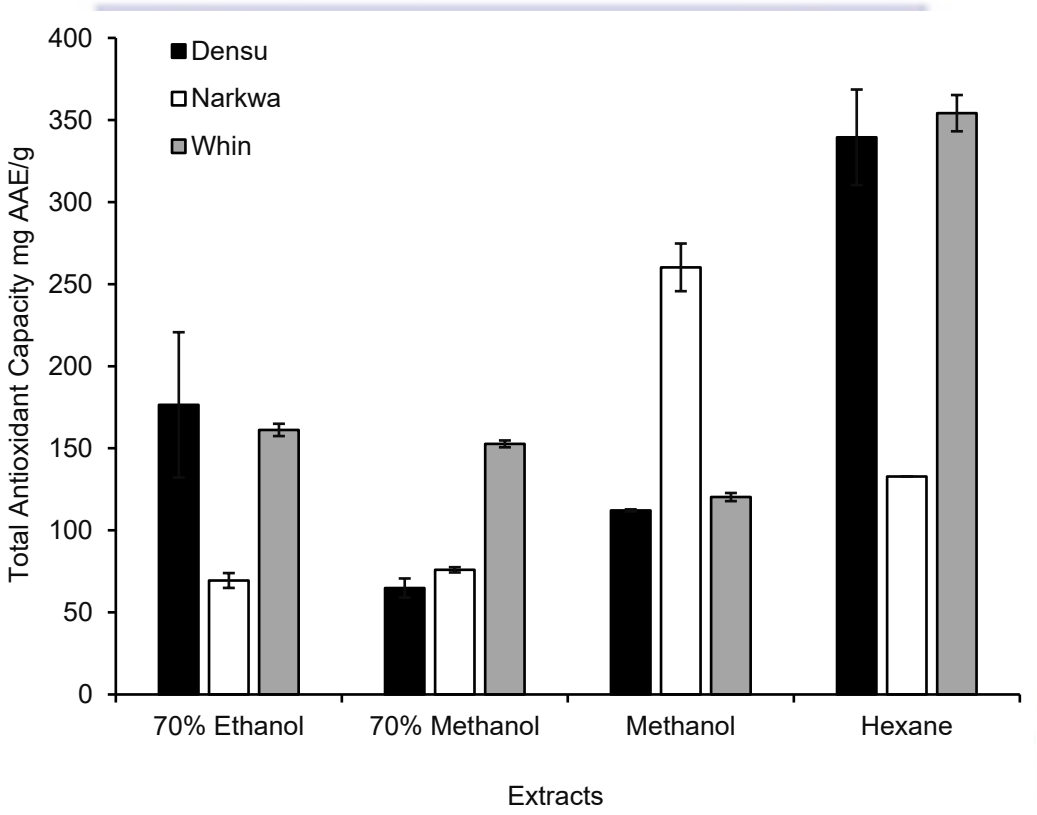

Figure 18: Total antioxidant capacity of different solvent extracts of *C. tulipa* from Densu estuary, Narkwa lagoon and Whin estuary. (Error bars = standard deviations of the means of duplicate determination)

4.7.5 Fatty Acid Profile

A total of 16 fatty acids were identified from the mass spectra (Appendix H) of *C. tulipa* oils and are shown in *Table 10,* however, docosapentaenoic acid was not identified for *C. tulipa* from Narkwa lagoon. Hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid) were the most abundant saturated fatty acids found in *C. tulipa* from all the three water bodies, whereas, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were the most abundant unsaturated fatty acids. The percentage proportion of unsaturated fatty acids in *C. tulipa* from the various water bodies were higher than those of their saturated fatty acids, however, *C. tulipa* from Narkwa lagoon had higher amounts of saturated fatty acids than their unsaturated fatty acids. Polyunsaturated fatty acids were also higher than monounsaturated acids in *C. tulipa* from all the water bodies.

With the exception of 9-hexadecenoic (palmitoleic acid), 13-docosenoic acid (erucic acid) and docosapentaenoic acid (DPA), there were no significant differences in the fatty acid composition of *C. tulipa* from the different water bodies (ANOVA, $P > 0.05$). Monounsaturated fatty acids in *C. tulipa* from Whin estuary (26.39 ± 1.73 %) were significantly higher than those from Densu estuary (18.60 ± 0.98 %) and Narkwa lagoon (16.38 ± 0.40 %) (ANOVA, $P < 0.05$), however, saturated (42.68 ± 0.34 – 56.55 ± 12.87 %) and polyunsaturated (27.08 ± 13.27 % – 36.18 ± 3.31 %) fatty acids were statistically similar (ANOVA, $P > 0.05$). DHA/EPA ratios were higher in *C. tulipa* from Densu estuary (1.88 ± 1.09 %) and Narkwa lagoon (1.38 ± 0.30 %) than in those from Whin estuary (0.74 ± 0.04 %). The atherogenic index of *C. tulipa* from Narkwa lagoon (0.69 ± 0.33) was also

higher than those of Densu (0.40 ± 0.04) and Whin (0.39 ± 0.01) estuaries, however, there were no significant differences between the atherogenic indices (ANOVA, $P > 0.05$).

Table 10*: Fatty acid composition (% area) of C. tulipa from Densu estuary, Narkwa lagoon and Whin estuary.*

Fatty acid	Concentration (% Area)			P value
	Densu	Narkwa	Whin	
Saturated Fatty Acids (SFA)				
Tetradecanoic acid, methyl ester	2.33 ± 0.14	3.40 ± 1.68	2.41 ± 0.06	0.543
Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	1.56 ± 0.04	1.67 ± 0.63	1.42 ± 0.05	0.798
Hexadecanoic acid, methyl ester	26.90 ± 0.67	35.20 ± 12.04	24.36 ± 0.06	0.387
Hexadecanoic acid, 15-methyl-, methyl ester	1.86 ± 0.18	1.49 ± 0.01	1.45 ± 0.11	0.079
Heptadecanoic acid, methyl ester	1.81 ± 0.00	1.88 ± 0.28	1.65 ± 0.12	0.489
Octadecanoic acid, methyl ester	10.77 ± 1.66	12.91 ± 1.21	11.41 ± 0.26	0.324
Monounsaturated Fatty Acids (MUFA)				
9-Hexadecenoic acid, methyl ester,	2.59 ± 0.09^{a}	3.22 ± 0.66^{ab}	4.39 ± 0.15^{b}	**0.042**
9-Octadecenoic acid, methyl ester	4.52 ± 0.55	6.00 ± 1.32	4.78 ± 0.56	0.335
13-Octadecenoic acid, methyl ester	4.03 ± 0.69	2.41 ± 0.24	4.59 ± 1.04	0.117
11-Eicosenoic acid, methyl ester	5.15 ± 1.12	2.72 ± 0.67	4.08 ± 0.11	0.105
13-Docosenoic acid, methyl ester	2.31 ± 0.78^{a}	2.03 ± 0.66^{a}	8.55 ± 0.10^{b}	**0.003**
Polyunsaturated Fatty Acids (PUFA)				
9,12-Octadecadienoic acid, methyl ester	1.86 ± 0.35	1.62 ± 0.30	1.35 ± 0.34	0.413
5,8,11,14-Eicosatetraenoic acid, methyl ester	4.12 ± 0.94	3.13 ± 1.03	3.26 ± 0.18	0.502
5,8,11,14,17-Eicosapentaenoic acid, methyl ester (EPA)	10.35 ± 2.51	9.13 ± 4.12	14.53 ± 0.88	0.281
7,10,13,16, 19-Docosapentaenoic acid, methyl ester	1.72 ± 0.10^{a}	-	1.06 ± 0.18^{b}	**0.046**
4,7,10,13,16,19-Docosahexaenoic acid, methyl ester (DHA)	18.13 ± 6.52	13.19 ± 8.41	10.72 ± 1.18	0.546
ΣSFA	45.22 ± 2.33	56.55 ± 12.87	42.68 ± 0.34	0.292
ΣMUFA	18.60 ± 0.98^{a}	16.38 ± 0.40^{a}	26.39 ± 1.73^{b}	**0.007**
ΣPUFA	36.18 ± 3.31	27.08 ± 13.27	30.92 ± 2.07	0.580
Total unsaturated fatty acids	54.78 ± 2.33	43.45 ± 12.87	57.32 ± 0.34	0.292
DHA/EPA	1.88 ± 1.09	1.38 ± 0.30	0.74 ± 0.04	0.343
Atherogenic index (AI)	0.40 ± 0.04	0.69 ± 0.33	0.39 ± 0.01	0.341

Values are means ± SD of duplicate determinations. Mean ± SD with different superscripts in a row are significantly different ($P < 0.05$). – means not identified. SFA = Saturated Fatty Acids, MUFA = Monosaturated Fatty Acids, PUFA = Polyunsaturated Fatty Acids

CHAPTER FIVE

DISCUSSION

The present study assessed the nutritional quality of *C. tulipa* in three water bodies (Densu estuary, Narkwa lagoon and Whin estuary) where there are vibrant oyster fisheries and thriving oyster populations. The impact of the physicochemical properties of these water bodies on the nutritional quality of the oysters was also investigated. In addition, some medicinal properties, including phenolic content, DPPH scavenging activity, antioxidant capacity and fatty acid constituents of *C. tulipa* were assessed. This chapter discusses the results with reference to previous studies.

5.1 Physicochemical characteristics of water bodies

Physicochemical characteristics such as temperature, pH, dissolved oxygen, salinity and availability of food highly influence the survival, growth and other physiological activities of aquatic organisms. Environmental conditions have also been shown to impact oyster biochemical compositions, such as amino acids, fatty acids, proximate and mineral compositions (Lemasson et al., 2019; Ivanina et al., 2020; Pourmozaffar et al., 2020; Silva et al., 2020; Mosca et al., 2021). The physicochemical properties reported for the three water bodies in this study were within the ranges of those previously reported by other researchers to support oyster culture for the same water bodies (Asare et al., 2019; Sowah, 2019; Chuku et al., 2020; Osei et al., 2021). The two estuaries showed similar physicochemical characteristics, as compared to those of the lagoon. The estuaries had higher chlorophyll-a and dissolved oxygen concentrations than the Narkwa lagoon, which

had higher salinities. High fresh water inflow from rivers associated with the estuaries may be responsible for the low salinities of the estuaries as compared to the lagoon. The fresh water inflows perhaps introduce nutrients into the estuaries and could also account for the high chlorophyll-a and dissolved oxygen concentrations of the estuaries as compared to the lagoon.

5.2 Morphometric characteristics of *C. tulipa*

C. tulipa from the different water bodies showed significant differences in their sizes, with those from the Densu estuary having the biggest sizes, whilst the smallest sizes were recorded for those from the Narkwa lagoon. On the contrary, cultured *C. tulipa* from Narkwa lagoon were reported by Chuku (2019) to have higher shell heights and growth rates than cultured *C. tulipa* from Densu and Whin estuaries. Asare et al. (2019) also reported higher shell heights for cultured oysters than wild oysters from Narkwa lagoon. The smaller shell sizes recorded for wild oysters from Narkwa lagoon may be due to high exploitation rates (Asare et al., 2019), which prevents the oysters from reaching larger shell sizes before being harvested. Other factors, including suitable substrates, could also account for the difference in sizes.

The meat yield of oysters, which correlates with their condition indices, describes the general health as well as the economic value of the oysters (Lemasson et al., 2019). The mean meat yield of *C. tulipa* from Densu estuary (11.69 ± 2.93 %), Narkwa lagoon (14.14 ± 2.99 %) and Whin estuary (12.01 ± 3.17 %) reported in this study were all higher than the acceptable minimum value for meat yield of oysters (4.9 %) as reported by the Cuban legislation (Suastegui, 2018) and are

classified as 'Special' (meat yield > 9 %) based on the classification for *Crassostrea gigas* reared in France (Soletchnik et al., 2001). The meat yield of bivalves are known to decline under unfavourable conditions (Lemasson et al., 2019). In the present study, temperature had a negative effect on the meat yield of *C. tulipa.* Mosca et al. (2021) reported a significant negative relationship between temperature and meat yield of *Crassostrea gigas* reared in Italy. An increase in temperature has been shown to increase the metabolic rates of oysters as well as decrease their feeding rates, which could explain the decrease in meat yield (Li et al., 2017; Lemasson et al., 2019). Other factors, such as the gametogenic cycle, also influence the meat yield of oysters (Austin et al., 1993; Yildiz et al., 2011; Mosca et al., 2021).

5.3 Proximate composition of *C. tulipa*

Proximate composition of a food involves the total protein, crude fat, fibre, carbohydrate, ash, and moisture expressed as the percentage composition of the food (Self, 2005). The major component of oysters is moisture and it depicts their succulence which is an important sensory characteristic (Lemasson et al., 2019). Moisture content of *C. tulipa* from the different water bodies were similar for *C. tulipa* from the three water bodies. Similar moisture contents have also been reported for *C. tulipa* from Densu estuary (Osei, 2019), Benya lagoon and Pra estuary in Ghana (Yankson et al., 1994). pH had a significant negative relationship, whereas chlorophyll-a had a significant positive relationship with the moisture content of *C. tulipa* in the present study. Silva et al. (2020) also reported negative relationships between pH, temperature, and a positive relationship between

precipitation and the moisture content of *C. gasar* cultured in Brazil. Oysters tend to close their valves under unfavourable conditions and could have accounted for the changes in their moisture content (Lombardi et al., 2013; Porter & Breitburg, 2016).

Ash contents, which signify the mineral or inorganic constituents of the oysters, were similar for *C. tulipa* from the different water bodies and were within the range of 0.95 ± 0.06 % to 2.53 ± 0.13 %. The ash contents reported in this study were closer to those reported for *C. tulipa* from the Densu estuary (3.04 ± 0.13 %) by (Osei, 2019). However, they were smaller than those reported for *C. tulipa* from Benya lagoon (12.7 ± 1.0 %) and the Pra estuary (14.4 ± 1.6 %) by Yankson et al. (1994). pH had a significant positive relationship, whereas chlorophyll-a had a significant negative relationship with the ash content of *C. tulipa* in the present study. Silva et al. (2020) also reported significant positive relationships between pH and temperature, and a significant negative relationship between precipitation and the ash content of *C. gasar*. The metabolic rates of biomineralization cells in *C. gigas* were reduced when exposed to low pHs (Ivanina et al., 2020), which could explain the positive association between pH and ash content in *C. tulipa*. Amino acids and minerals such as Na, Mg and K, which make up the ash contents of food products, are involved in the maintenance of acid-base balance and osmotic pressure of oysters (Haider et al., 2020; Ivanina et al., 2020) and could also explain the influence of the environmental factors on the ash content of *C. tulipa*.

Protein, fat and carbohydrate play important physiological roles and are the main energy reserves and sources of energy for oysters and other living organisms

(Lemasson et al., 2019). There were no significant differences in these energy reserves of *C. tulipa* from the various water bodies in this study. The estimates were in accordance with those reported by Osei (2019) and higher than those reported by Yankson et al. (1994); however, the protein contents reported in this study were lower than those reported by Yankson et al. (1994). The variations in the nutritional content of *C. tulipa* reported recently by Osei, (2019) and in the present study as compared to those reported about three decades ago by Yankson et al. (1994) might be explained by changes in climate conditions and other environmental factors that have occurred over the past three decades. For example, Ghana has had an average temperature increase of 0.21 ºC every decade since 1960 (MESTI, 2015).

Dietary fibre content consists of complex polysaccharides which are usually nondigestible by humans. Due to the several important functions of dietary fibre, such as decreasing intestinal transit time, increasing the volume of faecal bulk, as well as decreasing cholesterol and glycaemic levels, their consumption has been related to a decrease in the incidence of several types of diseases (Dhingra et al., 2012). The estimated fibre contents in *C. tulipa* were higher than those reported for *C. tulipa* by Osei (2019) but similar to those reported for *C. gasar* by Johnnie et al. (2020). The fibre contents were also comparable to those of rice and corn as reported by Prasad et al. (2018) and Prasanthi et al. (2017), respectively. Environmental factors did not have any significant effects on the energy reserves as well as the fibre content of *C. tulipa*. However, there was a significant positive relationship between the chlorophyll-a concentrations and the protein content of *C. tulipa. C. tulipa* may possess an adaptive coping strategy which prevents significant

alterations in their energy reserves by slight changes in environmental conditions as observed for bivalves elsewhere (Anacleto et al., 2014; Lemasson et al., 2019).

5.4 Mineral composition of *C. tulipa*

Minerals have a wide range of functions and potentials in the body's metabolism and homeostasis, including bone formation, hormone production, and nerve impulse transmission (Gharibzahedi & Jafari, 2017; Wang et al., 2021). Deficiencies in minerals can result in diseases such as osteoporosis, cardiovascular diseases and anemia. However, they can also be toxic at high concentrations (Han et al., 1998; Wang et al., 2021). The findings of this study are consistent with those of other studies, which have reported oysters as a rich source of Ca, Zn, Cu and Fe (Chakraborty et al., 2016; Bates et al., 2021; Catry et al., 2021). Also, using a daily consumption rate of 109 g wet weight of oyster meat per day (Essumang et al., 2018), all the minerals did not exceed their respective permissible limits for daily intake as defined for adults in Australia/New Zealand (Capra, 2006). Sowah (2019) also reported concentrations within permissible limits for Cu and Zn for *C. tulipa* in Ghana.

In the present study, significant differences in some mineral concentrations were obtained for *C. tulipa* from the different water bodies. According to previous studies (Juhna & Klavinš, 2001; Thomsen et al., 2018), fresh water and estuaries are usually lower in calcium ions as compared to seawater since salinity is positively correlated with calcium. In this study, the significantly higher concentrations of Ca and Na in *C. tulipa* from Narkwa lagoon, as compared to the others could be attributed to the relatively high salinity of the Narkwa lagoon.

Regression analysis in this study also showed a significant positive relationship between salinity and a negative relationship between pH and the Na content of the oysters.

C. tulipa from the Whin estuary had the highest Zn, Cu, and Fe concentrations. Sowah (2019) also reported higher zinc and copper concentrations of *C. tulipa* from the Whin estuary as compared to those from the Narkwa lagoon and the Densu estuary. Relatively high copper and zinc contents have been reported for oysters growing in areas associated with mining and harbour activities (Frías-Espericueta et al., 2009; Rizo et al., 2010) and could account for the relatively high amounts of trace elements in oysters from the Whin estuary, which is also being impacted by mining activities.

5.5 Amino acid composition of *C. tulipa*

Protein quality is an important aspect of the nutritional quality of foods. Protein quality is primarily determined by the amino acid constituents of the protein. The amino acids of *C. tulipa* in this study signified very good protein quality, and there were no significant differences in the amino acid composition of *C. tulipa* from the different water bodies. Essential amino acids formed 76 – 79 % of the total amino acids determined, and the amounts of these amino acids were all above the recommended amino acid pattern for adults as indicated by FAO/WHO/UNU (2007). There were no limiting amino acids and the chemical score of the oysters ranged from 101.3 to 104.9 %. Chemical scores above 100 % signifying no limiting amino acids have also been reported for a number of shellfishes, including the eastern oyster (Venugopal & Gopakumar, 2017). Previous

studies, on the other hand, found methionine to be the limiting amino acid in oysters, but this finding was attributed to methionine's easy oxidation during protein hydrolysis (Spindler et al., 1984; Qin et al., 2018; Jiang et al., 2019). In the present study, phenol, which is an antioxidant, was added to the digestion mixture, and the digestion tubes were also filled with nitrogen gas to displace oxygen before the hydrolysis to minimize the oxidation of methionine in the oysters. As estimated in this study, the essential amino acid index (EAAI), which indicates the quality of a protein based on all its essential amino acids, and the biological value (BV), which indicates the percentage of protein that can be utilized by the body (Oser, 1959; Hansen, 1975), showed that *C. tulipa* had superior protein quality. In this study, EAAIs were within 117.6 – 121.5 %, whereas BVs were within 116.5 – 120.7 % for *C. tulipa*. Similar EAAI and BV were also reported for *C. madrasensis* by Asha et al. (2014). This supports previous findings that oyster protein is comparable to egg protein in quality and that oyster protein is superior to finfish protein in quality (Asha et al., 2014; Venugopal & Gopakumar, 2017).

Amino acids also play important roles in maintaining intracellular osmotic pressure in marine bivalves (Silva & Wright, 1994; Berger & Kharazova, 1997; Haider et al., 2020; Ivanina et al., 2020) and are highly influenced by different environmental conditions. In the present study, different environmental factors (pH, temperature, precipitation and chlorophyll-a) had significant effects on the amino acid composition of *C. tulipa.* The effect of environmental factors on the amino acids of *C. tulipa* were more similar to those of the ash contents than most of the minerals evaluated in this study. This is in accordance with reports that amino acids

and other organic acids have a greater impact in maintaining osmotic balance of marine bivalves than inorganic ions (Ivanina et al., 2020). Some physiological conditions of oysters, such as gametogenesis and spawning, also affect their amino acid constituents (Qin et al., 2018; Qin et al., 2021), which could also explain the variations in the amino acid composition of *C. tulipa* during the period of study.

5.6 Medicinal properties of *C. tulipa*

According to Odeleye et al. (2019), bioactive compounds are the components or ingredients that make a food functional. In this study, the different solvents used for extraction of bioactive compounds in oysters from the three water bodies gave percentage yields ranging from 2.57 to 33.86 %. A better yield of extracts was obtained with extraction with absolute methanol as compared to the other solvents (70 % ethanol, 70 % methanol and hexane). Also, the various solvents showed different yields based on the oysters' environment and may signify that oysters from the different water bodies have different kinds of bioactive compounds since the chemical nature of samples are key determinants in an extraction process (Delazar et al., 2012; Odeleye et al., 2019).

Phenolic compounds are bioactive compounds with at least a phenol group and are divided into several classes such as tannins, phenolic acids, flavonoids, stibenes and lignans (Ayad & Akkal, 2019; Saranraj et al., 2019). Phenolic compounds were present in all the extracts of *C. tulipa,* with the 70 % ethanolic extracts yielding the highest phenolic content. Phenolic compounds in bivalves can be linked to their consumption of plant-derived materials like phytoplankton (Aligiannis et al., 2003; Krishnamoorthy et al., 2019). Krishnamoorthy et al. (2019)

also reported higher concentrations of phenolic contents in ethanolic extract of *Perna viridis* as compared to other solvent extracts. The phenolic contents of *C. tulipa* in this study, were higher than those reported for oysters in previous studies (Asha et al., 2016; Lee et al., 2018). Phenolic compounds are antioxidants and they play important roles such as decreasing inflammatory and oxidative stress-related diseases (Ayad & Akkal, 2019; Saranraj et al., 2019).

Several studies have reported antioxidant activities for oysters elsewhere (Watanabe et al., 2012; Asha et al., 2016; Lee et al., 2018; Peralta et al., 2018; Chakraborty & Joy, 2019). Isolated compounds from oysters such as 3,5-dihydroxy-4-methoxybenzyl alcohol (Watanabe et al., 2012), phenylacetyloxy-trimethylpicene-23-carboxylate derivatives (Chakraborty & Joy, 2019) as well as other polyphenols, carotenoids and some secondary metabolites (Chakraborty and Joy, 2020) are responsible for the antioxidant properties of oysters. In the present study, the various extracts of *C. tulipa* showed DPPH scavenging activities which were all dose dependent ($P < 0.05$). Trends in DPPH scavenging activities for the various extracts were similar to those of their phenolic contents. These signify that the phenolic compounds played major roles in the free radical scavenging activities of the extracts. Positive correlations between DPPH scavenging activity and the phenolic content of oysters have also been reported by Peralta et al. (2018). The free radical scavenging activities of extracts of *C. tulipa* from Densu and Whin estuaries were higher than those of Narkwa lagoon. This could be attributed to the high chlorophyll-a content of the estuaries, which signifies richness in phytoplankton as compared to that of the Narkwa lagoon.

Total antioxidant capacities (TAC) of *C. tulipa* extracts were estimated using the phosphomolybdate method, which determined the ability of the extract to reduce Mo (VI) to Mo (V). The total antioxidant capacity assay quantifies all antioxidants, including those that are water-soluble and fat-soluble (Mbinda & Musangi, 2019). Trends in TAC of the extracts of *C. tulipa* were not similar to those of their phenolic contents and DPPH scavenging activities. Here, hexane extracts of *C. tulipa* from Densu and Whin estuaries had the highest TAC, whereas methanol extracts had the highest TAC for *C. tulipa* from Narkwa lagoon. These findings suggest that *C. tulipa* may contain high concentrations of non-polar antioxidants as well as antioxidants with distinct modes of action (Mbinda & Musangi, 2019). Also, *C. tulipa's* antioxidant properties are perhaps influenced by their environment.

The fatty acid composition of *C. tulipa* demonstrated that *C. tulipa* is a major source of unsaturated fatty acids with important health benefits. Unsaturated fatty acids formed 43.45 ± 12.87 % to 57.32 ± 0.34 % of total fatty acids in *C. tulipa,* with polyunsaturated fatty acids (27.08 ± 13.27 % – 36.18 ± 3.31 %) being the most abudant. Chakraborty et al. (2016) and Qin et al. (2018) also reported similar high percentages of unsaturated and polyunsaturated fatty acids for oysters in India and China. Ecosapentaenoic (EPA) and docosahexaenoic (DHA) acids were the major unsaturated fatty acids in *C. tulipa* from the three water bodies. These long chain omega-3 fatty acids have essential health benefits and are needed in human diets. They serve as precursors for leukotrienes and prostaglandins which prevent inflammation, thrombosis and also act as hypolipidemics to inhibit atherosclerosis (Gordon & Ratliff, 1992; Guo et al., 2019). EPA and DHA have

also been shown to decrease the incidence of asthma in children, improve brain development and neurological functioning, as well as have strong antiarrhythmic and cardioprotective effects (Gordon & Ratliff, 1992; Lauritzen et al., 2016; Rees et al., 2019). According to Chakraborty et al. (2016), foods ingested by bivalves are directly reflected in their fatty acid composition. Budge and Parrish (1998) also reported DHA/EPA ratios > 1 indicating a diet dominated by dinoflagellates and ratios < 1 indicating a diet dominated by diatoms for oysters. In the present study, *C. tulipa* from Narkwa lagoon and Densu estuary had DHA/EPA ratios greater than 1, whereas that of *C. tulipa* from Whin estuary was less than 1, which may reflect variation in diets. Because of the high amount of unsaturated fatty acids in *C. tulipa*, they had atherogenic indices (AI) less than 1, indicating that they have less atherogenic tendencies (Ulbricht & Southgate, 1991). Docosapentaenoic acid was not identified in *C. tulipa* from Narkwa lagoon, which may have resulted in a higher AI than those from the estuaries. However, the AI observed for *C. tulipa* in this study were lower than 0.77 – 1.1 reported for *C. madrasensis* by Chakraborty et al. (2016) and 1.01 – 1.1 for *C. rhizophorae* by Lira et al. (2013).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The present study assessed the nutritional quality of *C. tulipa* in three water bodies, namely Densu estuary, Narkwa lagoon and Whin estuary. The impact of the environmental condtions of these water bodies on the nutritional quality of the oysters was also investigated. In addition, some medicinal properties, including phenolic content, DPPH scavenging activity, antioxidant capacity and fatty acid constituents of *C. tulipa* were assessed. Information on shell sizes (shell length, shell height and shell width), meat yield, proximate composition (moisture, ash, fat, protein, fibre and carbohydrate), macro-minerals (K, P, Na, Ca and Mg), micro-minerals (Cu, Zn and Fe), 16 amino acids, 16 fatty acids and some antioxidant properties have been provided to promote the popularity of oysters, culture of oysters and the potential of oysters as an alternative source of good quality protein. To maximize yield and maintain good physiological conditions of the oysters, spatiotemporal variations as well as effects of environmental conditions (pH, temperature, salinity, dissolved oxygen, chlorophyll-a and precipitation) on the meat yield, proximate, minerals and amino acid composition of the oysters were also investigated.

6.2 Conclusions

The nutritional quality assessment qualifies *C. tulipa* as a highly nutritious and potential functional food.

There were no significant differences in the proximate and amino acid composition of *C. tulipa* from the three water bodies. *C. tulipa* from Whin estuary had significantly higher Fe and Zn contents, whereas *C. tulipa* from Narkwa lagoon had significantly higher Ca and Na. There were significant temporal variations in the proximate, mineral and amino acid compositions of *C. tulipa* in each location.

The majority of the nutritional compositions of the oysters were significantly influenced by environmental conditions. pH had positive effects on ash, Fe, Cu and amino acids, and negative effects on moisture and Na. Temperature had negative effects on meat yield and positive effects on amino acids. Chlorophyll-a had negative effects on ash and amino acids and a positive effect on moisture. Precipitation had positive effects on amino acids, whereas salinity had positive effects on Na.

The different solvent extracts of *C. tulipa* all showed good medicinal properties. Phenolic compounds were present in *C. tulipa* and their concentrations varied depending on the extraction solvents and water body of the oysters. All the extracts showed DPPH scavenging activities, which were dose-dependent, and the 70% ethanol extracts had the highest DPPH scavenging activity. The total antioxidant capacity of *C. tulipa* suggested that they have high concentrations of non-polar antioxidants. Oils of *C. tulipa* were abundant in eicosapentaenoic and docosahexaenoic fatty acids (EPA and DHA).

6.3 Recommendations

The recommendations made from this study are;

1. Further studies should be conducted on the effect of the gametogenic stages of *C. tulipa* on their nutritional quality to help properly decide when to harvest high-quality oysters.
2. A nationwide promotion of oysters as a highly nutritious alternative source of protein, should be conducted to stimulate consumer demand.

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APPENDICES

Appendix A: Total monthly precipitation at Densu estuary, Narkwa lagoon and Whin estuary.

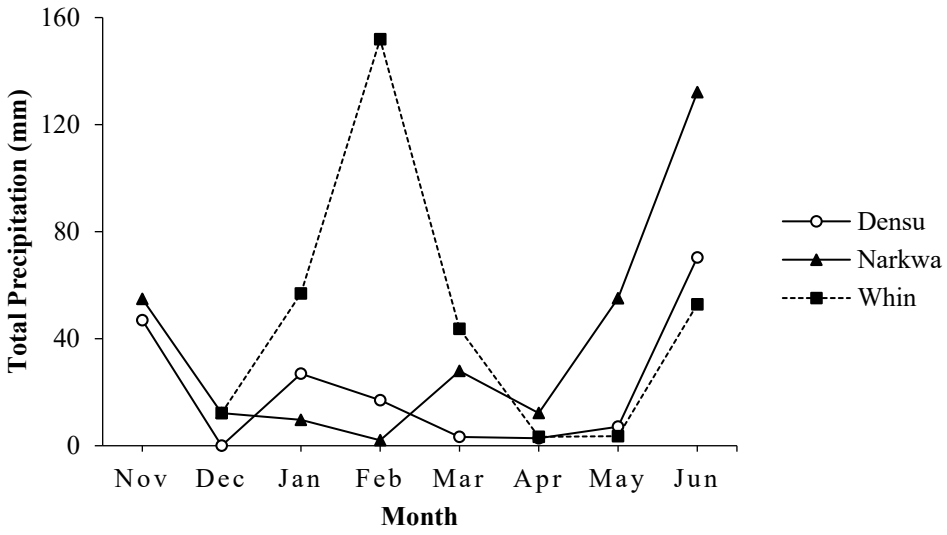


Figure 19: Total monthly precipitation for Densu estuary, Narkwa lagoon and Whin estuary during the period of study (November 2020 – June 2021) were retrieved from Tutiempo, a database for global climate data (https://en.tutiempo.net/climate/). Precipitation data for Accra, Saltpond and Takoradi were used for Densu estuary, Narkwa lagoon and Whin estuary, respectively.

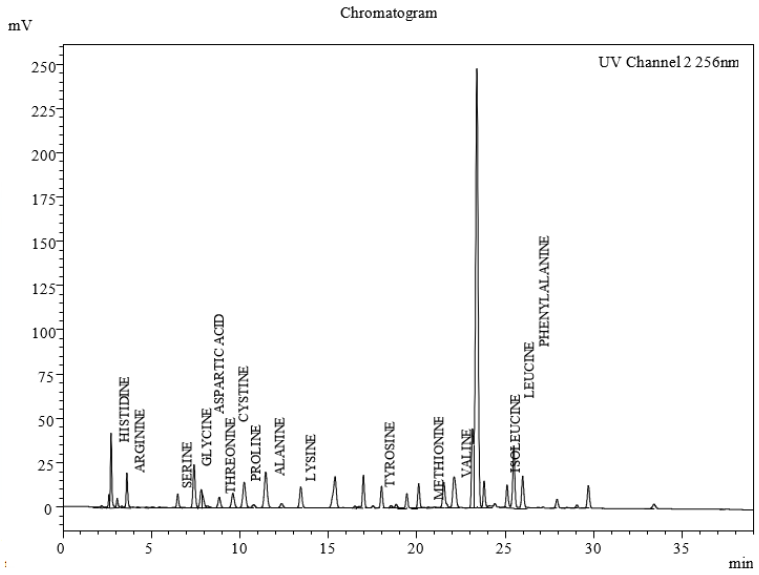
Appendix B: Quality control for amino acids composition determination

Table 11: *Retention time (RT), calibration linearity (R^2), precision (% RSD), limit of detection (LOD) and limit of quantitation (LOQ) of the ultrafast liquid chromatography method used for the amino acid analysis.*

Amino acid	RT (min)	R^2	% RSD	LOD µmol/mL	LOQ µmol/mL
Histidine	2.710	0.9979	10.933	0.20	0.60
Arginine	3.604	0.9953	15.063	0.04	0.12
Serine	6.478	0.9987	3.399	0.06	0.17
Glycine	7.408	0.9993	4.829	0.03	0.03
Aspartic acid	7.817	0.9958	18.961	0.08	0.10
Threonine	8.839	0.9955	21.096	0.07	0.21
Cystine	9.806	0.9954	8.534	0.03	0.07
Proline	10.234	0.9976	14.774	0.03	0.08
Alanine	11.446	0.9995	3.589	0.03	0.09
Lysine	13.424	0.9968	15.581	0.03	0.10
Tyrosine	17.985	0.9966	13.026	0.06	0.18
Methionine	20.084	0.9984	4.438	0.04	0.13
Valine	22.082	0.9987	15.686	0.03	0.08
Isoleucine	25.101	0.9996	12.801	0.03	0.09
Leucine	25.480	0.9974	14.547	0.02	0.06
Phenylalanine	26.029	0.9985	15.894	0.03	0.09

Appendix C: Some chromatograms for derivatized amino acids.

I



II

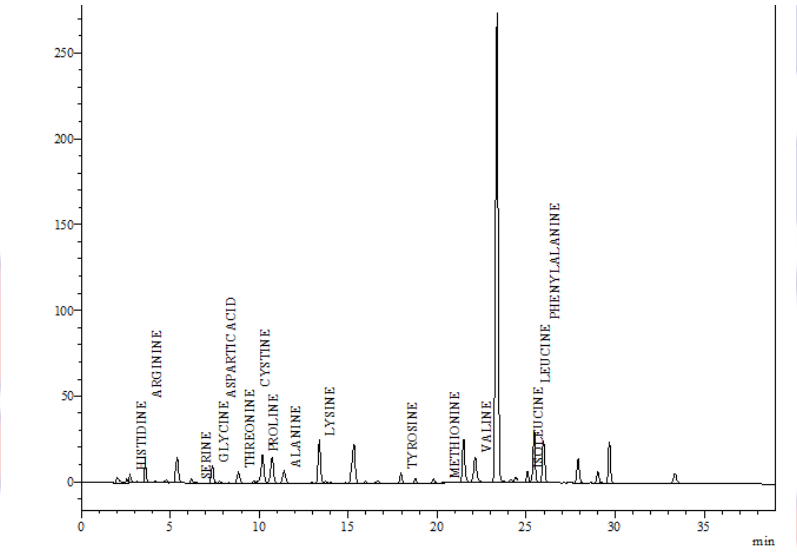


Figure 20: (I): Chromatogram of standards (II): Chromatogram of hydrolysed oyster meat

Appendix D: Some standard curves used for amino acid quantification

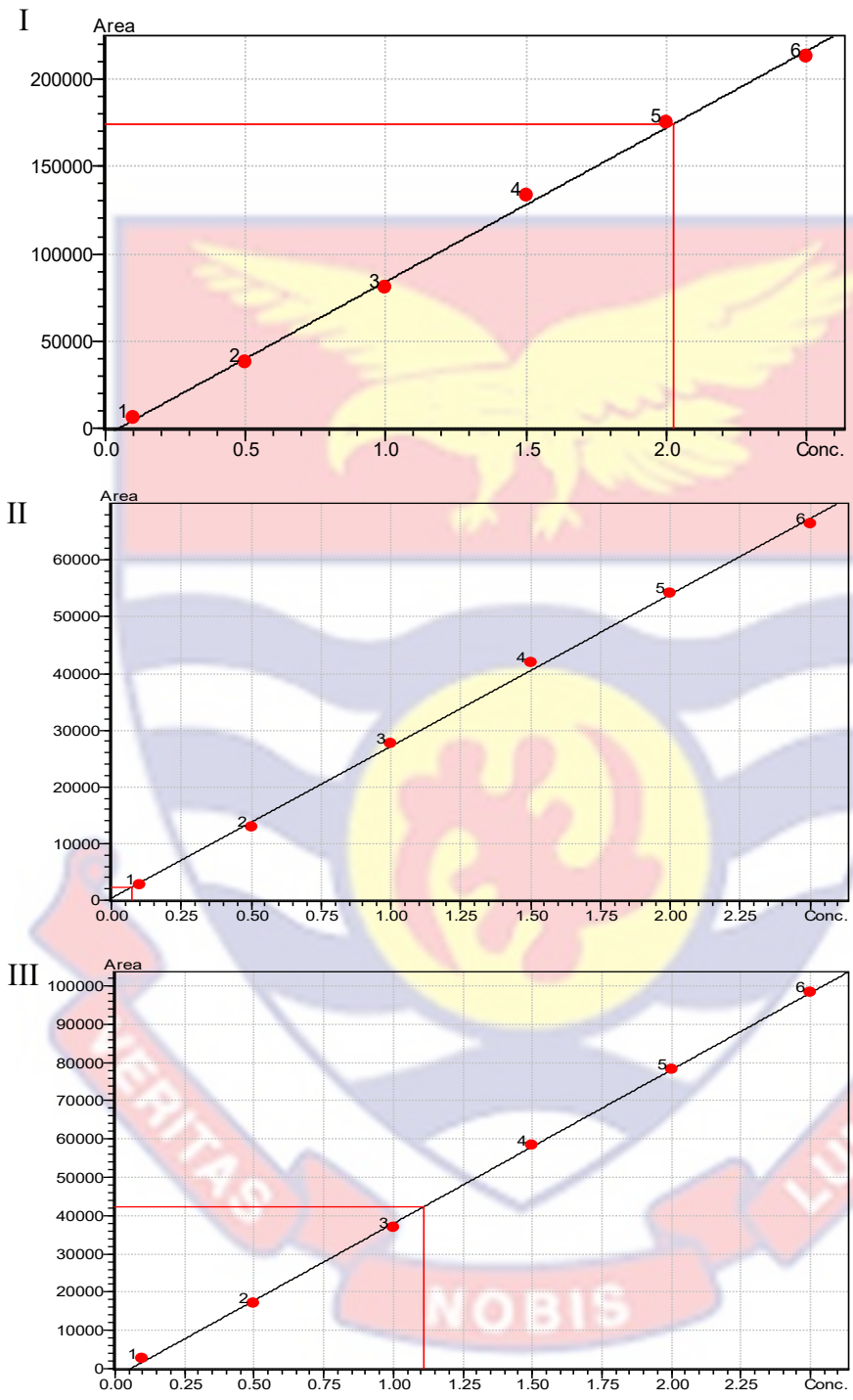


Figure 21: Standard curves for (I) histidine (II) serine and (III) isoleucine.

Appendix E: Standard curve for quantification of phenolic compounds in *C. tulipa* extracts.

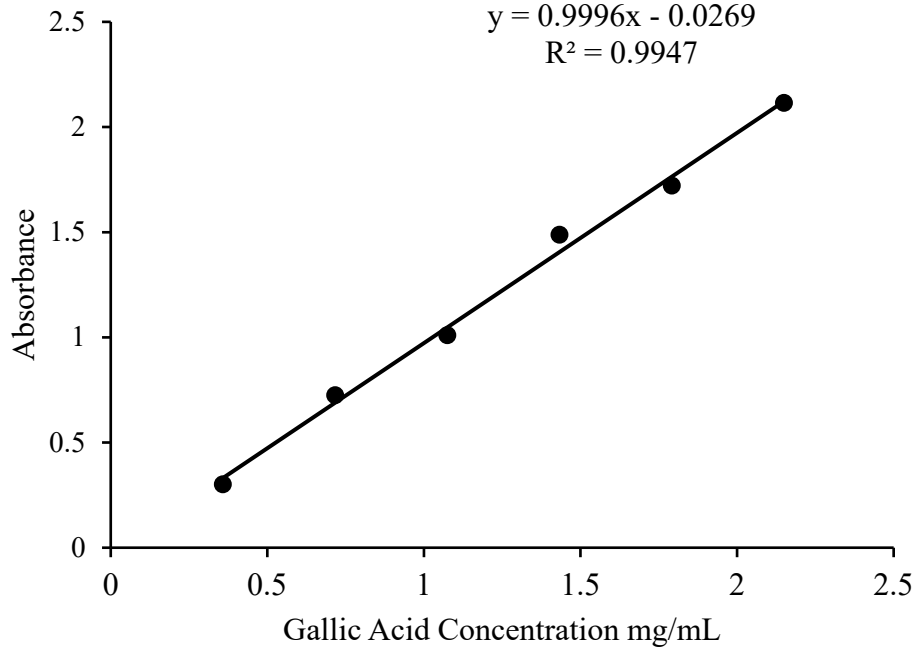


Figure 22: Gallic acid standard curve for phenolic content determination.

Appendix F: Standard curve for determination of the total antioxidant capacities of *C. tulipa* extracts.

s

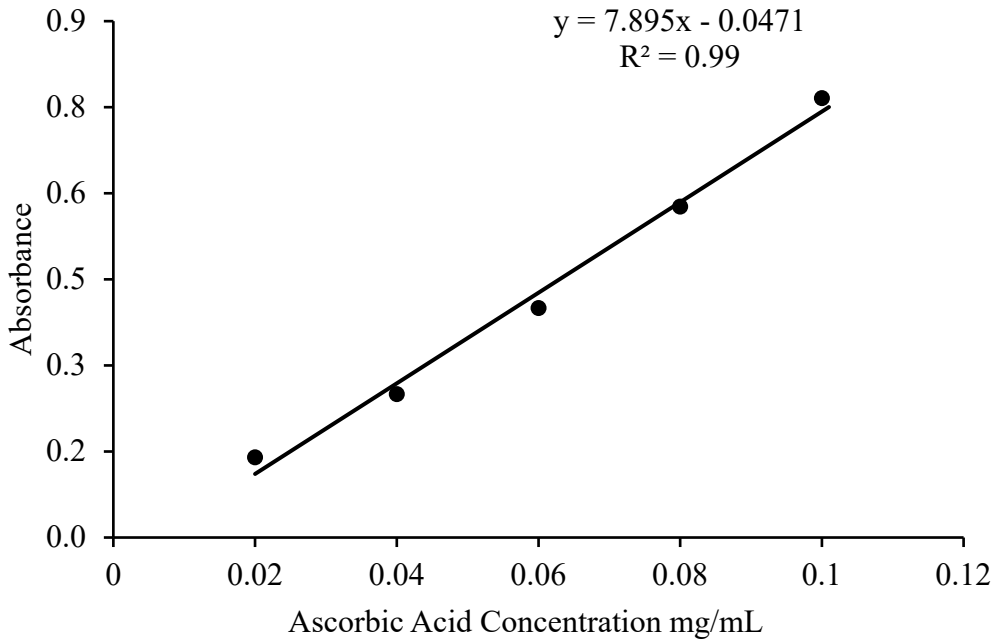


Figure 23: Ascorbic acid standard curve for total antioxidant capacity determination.

Appendix G: Some gas chromatograms for fatty acid methyl esters in oils extracted from *C. tulipa.*

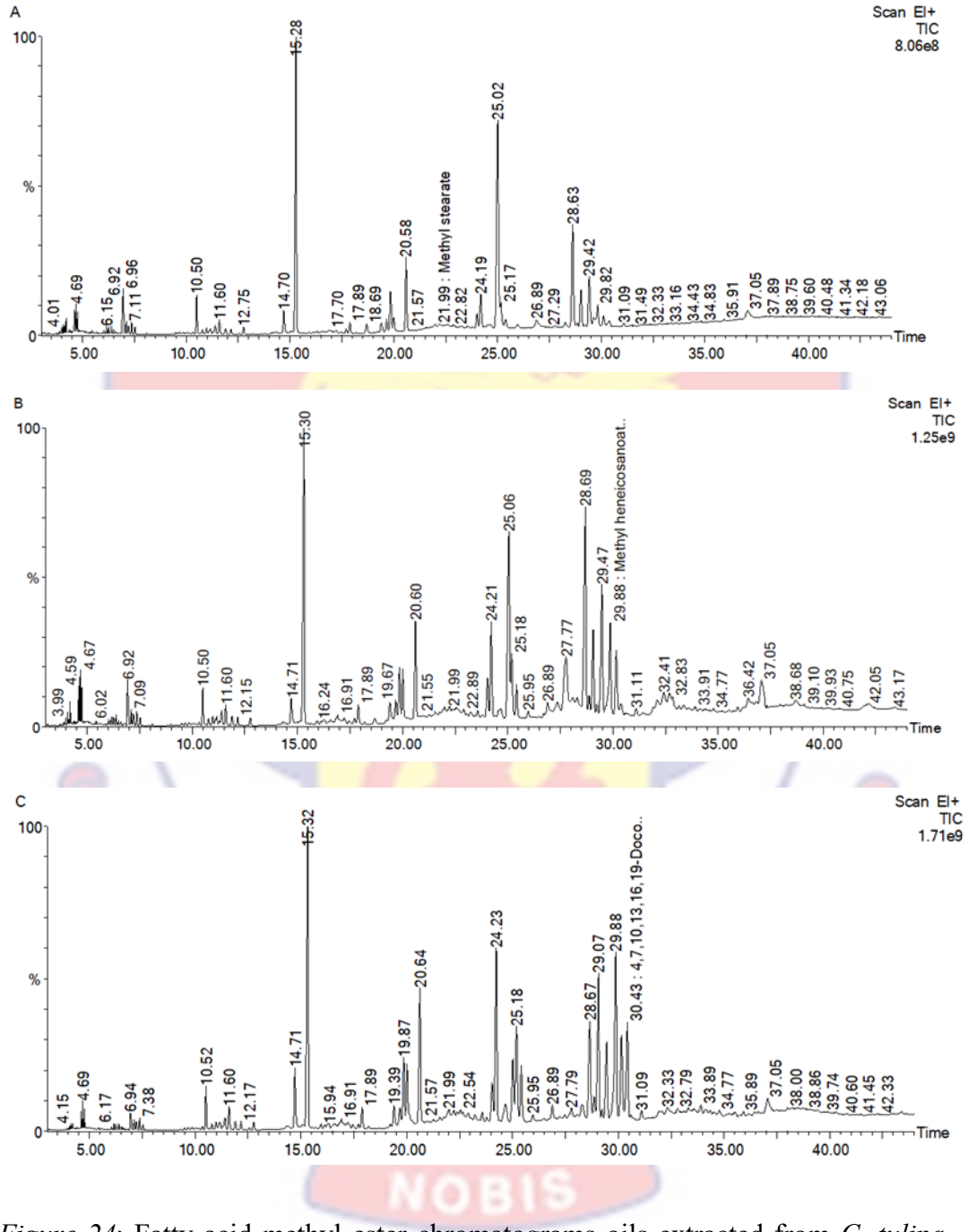


Figure 24: Fatty acid methyl ester chromatograms oils extracted from *C. tulipa* from **A**. Narkwa lagoon **B.** Densu estuary and **C.** Whin estuary.

Appendix H: Some mass spectra for the identification of fatty acid methyl esters in oils extracted from *C. tulipa.*

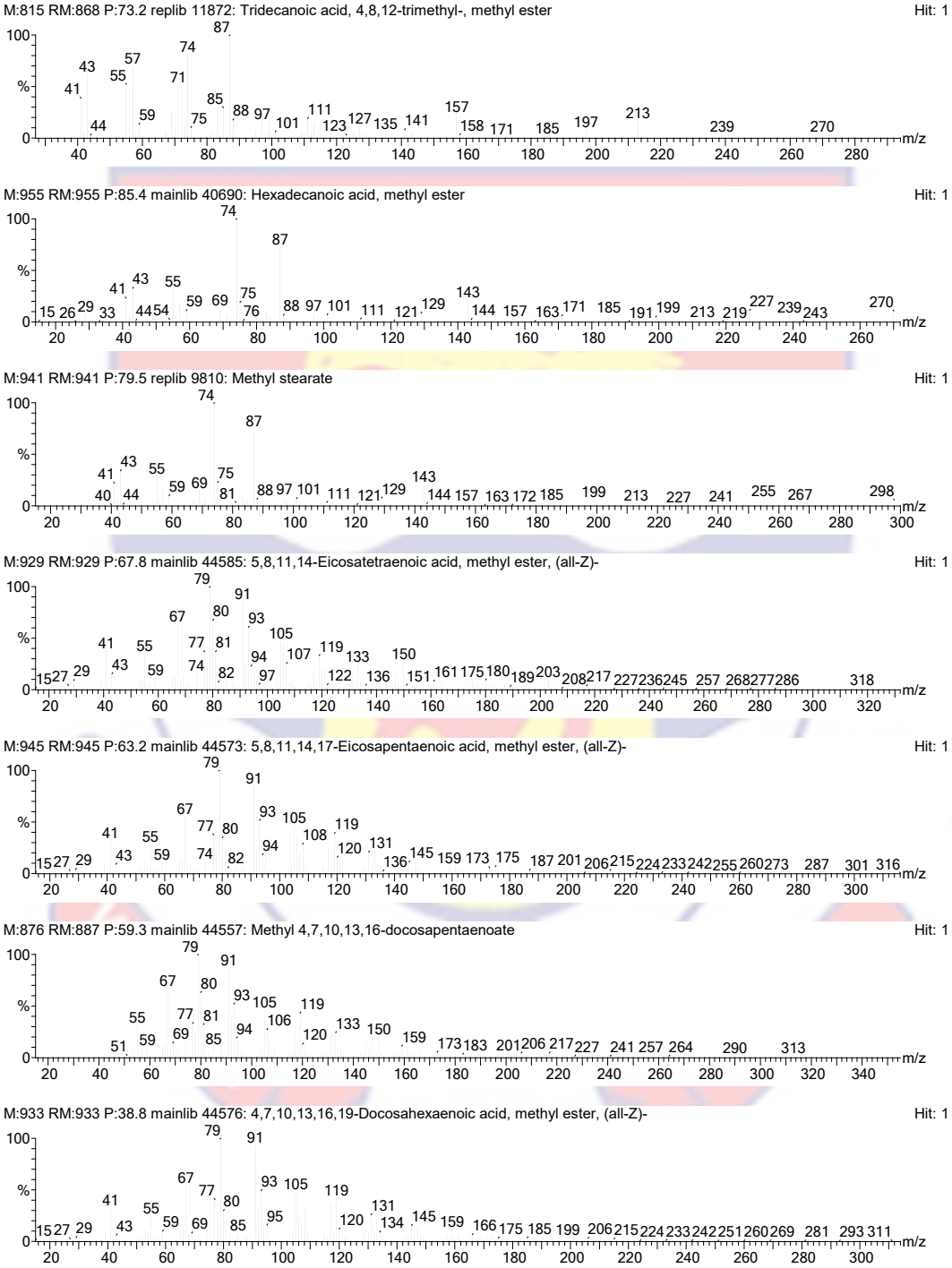


Figure 25: Some mass spectra for the identification of fatty acid methyl esters in oils extracted from *C. tulipa.*