UNIVERSITY OF CAPE COAST

ASSESSMENT OF SOME WATER QUALITY PARAMETERS OF THREE COASTAL WATER BODIES TOWARDS THE CULTURE OF OYSTERS IN GHANA

SUCCESS ADJELEY SOWAH

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BY

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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 fulfilment of the requirements for the award of a Master of Philosophy (M.Phil.) degree in Oceanography and Limnology

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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: ….SUCCESS ADJELEY SOWAH……...….

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: …….

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

Name: ….DR. NOBLE KWAME ASARE……..

ABSTRACT

Oyster culture as a supplementary livelihood for many coastal communities is a potential that is yet to be exploited in Ghana. Temperature, salinity, dissolved oxygen, pH, turbidity, bacteria (coliforms and total bacteria) and heavy metal loads are among the key environmental factors that influence the physiological wellbeing (also known as condition index) of oysters. This study investigated these environmental parameters in three coastal water bodies in Ghana and their influence on the condition index (CI) of the resident oysters, as a means of determining the suitability of the water bodies for oyster culture. Physicochemical parameters were measured *in situ* with different measurement tools, whereas microbial load and heavy metal concentrations were determined in the laboratory using the pour plate method and atomic absorption spectrophotometer respectively. The environmental parameters fluctuated in similar patterns in the water bodies during the study period, with values within limits that indicated good ecological health of the water bodies. The bacterial loads generally exceeded the acceptable limits, but heavy metal concentrations were within permissible limits. A linear regression analysis showed that physicochemical parameters had variable influence on the CI of oysters from the water bodies, with temperature having the strongest influence. Bacterial loads in water had variable influence on CI, but total coliform load in oysters had a positive significant influence on CI. The heavy metal concentration in water samples also had variable influence on the CI of oysters. It is concluded that all the three water bodies could be considered as suitable sites for oyster culture provided efforts are made to limit activities responsible for the high microbial load.

KEY WORDS

Oyster culture

Crassostrea tulipa

Condition index

Physicochemical parameters

Bacterial load

Heavy metals

LIST OF ACRONYMS

FAO	Food and Agriculture Organisation
UNEP	United Nations Environment Programme
WHO	World Health Organisation
NERR	National Estuarine Research Reserves
USEPA	United States Environmental Protection Agency
As	Arsenic
Cd	Cadmium
Cr	Chromium
Co	Cobalt
Cu	Copper
Pb	Lead
Hg	Mercury
Ni	Nickel
Zn	Zinc
CI	Condition Index
DO	Dissolved oxygen
NTU	Nephelometric Turbidity Unit
DFAS	Department of Fisheries and Aquatic Sciences

MBB	Molecular Biology and Biotechnology
UCC	University of Cape Coast
PC	Plate Count
TSI	Triple Sugar Iron
GAEC	Ghana Atomic Energy Commission
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
H_2O_2	Hydrogen Perioxide
ANOVA	Analysis of Variance
PCA	Principal Components Analysis
SPSS	Statistical Package for Social Sciences
STATA	South Texas Art Therapy Association
TC	Total Coliform
TVB	Total Viable Bacteria
LF	Lactose Fermenter
NLF	Non Lactose Fermenter
USFDA	United States Food and Drug Administration
USAID	United States Agency for International Development

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DEDICATION

To my mum (Angelina Sowah) and Amoako.

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CHAPTER ONE:

INTRODUCTION

This chapter gives a background to the entire research, including the significance of the study and the objectives to be achieved. It also presents the delimitations, limitation, definitions of terms and the organization of the study.

1.1 Background to the study

Fish, largely from marine origin, is the primary source of protein in Ghana (Komatsu & Kitanishi, 2015; Asiedu, Nunoo & Iddrisu, 2017). Approximately, the per capita consumption of fish in Ghana is 26 kg (Food and Agriculture Organization (FAO, 2016). This surpasses the per capita consumption of fish (20.5 kg) globally (FAO, 2018). However, marine fish landings in Ghana have been declining over the years. This has created an increasing gap between the demand and supply of fish. In recent years, aquaculture has been very supportive in trying to bridge the gap between demand and supply of fish in Ghana (FAO, 2016). According to Amenyogbe et al. (2018), productions from aquaculture in the country increased from over 32,000 metric tonnes (MT) to about 52,000 MT between 2013 and 2016. However, productions from aquaculture needs to be improved to levels that can adequately supplement the productions from capture fisheries. Amenyogbe et al. (2018), suggest that there is potential for shellfish culture in Ghana. In effect, diversifying the aquaculture industry to include the culture of shellfishes could be an appropriate step towards reducing the deficit in the Ghanaian fisheries sector.

According to Yankson (2004), oysters have shown culture potential in Ghana. However, there is limited information on the suitability of Ghanaian coastal water bodies for the culture of this species (Obodai & Yankson, 2002).

Oysters are aquatic invertebrates which belong to the phylum Mollusca and the class Bivalvia hence, they are referred to as bivalve molluscs. Other examples of bivalve molluscs include clams, mussels and scallops (Gosling, 2003). Oysters are distributed worldwide and they are of several genera (*Ostrea, Crassostrea, Saccostrea, Striostrea, Tiostrea, Hyotissa*) (Gosling, 2003; Yang, Sturmer & Baker, 2016). Oysters of the genus *Crassosstrea* are the most cultured around the world (Angell, 1986; Arakawa, 1990; Gosling, 2003; Yang et al., 2016). Angell (1986) reported that *Crasssostrea tulipa* can be found along the West African coast, from Senegal to Angola. This species thrives in most of the coastal water bodies in Ghana (Obodai & Yankson, 2002).

Lorio and Malone (1994) stated that the culture of oysters is the oldest form of bivalve culture. According to Yankson (2004), oysters from Ghana meet the culture requirements for aquatic organisms such as abundance, spat availability, capability to complete lifecycle in captivity, lower trophic level in food chain and high market value. This presents an opportunity for the Ghanaian fisheries industry to tap from the available skills and know-how on oyster culture in this country. In addition, there is market potential for the species, as has been reported by Asare, Obodai and Acheampong (2019).

A myriad of socio-economic, health and ecological benefits have been linked with oyster culture, as practised in other parts of the world. Oysters are among keystone species which are ecologically important. They provide beneficial ecosystem services such as water quality enhancement, carbon sequestration, habitat provision (for other aquatic organisms), shoreline stabilization and protection against coastal erosion (Grabowski et al., 2012; Kroeger, 2012).

The culture of oysters is beneficial for food provision (animal protein supplement). Oysters, like other shellfishes, also provide essential amino acids, omega-3 fatty acid, minerals, vitamins and glycogen to consumers (Dong, 2009; Chen, 2011; Mcmanus & Newton, 2011; Reames, 2012; Venugospal & Gopakumar, 2017). Oyster culture also provides other socio-economic benefits such as job creation and income generation. For instance, in America, the restoration of oyster reefs in North Carolina was associated with the creation of jobs and generation of income (Kroeger, 2012; Callihan et al., 2016). Also, in Africa, countries like South Africa, The Gambia and Nigeria have recorded some contributions of oyster culture to income generation (Afinowi, 1984; Adisa-Bolanta, 2012; Olivier, Heinecken & Jackson, 2013; Rice et al., 2015).

The shells of oysters have economic value, since they are useful in building and road constructions; preparation of paint, terrazzo, rough base for footpaths, fertilizer, liming of fish ponds, jewellery, poultry feed ingredient and traditional medicine (Yankson, 1990; Perkins, 1995; Obodai & Yankson, 1999; Yankson, 2004; Ansa & Bashir, 2007; Rice, Darboe, Drammeh & Babanding, 2012).

1.2 Statement of the Problem

The declining state of the world's fish stocks, which has generated a deficit between the demand for fish and the supply from wild capture fishery, is well documented (FAO, 2018). The support from aquaculture in alleviating fish demand deficits cannot be overemphasised (FAO, 2016). One major drawback in the contribution of aquaculture to fish supply in Ghana is the limitation of the industry to the culture of only two fin fish species- *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish). The need to diversify the industry to include the culture of shellfishes, and oysters in particular, has become crucial (Amenyogbe et al., 2018). Although the potential of oysters for culturing has been highlighted by several authors (Afinowi, 1984; Adisa-Bolanta, 2012; Rice et al., 2015), as indicated earlier, the suitability of coastal water bodies in Ghana for their culture has received limited attention; hence the need for such investigation.

1.3 Purpose of the Study

The West African mangrove oyster (*Crasssostrea tulipa*) thrives in abundance in many coastal water bodies in Ghana (Obodai 1997; Yankson 1990; Yankson, 2004). The species has shown culture potential and is noted to produce abundant spat, enough for commercial production (Yankson, 2004). However, owing to their feeding mechanism, oysters accumulate loads of contaminants which may be detrimental to their survival and by extension, may hamper the health of human consumers, as oysters are sometimes consumed raw (Perkins, 1995). The ecological health status of the aquatic ecosystems in which these organisms are

cultured is therefore a prerequisite for site selection. In addition, internationally recognised health organisations are particular about the conditions under which oysters are produced for human consumption, due to health concerns which have been traced to the consumption of shellfishes (Perkins, 1995). Consequently, this research assessed the ecological health status of three water bodies in attempt to ascertain their suitability for commercial oyster production.

1.4 Research Objectives

The aim of this research was therefore to assess the quality of three coastal water bodies in Ghana to determine their suitability for oyster culture. The specific research objectives were to:

I. undertake measurements of some physical and chemical parameters of the selected water bodies

II. determine the microbial load in the water bodies and oyster meat

III. investigate the heavy metal contamination of the water and oyster meat

IV. determine the condition indices of the oysters and possible relationship with the measured environmental parameters.

The study was conducted based on the following hypotheses:

1. H_0: Physicochemical parameters will not exhibit spatio-temporal variations in the three water bodies

H_1: Physicochemical parameters will exhibit spatio-temporal variations in the three water bodies

2. H_0: The microbial load of water samples and oyster meat from the three water bodies will not be higher than recommended levels

   H_1: The microbial load of water samples and oyster meat from the three water bodies will be higher than recommended levels

3. H_0: The concentrations of heavy metals will not be within the acceptable limits for oyster consumption and culture

   H_1: The concentrations of heavy metals will be within the acceptable limits for oyster consumption and culture

4. H_0: The condition index of oysters from the three water bodies will not be influenced by physicochemical parameters

   H_1: The condition index of oysters from the three water bodies will be influenced by physicochemical parameters.

5. H_0: The condition index of oysters from the three water bodies will not be influenced by microbial load

   H_1: The condition index of oysters from the three water bodies will be influenced by microbial load

6. H_0: The condition index of oysters from the three water bodies will not be influenced by heavy metal contamination

   H_1: The condition index of oysters from the three water bodies will be influenced by heavy metal contamination

1.5 Significance of the Study

It is of global concern that commercial oyster production must be done in pristine environments, because of issues of contaminations (microbial and heavy metal) which are related to aquatic pollution, and can pose health problems for human consumers (Perkins, 1995; Pillay and Kutty, 2005).

Available information on the water quality requirements for the optimum growth of bivalves in Ghana is limited (Obodai & Yankson, 2002). This limits bivalve farming as an alternative livelihood opportunity for most Ghanaian coastal communities which do not have suitable environment for the culture of freshwater animals (e.g tilapia and catfish species). This research is therefore necessary to help address this problem, particularly, for the culture of the mangrove oyster in Ghana. This is because *C. tulipa*, which occurs commonly in mangroves and other coastal ecosystems, provides several socio-economic benefits to coastal dwellers in the country (Yankson, 2004). In addition, previous studies have demonstrated that the oyster can be cultured for commercial purposes (Yankson, 2004). Obodai (1997) reported that some coastal water bodies in Ghana are suitable for oyster production on a large scale, based principally on the presence of thriving oyster populations. It

is imperative therefore, to assess the ecological health of some selected water bodies, as a baseline study for commercial production of oysters in Ghana.

1.6 Delimitations of the Study

The study did not cover all aspects of water quality with regard to oyster culture. Although the study focused on monitoring physicochemical parameters, bacterial and heavy metal contamination of the water and oyster meat and determining the condition indices of oysters, the microbial and heavy metal loads of the sediments, as well as the nutrient load and chlorophyll *a* concentrations, amongst others, in the selected ecosystems were not captured. Also, only three coastal water bodies were investigated in the study due to the limited time available for the work.

1.7 Limitations of the Study

The analysis for heavy metals was quite expensive; as a result, samples were collected and analysed quarterly, instead of monthly.

1.8 Definition of Terms

Physicochemical parameters

These comprise some physical and chemical properties of the water bodies that were measured during data collection. The physical parameters included temperature and turbidity whereas, the chemical parameters were salinity, dissolved oxygen and pH.

Bacterial load:

Bacterial load refers the total number of bacteria which were encountered in both water samples and oyster meat during the study.

Heavy metal load:

Heavy metal load refers to the concentrations of heavy metals in both water samples and oyster meat from the three water bodies assessed in this study.

Condition index:

Condition index refers to the "wellness" or "fatness" of shellfishes like oysters. It gives an indication of the physiological condition of the oysters.

1.9 Organisation of the Study

This thesis is organised into six chapters, as outlined below:

Chapter One introduces the study, with a brief background, problem statement, significance of the study, the research objectives, hypotheses as well as the delimitations, limitation, definition of terms and organization of the study. Chapter Two presents relevant literature that were reviewed to establish the theoretical basis of the research, pertaining specifically to water quality characteristics like physicochemical parameters, bacterial and heavy metal contamination and condition index which are relevant to commercial production of oysters and other related species. Chapter Three outlines the various materials, practical methods and procedures which were employed for this research - sampling

methods, laboratory analyses and statistical analyses geared towards the interpretation of the results obtained. Results obtained from the study are presented in Chapter Four. In Chapter Five, the results are interpreted, with reference to relevant information from literature and findings of other researchers. Finally, Chapter Six, the concluding section of the thesis, gives conclusions of the entire research and some recommendations on the way forward.

1.10 Chapter Summary

This chapter gives a brief overview of the importance of shellfish culture to Ghana's fisheries sector. It also presents the mangrove oyster as a potential candidate for shellfish culture and its benefits thereof. The problem which this research seeks to address has been elaborated upon and the purpose and significance of the study have been stated. The objectives and hypotheses upon which this research was conducted have also been specified.

CHAPTER TWO:

LITERATURE REVIEW

The key issues under consideration in this study; physicochemical parameters, bacterial and heavy metal loads and the condition index of oysters, were mentioned briefly in the previous chapter. This chapter however, presents a more detailed review of relevant literature on the aforementioned parameters to provide the appropriate theoretical framework for the research.

2.1 Water Quality

According to Chapman (1996), the term water quality indicates the suitability or otherwise of a water body to support uses or processes. In the context of this research, water quality refers to the suitability, or otherwise, of coastal water bodies for oyster culture in Ghana. It is noteworthy that, although oysters contribute to improving the water quality of aquatic systems, 'pristine' environments are required for their production on a commercial scale (Pereira et al., 2006; Peralta & Andalecio, 2011). Therefore, it is important to assess the quality of any water body earmarked for large scale production of oysters.

Several factors play significant roles in the growth and development of oysters and these are often used as indicators of water quality in oyster growing areas. Factors such as physicochemical parameters (temperature, salinity, dissolved oxygen, pH and turbidity), microbial (coliform) load and heavy metal concentration

are among the key determinants of water quality in oyster habitats (Gosling, 2003; Pillay & Kutty, 2005). Therefore, the determination of their levels in coastal water bodies or oyster habitats is a prerequisite for selecting sites for commercial oyster culture.

According to William (1996) most coastal areas globally, have been degraded by pollution and the resulting effect leaves a negative impact on commercially grown marine organisms. According to the author, the major pollutants of global concern include, but not limited to, organic pollutants, nutrients, radionuclides, oils, pathogens (microbes) and heavy metals. These pollutants are the constituents of pesticides, sewage and fertilizers among others.

Islam and Tanaka (2004) have also noted that in terms of volume, sewage is the most discharged waste in marine ecosystems. Sources of sewage originate from industrial, agricultural, municipal or domestic waste waters (Walters, Thebo & Boehm, 2011). Sewage is biodegradable and is therefore subjected to bacterial decay (William, 1996). As sewage effluents make their way into coastal water bodies, they carry along pollutants including pathogens and heavy metals (Quayle & Newkirk, 1989).

However, in developing African countries, sewage is not properly treated before disposal into the aquatic environment (Kronkvist, 2006), and Ghana is no exception. This problem has a deleterious effect on water quality. The quality of water in oyster growing areas is critical to oyster culturists, as it influences the health status of consumers of the shellfish. The foregoing notwithstanding, Obodai and Yankson (1996), on the basis of the presence of thriving oyster populations,

reported that most coastal water bodies in Ghana were suitable for oyster culture. Work done by Obodai and Yankson (2002), also showed that some Ghanaian coastal water bodies can be used for oyster culture. Some limitations of that study however were that, only a few water bodies were assessed and only physicochemical parameters were considered. In addition, key parameters such as microbial and heavy metal contaminations were not included. It is also noteworthy that, the pollution menace has worsened in the country over the last two decades (see Armah et al., 2010; Adokoh et al., 2011).

2.1.1 Physicochemical parameters

Brackish water ecosystems like estuaries and lagoons usually have dynamic characteristics. Comparing these systems to other aquatic ecosystems, fluctuations in physicochemical parameters are more pronounced in brackish systems, especially in estuaries (Edmunds, 1978). In the tropics for instance, of the two major seasons (dry and wet), during the rainy season, fresh water from land drians into brackish waters en route to the sea. These, coupled with other factors (tides and seasons, amongst others), induce variations in the hydrographic properties of water bodies (Bonsdorff, Blomqvist, Mattila & Nokko 1997; Das & Acharya, 2003).

The consistent changes in these ecosystems determine the type of organisms which can survive in them. However oysters are sessile thus, they are at the mercy of the changing conditions in water bodies that support their growth. As such, they are vulnerable to the changes that occur in their habitats (Lenihan, 1999).

Physicochemical parameters contribute significantly to the distribution of aquatic organisms. According to Quayle and Newkirk (1989), temperature, salinity, DO, pH and turbidity are part of the key factors to consider in the culture of bivalves.

Temperature

Temperature is of critical importance in aquatic ecosystems, because of its role in metabolic reactions and the physiology of the organisms therein (Pillay & Kutty, 2005). It influences the overall physiology of bivalves including feeding, respiration, osmoregulation, spawning, growth, spat setting, length of larval period and relationship with parasites (Lorio & Malone, 1994; Gosling, 2003; Resgalla, Elisângela, Brasil, & Salomão, 2007). Temperature also influences filtration rates in bivalves, by which the accumulation of bacteria is made easy through feeding (Campos, Kershaw & Lee, 2013). Kershaw, Campos and Kay (2012), also reported an inverse relationship between temperature and coliform load of shellfishes. In addition, the protandrous characteristic of oysters is induced by the temperature and salinity of the water overlying their habitats (Yankson, 1990; Gosling, 2003).

The tolerance range for temperature in organisms is subject to the type of species, but generally, bivalves survive within the range of -3 ^{o}C and 44 ^{o}C (Vernberg & Vernberg, 1972). Ajana (1979) reported that the mangrove oyster (*Crassostrea gasar*) was in good condition when water temperature was between 20 ^{o}C and 30 ^{o}C. Furthermore, Yankson (1990), observed that *Crassostrea tulipa* spat developed and settled more successfully within the temperature range of 20 ^{o}C and 30 ^{o}C. Obodai and Yankson (2002) reported 28.3, 30.8 and 30.7 ^{o}C as average

temperatures of three Ghanaian coastal lagoons with thriving oyster populations. Pillay and Kutty (2005) also reported that temperature range between 10 ^{o}C and 30 ^{o}C is ideal for the growth of Mangrove oysters.

Salinity

Salinity is an important factor that controls development and reproduction in oysters (Angell, 1986; Yankson, 2000). Gosling (2003) also posits that salinity may be the most important limiting factor for oyster populations in coastal (brackish) water bodies. In support, Quayle and Newkirk (1989), also stated that salinity is the main variable of influence in tropical bivalve culture. Salinity influences filtration rates, feeding and the sexual cycle of oysters (Gosling, 2003; Kershaw, Campos & Kay, 2012; Sutton, Yankson & Wubah, 2012; Hemachandra & Thippeswamy, 2008).

Oysters of coastal origin are euryhaline organisms, as such, they are able to survive the wide range of salinity fluctuations in brackish water bodies (Gosling, 2003). According to National Estuarine Research Reserves (NERR) (1997) and Gosling (2003), variations in salinity could be induced by tidal fluctuations, location, evaporation, volume of freshwater influx (from rivers and rainfall) and seasons. High precipitation during the wet season reduces salinity, whereas evaporation during the dry seasons causes an increase in salinity (Edmunds, 1978). Dzakpasu and Yankson (2015) also reported that salinity measurements in two coastal water bodies (Kakum and Nyan Estuaries) in Ghana were lower in the rainy season than in the dry season, especially in estuaries. The lower salinities were attributed to the intrusion of freshwater into estuaries and coastal lagoons during

heavy rainfalls, which dilutes the brackish waters. Massive deaths of bivalves (cockles) have been reported in periods of higher rainfall and the resulting lower salinities (Andrews, 1982; Pillay & Kutty, 2005). This may not be a major concern in tropical oyster culture, especially when the oysters can be cultured and harvested prior to the onset of the rainy season, because of the relatively shorter time taken to attain market size (Asare et al., 2019).

Oysters from the tropics, particularly Nigeria, perform well within the salinity range of 20-30 ppt (Ajana, 1980). Yankson (1990), in a laboratory experiment in Wales, found 20-30 ppt as the optimum salinity range for fertilization and larval development of *C. tulipa*. However, Pillay and Kutty (2005) reported that generally, mangrove oysters can withstand wide variations in salinity, often between 5 ppt and 45 ppt, although optimum levels should be below 37 ppt.

Dissolved oxygen (DO)

Dissolved oxygen is required by aquatic organisms for survival. Oxygen is introduced into water bodies through photosynthesis, mixing of surface waters (by wind) and atmospheric diffusion, amongst others (Kamer & Stein, 2003). Other factors like respiration and biodegradation of organic matter reduce DO concentration in aquatic systems (Kamer & Stein, 2003; Xu & Xu, 2016). Bello, Hashim and Haniffah (2017) reported that reduction in precipitation and increase in ambient temperature affect DO availability in aquatic systems.

Deficiency in DO concentration in water can reduce ecosystem productivity by posing survival threats (susceptibility to diseases, reduction in

reproduction and growth) on aquatic organisms or in extreme cases, death (Kamer & Stein, 2003; Xu & Xu, 2016). In addition, anoxic conditions could result in the depletion of species populations and by extension, may affect the aquatic food web.

Generally, DO level of 5 mg L^{-1} and above is suitable for growth and development of aquatic organisms (Pillay & Kutty, 2005), and levels below 5 mg L^{-1} are believed to be detrimental to organisms (Diaz & Rosenberg, 2008). Although Bello et al. (2017) noted that DO between 2 and 3 mg L^{-1} could induce the death of aquatic organisms, DO values within the range of 2 and 4 mg L^{-1} have been reported for some coastal water bodies in Ghana with thriving aquatic biota (Aheto et al., 2011; Dzakpasu & Yankson, 2015). Notably, the water bodies (Butuah Lagoon, Whin, Kakum and Nyan Esturies) which were studied by the aforementioned authors are coastal water bodies which are subjected to daily and seasonal fluctuations in physicochemical parameters. Furthermore, oysters have been found to be tolerant of DO as low as 1 mg L^{-1} (Andrews, 1982). Moreover, Quayle and Newkirk (1989) also stated that the occurrence of bivalves in a given habitat is an indication of satisfactory supply of dissolved oxygen.

pH

pH is the measure of hydrogen ion concentration in aquatic mediums. Carbon dioxide influences pH a great deal (Range et al., 2013). In addition, acidification, brought about largely by global warming, induces reduction in pH in aquatic ecosystems (especially the ocean). The resultant effect is reduced calcification (in shelled organisms), damaged shells, and reduced ingestion and

respiration (Cumming et al., 2011; Range et al., 2013). Contrary to this, in a report by Mackenzie et al. (2014), warming (by high temperatures) rather than ocean acidification reduced shell strength in bivalves. Again, Range et al. (2014) posit that the reduction in pH, in addition to temperature and salinity values outside optimal ranges, may be catastrophic to the survival of bivalves. pH is therefore an important factor in water bodies, especially for shell forming organisms like the oyster (Cumming et al., 2011; Range et al., 2013).

According to NERR (1997), most aquatic organisms survive within a pH range of 5.0 - 9.0. However, Ekubo & Abowei (2011) reported that the suitable pH for biological productivity is between 7.0 and 8.5. For aquaculture, a pH of 6.7- 8.6 is ideal. However, pH levels could reach 9-10 in highly productive water bodies, due to the uptake of carbon dioxide during photosynthesis (Pillay & Kutty, 2005). In addition, the USEPA (1976) reported that, elevated pH values as high as 9.5 in tropical marine water bodies is mainly due to photosynthetic activities.

Works done in Ghana show that the pH of most coastal water bodies in the country are within suitable ranges for aquatic life. Aheto et al. (2011), reported a mean pH value 7.6 in Butuah Lagoon and 8.1 in Whin Estuary. Also, Dzakpasu and Yankson (2015) reported a mean pH range of 5.30 - 6.77 in Nyan Estuary and 6.1-6.8 in Kakum Estuary. It is important that the pH levels in water bodies are within the prescribed suitable ranges because, values of pH less than 4.0 or greater than 10.5 could be lethal for marine organisms (Bhatnagar et al., 2004).

Turbidity

Turbidity in water bodies is due to the presence of suspended particles, brought about by natural (rainfall, influx of freshwater from rivers, land runoffs and bioturbation) and man-made influences (dredging and mining activities) (Mignani, Barbieri, Marques & Oliveira, 2013; Dzakpasu & Yankson, 2015; Okyere, 2019). High turbidity is attributed to the suspension of silt, organic matter and plankton, or a combination of all three, in water bodies (Quayle & Newkirk, 1989; Mignani et al., 2013). Tropical water bodies, especially estuaries are said to have relatively high turbidity (Blaber, 2000, as cited by Dzakpasu & Yankson, 2015).

Extremely high turbidity levels could affect the physiology of bivalves, resulting in reduced respiration, feeding efficiency, growth, reproduction and condition index (Grant & Thorpe, 1991; Ellis, Cummings, Hewitt, Thrush & Norkko, 2002; Norkko, Hewitt & Thrush, 2005) and in worse cases, death (Brick, 1970, as cited by Angell, 1986). In a report by Okyere (2019), high turbidity levels, exceeding the recommended values for water bodies, may have resulted in the decline of *C. tulipa* from the Pra River Estuary (Ghana), probably because the gills of the filter feeding oysters were clogged with silt. In view of such occurrences, intermediate turbidity levels, instead of higher ones are recommended for effective growth of oysters (Dutertre et al., 2009).

A turbidity range of 75 - 240 NTU has been recommended by Zweig, Morton and Stewart (1999) as suitable for aquatic organisms. Reports by Aheto et al. (2011) and Dzakpasu and Yankson (2015) reveal that turbidity levels in some

lagoons and estuaries in Ghana are within this range. However, there are some exceptional cases where turbidity has exceeded recommended levels for coastal waters due to mining activities upstream, as is the case of the Pra Estuary (Okyere, 2019). Quayle and Newkirk (1989) proposed that turbidity may not be of concern to bivalve culture if the levels are not high enough to cause silt deposition and that oyster culture is possible in areas of high turbidity levels.

2.1.2 Microbial contamination

Microbial contamination of water bodies is attributed largely to anthropogenic activities, especially through improper disposal of sewage (Jung et al., 2014). In addition, the contamination of coastal water bodies by bacteria is due to the presence of organic matter from domestic and industrial sewage effluents, urban drainage, run offs from land masses, poor sanitation, domestic animals and roosting birds (Geldreich, 1975; Manna, Das & Manna, 2008; Biancani, Carmichael, Daskin, Burkhardt & Calci 2011; Mignani et al., 2013; Jana et al., 2014; Sorio & Peralta, 2018). Likewise, natural processes like river discharge, erosion, as well as rainfall, augment bacterial load in coastal water bodies (Mill, Schlacher & Katouli, 2006; Huey & Meyer 2010; Walters, et al., 2011; Mignani et al., 2013).

The delivery of nutrient-rich and bacteria-loaded discharges into water bodies via run off, soil leaching, sewage influx and flooding from land origin is high after rainfall (Mill et al., 2006). This is because, there is re-suspension of particulate matter, microorganisms, detritus, etc. from sediments and submerged

aquatic plants to the water column, and these contribute to the elevation of bacteria (including coliforms) in water bodies (Mill et al., 2006; Badgley, Nayak & Harwood, 2010). According to Kirchman, Peterson and Juers, (1984), bacterial loads in a coastal ecosystem was found to be higher during low tides than at high tides. Also, fresh water influx into coastal water bodies during rainy seasons contributes to bacteria abundance in coastal water bodies (Vignesh et al., 2015).

Microbial contamination is very common in Africa, particularly due to poor treatment of waste water before release into water bodies (Kronkvist, 2006). Organic matter from the discharged sewage is biologically degraded by bacteria (William, 1996). Heterotrophic bacteria in aquatic ecosystems comprise all the types of bacteria, including total and faecal coliforms.

Although some coliforms may be non-pathogenic, pathogenic bacteria are mostly classified as coliforms. According to Bartram and Ballance (1996), coliforms are a type of bacteria that are identified as either thermo-tolerant or of faecal origin. They are gram-negative and rod-shaped and tend to share similar characteristics. Also, the presence of coliforms in water may not be critical to the health of other organisms, especially humans, but is however indicative of the presence of pathogenic species which endanger human health. Faecal coliforms are usually found in the intestinal tract of warm-blooded animals, including humans and they are mostly used as indicators of microbial water quality (Bartram & Ballance, 1996). Commonly encountered coliform bacteria in water bodies and oysters are *Escherichia coli, Shigella spp, Salmonella spp, Klebsiella spp, Vibrio*

spp, amongst others (Garnier et al., 2006; Pereira et al., 2006; Saulnier et al., 2010; Trabal et al., 2014; Neta et al., 2015). These bacteria are associated with some illnesses like abdominal pain, cramps, nausea, vomiting, diarrhoea, fever, dehydration and gastroenteritis (Hariharan & Amadi, 2016; Venugopal & Gopakumar, 2017).

While bivalves are useful indicators of microbial water quality, it is worthy to note that the consumption of contaminated bivalves results in both acute and chronic diseases (Perkins, 1995; Pillay & Kutty, 2005). This is because, bivalves accumulate loads of microorganisms like bacteria, through feeding (Pereira et al., 2006; Venugopal & Gopakumar, 2017; Sorio & Peralta, 2018). As such, much attention is given to the levels of coliforms in water bodies and shellfish habitats, especially for coliforms of faecal origin (Pereira et al., 2006; Peralta & Andalecio, 2011; Hariharan & Amadi, 2016; Venugopal & Gopakumar, 2017; Sorio & Peralta, 2018). Therefore, bacteria accumulation in bivalves is used to assess the health risks associated with the consumption of contaminated bivalves (Anacleto, Barrento, Nunes, Rosa & Marques, 2014). In support, Neta et al. (2015) indicated that microbial quality evaluation is among the essential parameters in oyster culture.

According to Anacleto et al. (2014), anthropogenic factors, climate and season determine the types of bacteria that are found in marine systems. Also, environmental factors like temperature, dissolved oxygen, salinity and turbidity influence bacteria retention in bivalves.

Some works related to microbial contamination in food have been reported in Ghana (Saba & Gonzalez-Zorn, 2012; Ameko, Achio & Kassim, 2012; Esena & Owusu, 2013). Kombat, Nunoo, Ampofo and Addo (2013) also reported on bacterial contamination of some fresh fishes landed in Ghana. Studies on dry fishes have also been reported (Lu, Pace & Plahar, 1998).

In terms of the quality of oyster meat and in water from which they were harvested, several reports from other African countries are available: In Nigeria, studies have been conducted on the effect of depuration on the microbial load of mangrove oysters (Okereke, Ezeama, Davis & Ezeonyejiaku, 2017); mortality rate and microbial load in fresh, processed and stored oysters (Amadi, 2016); the effect of preservatives on oysters (Efiuvwevwere & Amadi, 2015); bacterial and PAHs accumulation in oysters (Eduok, Ebong, Udoinyang, Njoku & Eyen, 2010) and the effect of sun and oven drying on the nutritional composition of oysters (Adebayo-Tayo & Ogunjobi, 2008). In Sierra Leone, Gordon and Davey (1935) reported on bacterial contamination in oysters. Studies have been conducted on the sanitary quality of oysters from The Gambia (Rice et al., 2015). Coly, Sow, Seydi and Martinez-Urtaza (2013) reported on bacterial contamination in oysters from Senegal. In South Africa, Watling (1982) also reported on the effect of some heavy metals on the growth of oysters. However, in Ghana, little has been done concerning microbial load in oysters. For example, Obodai, Nyarko and Amponsah (2010), reported some findings on microbial (fungal) load in oysters. However, to the best of my knowledge, no work has been done in detail, to assess the bacterial load in Ghanaian oysters. Nonetheless, work done by Adjei-Boateng, Amisah and

Quagrainie (2009), confirmed that the Volta estuary is contaminated by bacteria and in effect, the clam species (*Galatea paradoxa*) found there are equally contaminated.

Owing to the effect of microbial load contamination in oysters on the health of human consumers, some methods have been suggested for ridding oysters of bacterial contaminants. Wittman and Flick (1995), suggested depuration and relaying as effective methods for decontaminating oysters. According to Anacleto, Pedro, Nunes, Rosa and Marques (2013), depuration was effective in reducing *E. coli* contamination in clam species. Obodai et al. (2010) further showed that there was both a reduction and complete removal of fungal contaminants in oysters from Benya Lagoon in Ghana. The success of depuration on the decontamination of bacteria contaminated oysters gives hope for commercial production of the species in Ghana. This may however come at a cost. Hence studies to identify contamination-free areas or prevention of contamination of water bodies to be used for oyster culture is a cost effective venture.

2.1.3 Heavy metal contamination

The term 'heavy metals' is given to metal or metalloid elements which have relatively high densities (3.5 - 7 gcm^{-3}). Examples of heavy metals are mercury (Hg), arsenic (As), cadmium (Cd), cobalt (Co), lead (Pb), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn). Heavy metals in the environment could be toxic at low or high concentrations, especially when their levels exceed tolerable thresholds. Usually, the most destructive metals like Hg, As, Cd, Pb and Ni are

toxic at low concentrations. However, because Cu and Zn are essential elements and are thus required for growth and development in humans, they only become toxic at higher concentrations (Islam & Tanaka, 2004; Gautam, Sharma, Mahiya, & Chattopadhyaya, 2014).

Heavy metals occur naturally and are largely found in the earth's crust (Camusso & Gasparella, 2006). Natural processes (geological weathering of rocks and atmospheric deposition) are usually pathways for these metals into the environment. However their availability may be augmented by anthropogenic processes (Jan et al., 2015). Some of the anthropogenic activities include mining, electroplating, electric and metal finishing, industrial discharge and domestic effluents, landfill leachates, fishing and boating activities have been identified as potential routes for metals into the environment (Thomson, Luoma, Johansson & Cain, 1984; Gautam et al., 2014). The mode of entry of heavy metals into water bodies include runoffs from agricultural, industrial and urban land use, anthropogenic activities and atmospheric deposition, among others (Tomlinson, Wilson, Harris & Jeffery, 1980; Biswas, Bandyopadhyay & Chatterdee, 2013; Farrell et al., 2018).

In aquatic systems, metals are re-suspended into water column from the sediments and are ingested by aquatic organisms through feeding (by assimilation of bioavailable metals, especially through their gills and alimentary canal). As a result, the metals accumulate in the tissues of living organisms and magnify in food chains (WHO, 2007; Peer, Safahieh, Sohrab & Tochaii, 2010; Dabwan & Taufiq,

2016), because they are usually not biodegradable in nature (Camusso & Gasparella, 2006). Biomagnification of these metals, due to the inability of living organisms to metabolize them, causes them to become toxic through long term accumulation (Bharti, Tyagi & Singh, 2014; Salem, Eweida & Farag, 2000).

Bivalves are known to accumulate heavy metals at varying degrees. For instance, in a report by Dabwan and Taufiq (2016), *Anadara granosa* was found to accumulate more Cd and Pb, whereas *Polymesoda expansa* accumulated more Cu and Zn. Also, Delshab, Farshchi, Mohammadi and Moattar (2017) reported that the Cd and Pb loads in the oyster, *Saccostrea cucullata*, were below allowable limits whereas those of Cu and Zn exceeded the limits. However, researchers have different opinions on this matter, because the exact pathway for these metals is not fully understood. Cadmium, for example, is believed to be absorbed in solution (Van Hattum et al., 1989), whereas copper is assimilated through feeding (Han & Hung, 1990). Again, Guzman-Garcia, Botello, Martinez-Tabche and Gonzalez-Marquez (2009) and Gautam et al. (2014), reported that heavy metals accumulated in oysters were not eliminated during depuration thus, the tendency for them to pose physiological and metabolic stresses on the bivalves is high. Contrary to this, Lim, Lee and Din (1998), reported that depuration has the possibility of lowering metal contamination in oysters. In order to ascertain the levels of heavy metals in aquatic ecosystems, measurements are usually done in water, sediment and fish samples (Camusso & Gasparella, 2006).

In Ghana, several studies on heavy metals have been conducted on various water bodies (Bamford, Osae, Aboh, Biney & Antwi, 1990; Adomako et al., 2008; Tay, Asmah & Biney, 2008; Armah et al., 2010; Obodai et al., 2011). Also, some studies have been conducted to determine the level of heavy metal concentration in oysters (Adokoh et al., 2011; Obodai et al., 2011). In addition, studies by Otchere (2003) revealed that oysters, mussels and clams were contaminated with heavy metals. Furthermore, Adjei-Boateng, Obirikorang and Amisah. (2010) and Amisah, Obirikorang and Adjei-Boateng (2011) reported heavy metal contamination in clams.

Heavy metal contamination (poisoning) is followed by associated health risks such as interference with metabolic activities, damage to the skeletal and peripheral nervous systems and memory loss (Habte et al., 2015; Jaishankar et al., 2014). Also, reports by Khayatzadeh and Abbasi (2010) showed that, heavy metal contamination caused growth and developmental anomalies, as well as a reduction of survival in fishes. The heavy metals of interest to the present study are cadmium, lead, zinc and copper.

Cd and Pb were selected for this study because of their toxicity at low concentrations. Cu and Zn on the other hand, are useful at low concentrations, but were selected because of their toxicity at higher concentrations; and oysters have been reported to accumulate these metals in higher concentrations. Higher concentrations of these metals in oysters could be detrimental to human consumers. As and Hg were removed from this study due to the cost of analysis.

Cadmium

Cadmium is considered a pollutant because it has no essential contribution to ecosystem health, yet its presence in aquatic systems only brings havoc (Sagyi, Deniz, Kutsal & Vural, 1991). One way through which cadmium enters aquatic systems is by binding with calcium, and upon entry, it becomes lethal to aquatic organisms (Verbost et al., 1989). Additionally, cadmium contamination can be associated with fertilizer contamination from farm lands. As a result, the metal is absorbed by phytoplankton, and subsequently, the phytoplankton is ingested by oysters (Butler & Timperley, 1996). It was evident from the work of Sokolova, Ringwood and Johnson (2005) that cadmium accumulation in oysters resulted in some physiological effects on their gills and hepatopancreas. According to Thrower and Euatace (1973), the consumption of cadmium contaminated oysters may also cause nausea and vomiting. WHO (2007) and Luo et al. (2018) also reported bone and kidney damage and cancer of the lungs as diseases associated with the consumption of cadmium contaminated oysters.

Lead

Lead contamination in the environment is of global concern. In water bodies, lead pollution originates from automobile and leaded fossil fuels during fishing and leisure activities (Zaroogian, Morrison & Heltshe, 1979; Hossen, Hamdan & Rahman, 2015). Gill function in fish is affected by lead pollution and fry and embryo of fishes suffer the most effect (Solomon, 2008). Contamination by lead has been associated with lethal effects throughout the stages of human life,

beginning with developmental and neuro-behavioural damage in foetuses, infants and children, to blood pressure elevation in adults (WHO, 2007). In addition, musculoskeletal anomalies, oral cleft and other birth defects have been attributed to lead pollution (Vinceti et al., 2001).

Zinc

Zinc contamination in water bodies has harmful effects on the aquatic system. Naito, Kamo, Tsushima and Iwasaki (2010) attributed zinc contamination to corrosion and storm water runoffs. Brereton, Lord, Thornton and Webb (1973), reported that it is imperative to assess zinc concentration in oyster farming site because of its ability to severely affect oyster breeding. The same report also attributed larval mortalities and decrease in growth of oysters to zinc contamination. Furthermore, supressed spat growth due to zinc contamination in oysters was reported by Boyden, Watling and Thornton (1975). In humans, zinc, together with copper, have been reported to cause cancer-induced mortalities (Leone, Courbon, Ducinmetiere & Zureik, 2006).

Copper

Copper contamination in the aquatic environment results from anthropogenic activities like mining, copper ores refining, milling, industrial smelting and fertilizer application (Shrivastava, 2009). Like other heavy metals, copper is assimilated by aquatic organisms via feeding and exposed body parts (Lias, Jamil & Aliaa, 2013). Reports by Liu et al. (2014) revealed that, in bivalves which were exposed to heavy metal contamination, copper was found to cause a

decrease in their gonado-somatic index. Also, DNA damage and abnormalities in larval developments were attributed to copper contamination in oysters (Mai et al., 2012). According to Thrower and Euatace (1973), copper contamination from oyster consumption led to nausea and vomiting in humans.

2.1.4 Condition index

According to Drexler et al. (2014), condition index refers to the physiological condition of oysters. It may also be described as the degree of plumpness or fatness of an oyster meat or how well the oyster meat fills the inner cavity of their shells (Quayle, 1980). CI is a good indicator of sexual maturity in bivalves, because most bivalves have better condition when they are spawning (Li, Qin, Li & Benkendorff, 2009). CI also determines commercial quality for most cultured organisms (Davenport & Chen, 1987), and is thus an instrumental factor which indicates the best time of harvest for commercially produced bivalves (Yankson, 2004). Lagade et al. (2011) reported that a good condition of oysters was dependent on their nutritive status. Oysters tend to have better CI during their reproductive cycle because of the induced bulkiness in their visceral mass (Gosling, 2003; Hemachandra & Thippeswamy, 2008; Freites et al., 2010). Krampah, Yankson and Blay (2016), also reported that the brown mussels *Perna perna* had better condition index during gonadal development than during spawning.

Mercado-silva (2005) indicated the degree to which environmental factors impact the condition of oyster meat. For example, parasites like pea crabs reduced oyster condition index significantly and oysters which remained submerged had

good condition index. Bacterial contamination of oysters have shown deteriorating effect on oyster condition indices (Hood, Ness, Rodrick & Blake, 1984; Jana et al., 2014). Heavy metals have been reported to have variable influence on CI. For instance, the report by Rebelo, Amaral and Pfeiffer (2005) showed that heavy metal contamination did not affect the CI of oysters. In contrast, Yap and Al-Barwani (2012) reported that heavy metal accumulation in mussels from Muar in Egypt negatively impacted their CI. Furthermore, Ismail and Yap (1999) also reported that heavy metals had a significant negative correlation with the CI of green-lipped mussels (*P. viridis*) from Malaysia.

It is evident from the foregoing literature that prior to embarking on commercial production of oysters in Ghana, it is important to concurrently evaluate relevant physicochemical parameters, microbial and heavy metal loads, and oyster condition in coastal water bodies. The outcome would facilitate site selection, as well as suggest remedial measures, where necessary, to salvage potential sites for mass production of oysters.

2.2 Chapter Summary

This chapter elaborated on the areas of interest in this study- water quality of oyster habitats. Specifically, the works of other researchers on suitable physicochemical parameters, the effects of bacterial and heavy metal loads on oysters and consumers, and the importance of condition index in bivalve culture have been reviewed, as a theoretical basis for this research.

CHAPTER THREE:

MATERIALS AND METHODS

The previous chapter presented a review of the relevant literature to the present study focusing on physicochemical parameters, microbial and heavy metal loads and the condition index of oysters. This chapter describes the study areas and the materials and methods used for executing the work, as well as an outline of how the data were analysed statistically.

3.1 Study Sites

The study was carried out in three water bodies along the coast of Ghana namely; Whin Estuary in Takoradi (Western Region), Nakwa Lagoon at Ekumfi Nakwa (Central Region) and the Densu Delta at Tsokomey (Greater Accra Region). The selected water bodies have thriving oyster populations and the surrounding communities actively harvest the oysters for food and income.

3.1.1 Whin Estuary

The Whin Estuary is located near Takoradi (Western Ghana), approximately 5° N, 1° 46′ 30″ W (Figure 3.1A). The estuary spans an area of 652,202 km^2 and takes its source from the Whin River. The river has two tributaries which pass through some suburbs of Takoradi, before joining the estuary. The sediment at the mouth of the estuary consists of two different substrates - sand on the west and rocks on the east. A vegetation of mangroves fringes the banks of the

estuary. Oysters were found attached to the stilt roots of mangroves and on the bottom sediment, however the oysters were mostly exposed at low tide (Appendix A). Birds, crabs and juvenile fishes were among other fauna inhabiting the mangroves. The estuary is exploited by riparian communities primarily for fishing, harvesting of oysters (Appendix A), fuel wood (mangrove tree) and transportation (personal observation).

3.1.2 Nakwa Lagoon

Nakwa Lagoon is an intermittent open lagoon which is located in the Ekumfi District of the Central Region of Ghana. The lagoon lies within the coordinates 5° N, 0° 55′ 30″ W (Figure 3.1B). The Nakwa River drains into the sea via the Nakwa lagoon. According to Amoah (2015), the people of Nakwa are involved in both fishing and harvesting of shellfishes like oysters and cockles (Appendix B). Salt mining (Ghana Statistical Service, 2014) and sand winning (Amoah, 2015) are also part of the activities around the lagoon. Homesteads, mixed vegetation of grasses and trees, including very few mangrove shrubs were seen along the landward bank of the lagoon. The seaward bank is characterised by a few coconut trees and some patches of grass, along parts of the sandbar that separated the sea from the lagoon (personal observation). The oyster bed is usually exposed at low tide (Appendix B).

3.1.3 Densu Delta

The Densu Delta is located about 11 km south west of Accra and lies approximately 5° N, 0° 19′ 10″ W (Figure 3.1C), in the Greater Accra Region of

Ghana. The dammed Densu River feeds into the delta and eventually enters the sea via two openings. Residential buildings are located around the water body. The inhabitants from the surrounding communities utilize the water body for fishing (both finfish and shellfish including oysters (Appendix C) and crabs) and also for salt mining (Osei et al., 2010). The oyster bed is usually exposed during low tides (Appendix C). A few mangrove trees were seen around the delta during the sampling period. The sparse mangrove vegetation may be due to the consistent harvesting of the mangrove trees by fishermen for the Acadja fishing practice (personal observation). A close observation of the vegetated areas surrounding the delta suggests that the mangrove vegetation may have been denser in the past.

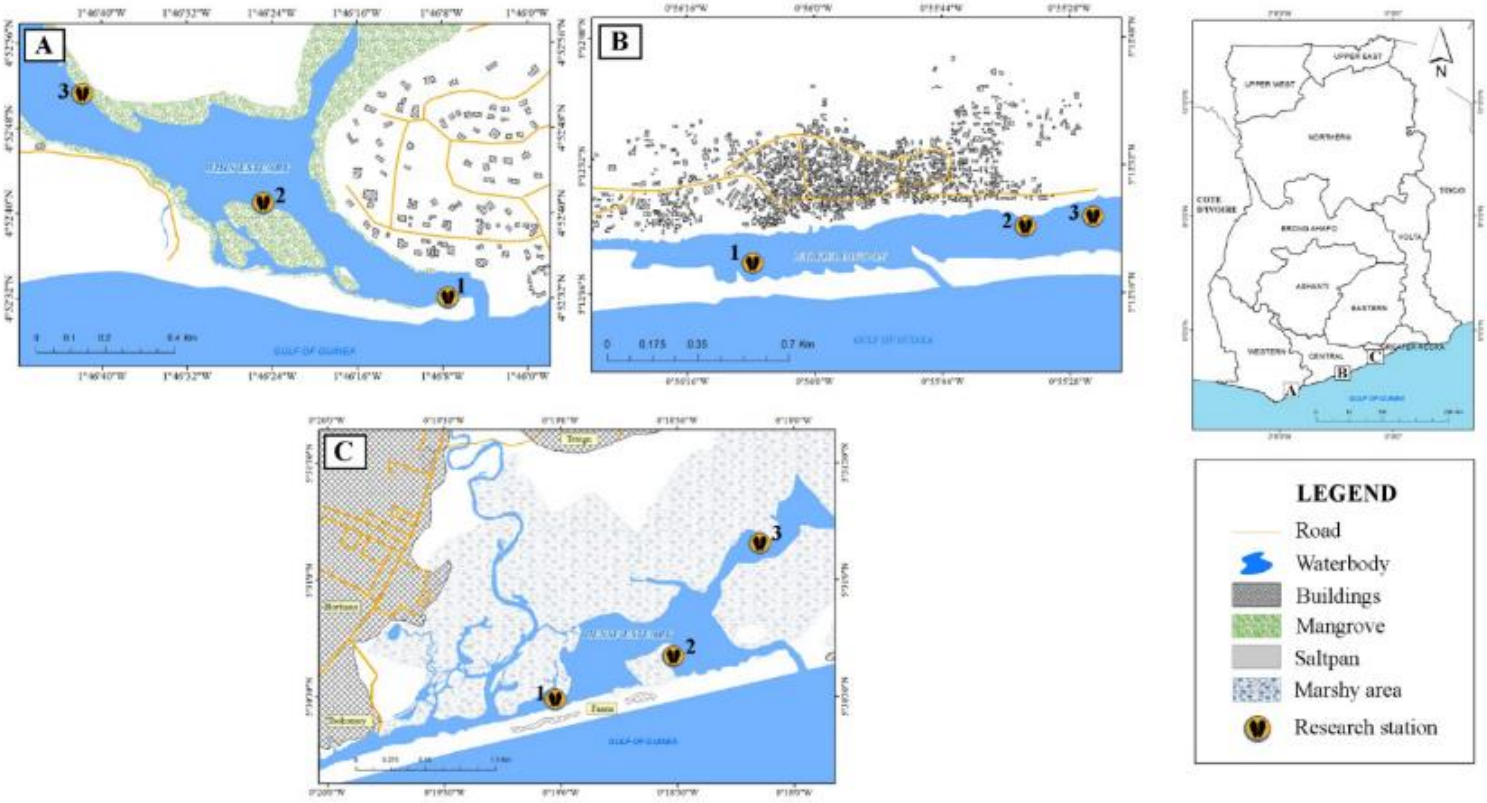

Figure 3.1: Maps of the three water bodies assessed for the culture of the mangrove oyster (A: Whin Estuary, B: Nakwa Lagoon and C: Densu Delta)

3.2 Sampling Procedure and Laboratory Analyses

Monthly samples were collected from all three sampling sites from October, 2017 to July, 2018. Within each site, three stations were established namely, Station 1 (close to the mouth), Station 2 (around the middle) and Station 3 (around the head i.e. towards the river). However, due to the dynamic nature of the water bodies, this demarcation was not strictly applied in all the water bodies. In Nakwa for instance, the mouth of the lagoon got closed naturally in April 2018. Thus, there was a change in the location of the mouth after the sand bar was artificially breached. Densu Delta also had more than one opening into the sea (one around Station 1 and the other around Station 2). However, as much as possible, the stations were representative of the estuarine portions where oysters were found.

Unlike Whin and Nakwa water bodies, in the Densu Delta, the areas where oysters are harvested had earlier been demarcated (Janha, Ashcroft & Mensah, 2017). Based on the demarcation, Station 1 was located at Kpovoduvo around Faana locality. Stations 2 and 3 were located along the Kele/Wegame section. However, whereas Station 2 was closer to the mouth of the delta which is situated along that section, Station 3 was located towards the head.

The monthly sampling and measurements were done at low tide from the three sampling stations within the sampling sites. Measurements of physicochemical parameters were done in triplicates and water samples were also collected in triplicates from each station. However, oyster samples were collected as a composite sample for each station. Sterile hand gloves were worn before samples were taken, to prevent contamination of samples. Water samples

were collected in sterile 250 ml bottles, whereas the oyster samples were hand-picked into sterile plastic bags.

3.2.1 Physicochemical parameters

The measurements of temperature, salinity, dissolved oxygen (DO) and turbidity were done *in situ*. DO and temperature readings were taken with the DO meter (YSI Environmental EcoSence® DO200A). Salinity was measured with a hand-held refractometer (E-LINE Refractometer 44-8080) with readings in parts per thousand (ppt). The turbidity measurement was taken with a turbidimeter kit (OAKTON® Turbidimeter T-100), which measures in Nephlometric Turbidity Unit (NTU). For pH, water samples were collected in small plastic bottles and transported (on ice) to the Department of Fisheries and Aquatic Sciences (DFAS) laboratory for measurement using an OAKTON® pH 700 meter.

3.2.2 Microbial load determination

Sample collection

The microbial load analysis conducted in the study focused solely on bacteria. Water samples were collected into 250 ml sterile bottles from all three sampling stations of each sampling site. Samples were collected from about 5 cm below the water surface. Oyster samples were collected from only two stations (stations two and three) in Whin estuary and Nakwa lagoon and from all three stations in the Densu Delta. This was because oysters were not present at Station 1 of the first two water bodies. Oysters were handpicked from the sediments of the three sampling sites at low tide (Appendix C). At Station 3 in

the Densu Delta however, the oysters were collected by diving, as the water was deep even at low tide. The oyster samples were placed in sterile polythene bags and were transported (on ice) to the laboratory of the Department of Molecular Biology and Biotechnology (MBB), University of Cape Coast.

Methods of sterilization

Glassware, culture media and pipette tips were sterilized using an autoclave at 121°C for fifteen minutes. The sterilized glassware and pipette were oven dried and allowed to cool before use. The culture media was also allowed to cool before use.

The laminar flow cabinet was thoroughly wiped with cotton soaked in 70% ethanol, in order to disinfect the work area. Sterile hand gloves were used throughout the procedure and disinfected with 70% ethanol intermittently.

Sample processing

All samples were prepared under aseptic conditions, within a laminar flow cabinet. An outline of the preparation of the culture media has been presented in Appendix D.

Water samples

A composite sample was prepared from the replicates from each station. This was done by pipetting 2 ml of water from each replicate sample into a sterile test tube, using a sterile pipette. The composite sample was thoroughly mixed and serially diluted based on the degree of turbidity.

Oyster samples

The procedure by Adjei-Boateng et al. (2009) was adopted with modification as follows: The oyster specimens were thoroughly washed with sterile distilled water and air dried. Thereafter, a sterile scalpel was used to aseptically shuck open the oysters. Subsequently, approximately 1 g of the oyster meat was aseptically transferred into a sterile plastic bag. Nine ml of sterile normal saline was added to the oyster meat, after which the meat was gently macerated. The homogenate obtained was subjected to 10-fold serial dilutions, following initial trials, and used as inocula for subsequent analysis.

Inoculation

The Pour Plate method of inoculation was adopted using two bacteriological media - MacConkey agar, for the isolation of coliform bacteria and Plate Count (PC) agar, for the isolation of viable bacteria, to estimate the total coliform and total viable bacterial respectively. This method was used for both water samples oyster meat as follows:

A sterile pipette was used to transfer 1 ml of inoculum into empty sterile petri dishes, after which approximately 15 ml of cooled molten agar medium were added to each. In order for the inoculum and the agar to mix thoroughly, the petri dishes were swirled clockwise and anticlockwise (three times each), after which the medium was allowed to solidify. This was repeated in triplicate for each sample. Thereafter, the petri dishes containing the same media were packed in sets into polythene bags and were incubated in the Gallenkamp Plus II incubator for 12 to 18 hours, at 35 ^{o}C.

Inspection of cultures

After the incubation period, the colonies that grew on the agar plates were counted and recorded. The bacterial counts were expressed as the number of colony forming units per ml of water or per gram of shellfish, for water and oysters respectively. The bacterial load was established based on the colony counts.

Storage of bacterial isolates

Colonies were randomly picked from selected plates using a sterile inoculation loop. The picked colonies were aseptically transferred into sterile nutrient agar slants contained in Eppendoff tubes and labelled accordingly. The inoculated nutrient agar slants were incubated at 35 ^{o}C for 12-18 hours, to allow for the growth of the inoculated bacteria (Appendix E), after which they were stored in a refrigerator at 4 ^{o}C for subsequent identification.

Sub-culturing

Stored isolates were aseptically and individually sub cultured on MacConkey agar by streaking with an inoculation loop. The plates were then incubated at 35 ^{o}C and the culture plates were examined at 18 hours post incubation (Appendix E).

Identification of stored bacterial isolates

The pure culture obtained were subjected to biochemical test, using four different media namely, peptone water, triple sugar iron (TSI, also known as kliger iron agar (KIA)), Simmon citrate agar and urea base agar.

Biochemical tests

Single colonies were aseptically transferred from the plates into test tubes containing 4 ml of sterile normal saline. The bacterial suspensions obtained were vortexed in order to get the bacterial cells evenly distributed in the solution. Using a sterile inoculation needle, the bacterial suspensions were inoculated unto all four media slants, by aseptically stabbing the needle through the medium and streaking on the slant surface afterwards. In the case of peptone water, after picking the colonies, the inoculation needle was used to stir the media, to release the colonies. After streaking, the media were incubated for 18 hours at 35 ^{o}C. Thereafter, samples were examined for indicator colour changes (Appendix F).

The TSI agar was used to detect the fermentation of glucose, lactose and sucrose, which is indicated by colour changes (yellow or red) on the slant and in the butt of the medium and also, for gas production and the presence or absence of hydrogen sulphide (indicated by black colour) (Appendix F). A blue colour on the Simmon citrate agar was recorded as positive whereas, no colour change was recorded as negative (Appendix F). The citrate test was carried out to determine the possibility of the bacterial isolate surviving on citrate as the sole source of carbon.

The urea test shows which organism is capable of producing urease and thus, can breakdown urea into ammonia. For this test, the positive colour indicator was pink while a negative reaction was indicated by the absence of a colour change (Appendix F). For the indole test with peptone water, 0.5 ml of Kovac's reagent was added to the solution. The solution was gently swirled and allowed to stand for about 5 minutes. The occurrence of a red ring on the surface of the inoculum indicated a positive colour and a negative reaction did not induce any colour change (Appendix F).

Based on the four tests conducted, the results obtained from the biochemical test were compared to a chart described by Cheesbrough (2006) and subsequently used to generate a list of identified bacterial species encountered in this study (Appendix G).

3.2.3 Heavy metal load determination

Sample collection

Water samples, from about 5 cm below the water surface, were collected into labelled plastic bottles in triplicate. The samples were preserved on ice and transported to the laboratory.

Oyster samples were hand-picked from each station into labelled plastic bags, placed on ice and conveyed to the laboratory. Oyster samples were not collected in triplicates like the water samples, rather, about five oyster individuals were picked from each station and treated as a composite sample for the respective station.

Sample preparation

Water samples

Five drops of nitric acid were added to samples for preservation, to prevent the deterioration of the samples, in the event of prolonged storage for analysis. The samples were later transported to Ghana Atomic Energy Commission (GAEC) for analysis.

Oyster sample:

Samples were thoroughly washed and air dried. Oyster individuals (measuring about 5 mm) were selected and then shucked and the meat was placed on aluminium foil and oven dried at 70°C for three days. The dried meat was then crushed in a laboratory (porcelain) mortar with a pestle. The powdered meat was weighed, bagged and thereafter sent to GAEC for analysis.

Digestion

Water digestion

Forty ml of the water sample was weighed and transferred into a 100 ml class A beaker. Aqua regia solution was prepared by mixing concentrated hydrochloric acid (HCl) and nitric Acid (HNO_3) in the ratio of 3:1 (3 ml HCl and 1 ml HNO_3). Thereafter, 5 ml of the aqua regia solution was added to the water sample in the beaker. The beaker was covered with a cling film and placed on a hot plate for digestion for 3 hours at a temperature of 45 °C. After digestion, the digestate was transferred into a 50 ml measuring cylinder and topped to the 30 ml mark with double distilled water. The solution was then transferred into

a test tube for analysis, using the Varian AA 240FS- Atomic Absorption Spectrophotometer (AAS).

Oyster digestion

Two grams of the powdered oyster meat were transferred into a 100 ml borosilicate beaker. Thereafter, in a film chamber, 20 ml of concentrated nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2) were added to the beaker containing the powdered oyster. The beaker was covered with a cling film and placed on a hot plate at a temperature of 45 °C. Digestion was done for 3 hours, after which the digestate was transferred into a 100 ml measuring cylinder. Distilled water was added to top the solution to the 20 ml mark. The solution was then transferred into a test tube for AAS analysis.

Heavy metal analysis

The water and oyster digestates were then assayed in duplicated blanks for the presence of cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn). The analysis was done with the AAS in an acetylene-air flame, using the following quality control and quality assurance techniques:

Blanks: To check the sensitivity of the instrument and determine the detection limit of the instrument.

Duplicates: To check the reproducibility of the method used.

Standards: To check the accuracy of the equipment being used.

Blanks and duplicates of samples were digested in the same conditions as the samples. These served as internal positive controls. Reference standards were

used for the elements of interest. The reference standards used are from Fluka analytical and Sigma-Aldrich Chemie GmbH, and product of Switzerland (Appendix H).

3.2.4 Condition Index

The condition index (CI) was determined using the procedure and formula adopted by Yankson (1986).

Thirty individual oysters were collected from each sampling site. The specimens were bagged and transported on ice to the DFAS laboratory. In the laboratory, they were thoroughly washed with a brush to remove all debris and sediment deposits adhering to the shells of the oysters. Fouling organisms which were exposed after washing were scraped off with a scalpel and washed off with water. The cleaned oysters were sequentially arranged on a tray and allowed to air dry. Drying was facilitated with paper towels, where necessary. After about fifteen minutes of drying, the weight (in grams) of the oysters was determined with an electronic weighing scale.

The whole volume of each oyster was determined by the displacement method, whereby individual oysters with tightly closed valves were carefully dropped in a measuring cylinder containing water. The initial and final water levels were recorded during the procedure. The difference between the two sets of measures was recorded as the volume of the whole oyster. The oysters were thereafter shucked open with a knife to remove the meat. The meat was picked with a pair of forceps, dried (with a paper towel) and displaced in like-manner, to determine the volume. The volumes of empty shells of each oyster were

similarly determined by displacement. The CI was then calculated using the formula below.

$$C.I = \frac{\text{Meat volume}}{\text{Whole volume} - \text{Shell volume}} \text{ X } 100$$

3.2.5 Statistical analyses

The results obtained from the various laboratory analyses were recorded using spreadsheet (Microsoft Excel). Significant differences between the parameters recorded during the study were statistically tested by Analysis of Variance (ANOVA), using Minitab 17 software. *P*-values $\leq$ 0.05 were considered as statistically significant at a confidence level of 95%, whereas *p*-values > 0.05 were considered as not statistically significant. Principal Component Analysis (PCA) was conducted to identify significant physicochemical parameters that could influence the oyster. The PCA was done with Statistical Package for Social Sciences (SPSS) software. Multiple linear regression analysis was conducted to determine the relationships between physicochemical parameters, microbial load, heavy metal load and the condition index of oysters. Negative binomial regression analysis was also conducted to determine the effects of physicochemical parameters on the microbial load of water samples from the three water bodies. The regression analyses were extrapolated using the South Texas Art Therapy Association (STATA) software. Line graphs and bar charts were plotted using Microsoft Excel.

3.3 Chapter Summary

In this chapter the sampling sites were described. This was followed by a detailed description of the materials and methods used to collect the data from the field and the laboratory, where necessary. Finally, the statistical tools that were used to analyse the data were stated.

CHAPTER FOUR:

RESULTS

The results obtained for the entire period of study (October, 2017 to July, 2018) are presented in this chapter. The data were analysed using statistical tools, as outlined in Chapter three and the outcome presented in graphs and tables where necessary. The results have been outlined according to the research objectives.

4.1 Physicochemical Parameters

Monthly variations in physicochemical parameters- temperature, salinity, DO, pH and turbidity are presented below. The mean values of the parameters have been presented as mean ± standard error of the mean.

4.1.1 Temperature

Surface water temperature trends were generally similar in the three water bodies (Figure 4.1). The highest temperatures for the Nakwa Lagoon and Densu Delta were observed in October 2017 as 32.0 ± 0.7 °C and 31.6 ± 0.2 °C respectively and in February 2018 for Whin Estuary as 32.0 ± 0.7 °C. The lowest temperatures were recorded in July 2018 as 25.2 ± 0.1 °C, 24.9 ± 0.3 °C and 26.8 ± 0.1 °C for Whin, Nakwa and Densu respectively. In general, the highest and lowest temperatures for the study period were recorded in the Nakwa Lagoon. The mean temperatures recorded were 29.5 ± 0.3 °C for Whin, 29.7 ± 0.3 °C for Nakwa and 29.6 ± 0.2 °C for Densu. There were no significant differences ($p \geq 0.05$) in temperatures between the water bodies from November 2017 to April 2018 (Appendix I).

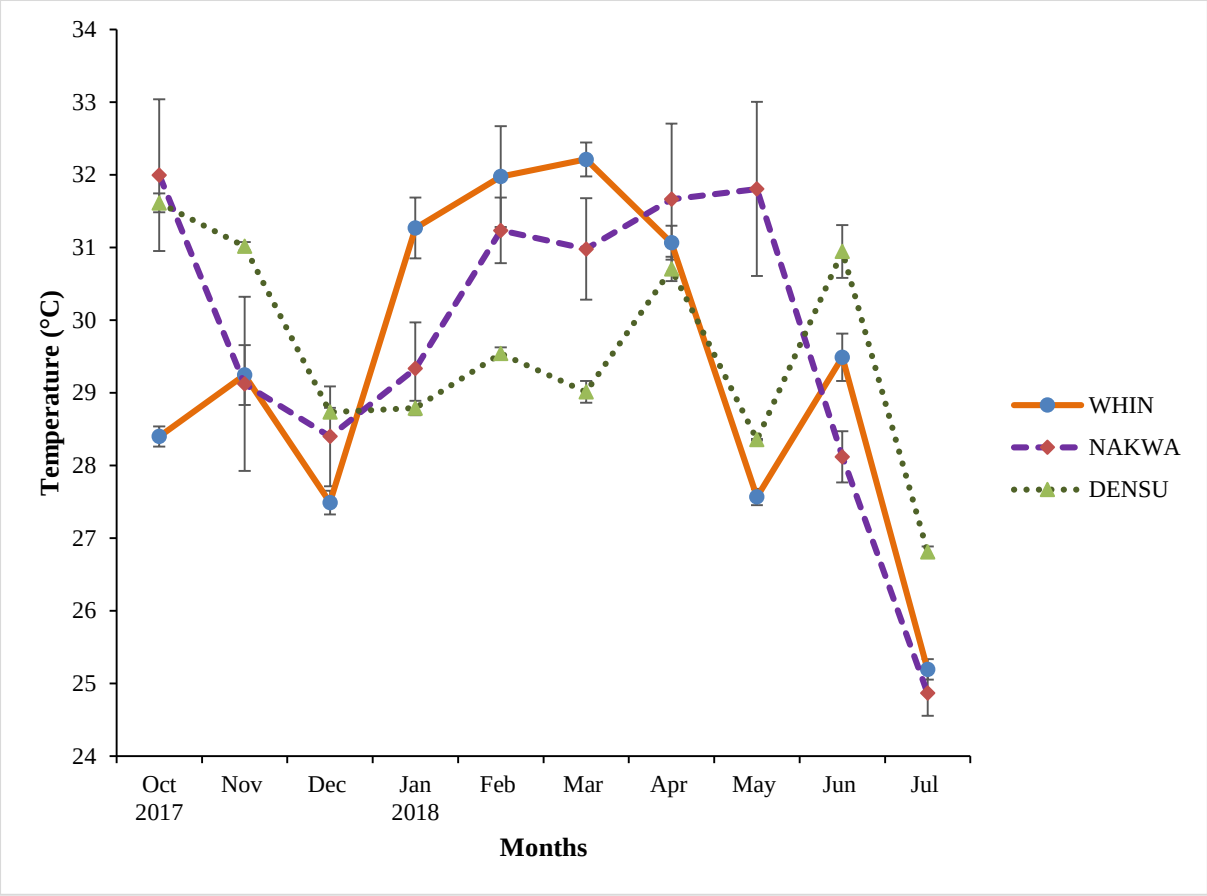

Figure 4.2: Monthly variations in water temperature in Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

The ambient monthly air temperatures (Appendix J), for Takoradi, Saltpond and Accra, representative of Whin, Nakwa and Densu, respectively, showed similar trends that more or less mirrored the trends in the respective water bodies.

4.1.2 Salinity

Monthly variations in the salinities of the water bodies during the period of study are illustrated in Figure 4.2. It is evident that the salinities showed distinct fluctuations during the study period. Densu Delta and Nakwa Lagoon showed similar trends in the salinity fluctuations except in May and July 2018 when the trends changed. These two water bodies generally had higher salinities with mean values of 18.19 ± 1.20 ppt and 20.42 ± 0.98 ppt respectively compared with 16.55 ± 1.04 ppt in Whin. Densu had the highest salinity of 31.96 ± 0.89 ppt in May 2018 and lowest value of 2.67 ± 0.82 ppt in July 2018. Nakwa recorded the highest salinity of 29.00 ± 0.28 ppt in March 2018 and the lowest of 8.07 ± 2.94 ppt in June 2018. For Whin, the highest salinity of 27.79 ± 0.43 ppt was recorded in October 2017 and the lowest 4.11 ± 2.09 ppt in May 2018. Between January and April 2018, all the three water bodies had salinities higher than 15 ppt. Densu had the overall highest and lowest salinities. The mean salinities recorded were 16.55 ± 1.04 ppt, 20.42 ± 0.98 ppt and 18.19 ± 1.20 ppt for Whin, Nakwa and Densu respectively. The salinity measurements from the water bodies differed significantly ($p < 0.05$) in October 2017 and in January, May and July 2018 (Appendix I).

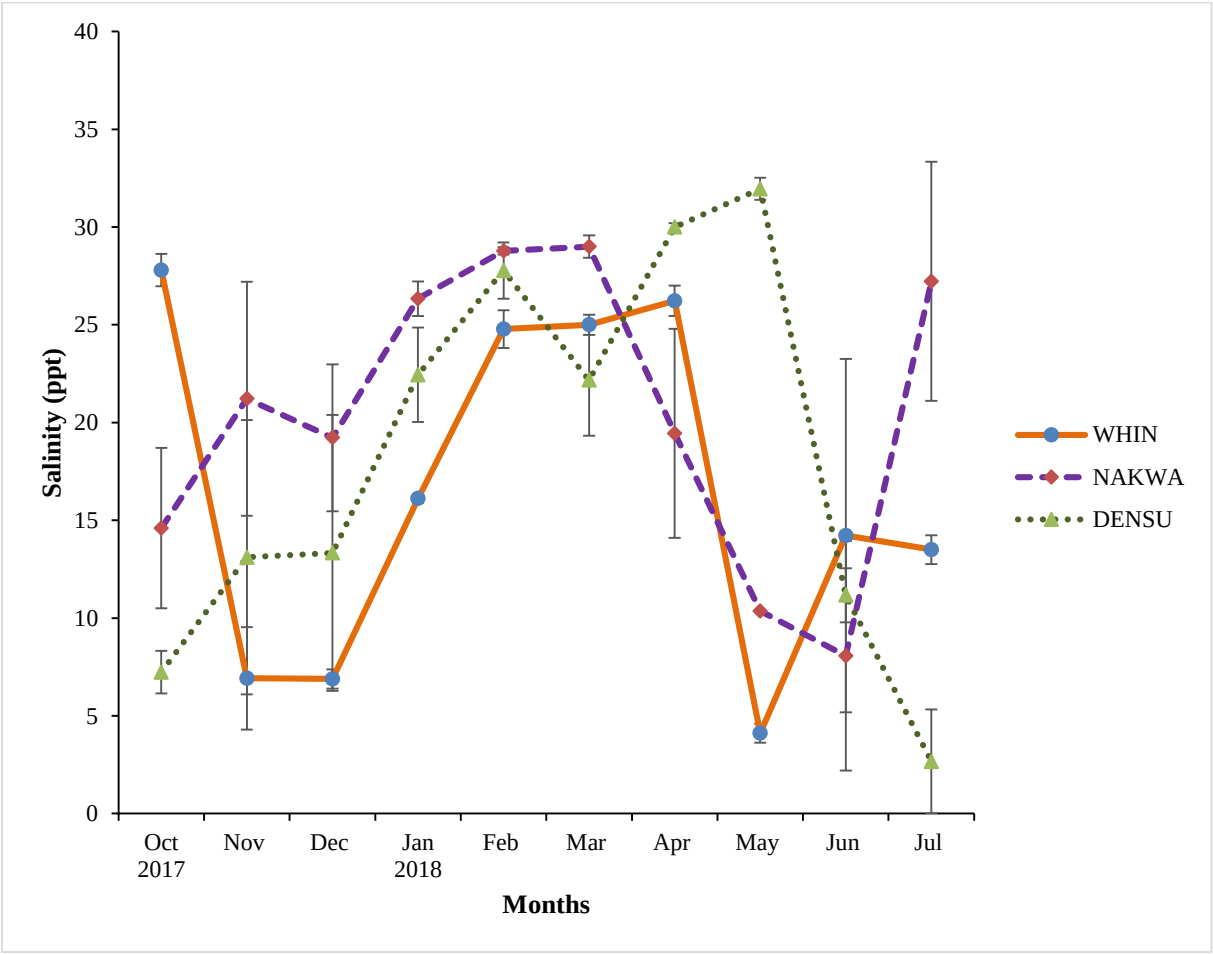

Figure 4.3: Monthly variations in salinity in Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

4.1.3 Dissolved oxygen (DO)

From Figure 4.3, the DO trends were similar in the three water bodies during the study period, except in November and December 2017 and May 2018 when significantly lower DO values were recorded in the Whin Estuary than in Densu Delta and Nakwa Lagoon. The highest recorded DO for Whin was 8.6 ± 0.5 mg L^{-1} in January 2018. For Nakwa and Densu 8.1 ± 0.5 and 8.4 ± 0.5 mg L^{-1} respectively, were the highest DO values recorded in February, 2018. The least DO levels were 1.2 ± 0.0 mg L^{-1} in May, 2018 for Whin and 1.2 ± 0.1 and 1.3 ± 0.2 mg L^{-1} in October, 2017 for Nakwa and Densu respectively. Whin had the highest measured DO whereas, Nakwa had the least. The mean DO values were 3.6 ± 0.3 mg L^{-1}, 4.1 ± 0.3 mg L^{-1} and 3.9 ± 0.3 mg L^{-1} for Whin, Nakwa and Densu respectively. There were statistically significant differences ($p \geq 0.05$) between the DO concentrations in the water bodies in December 2017 and March, May and July 2018 (Appendix I).

4.1.4 pH

The pH values in all the three water bodies were fairly stable throughout the study period, ranging between 7.0 and 8.5 for most months except in November, 2017, when 9.90 was recorded in Densu Delta. There was also a slight decrease in pH in Nakwa Lagoon in March 2018, whereas the corresponding values in Whin Estuary and Densu Delta showed increases (Figure 4.4). The highest pH values were 8.6 ± 0.1 and 8.7 ± 0.0 for Whin and Nakwa respectively, in January 2018, whereas that of Densu was 9.9 ± 0.3 in November 2017. Nakwa had the least overall pH value whiles Densu had the highest.

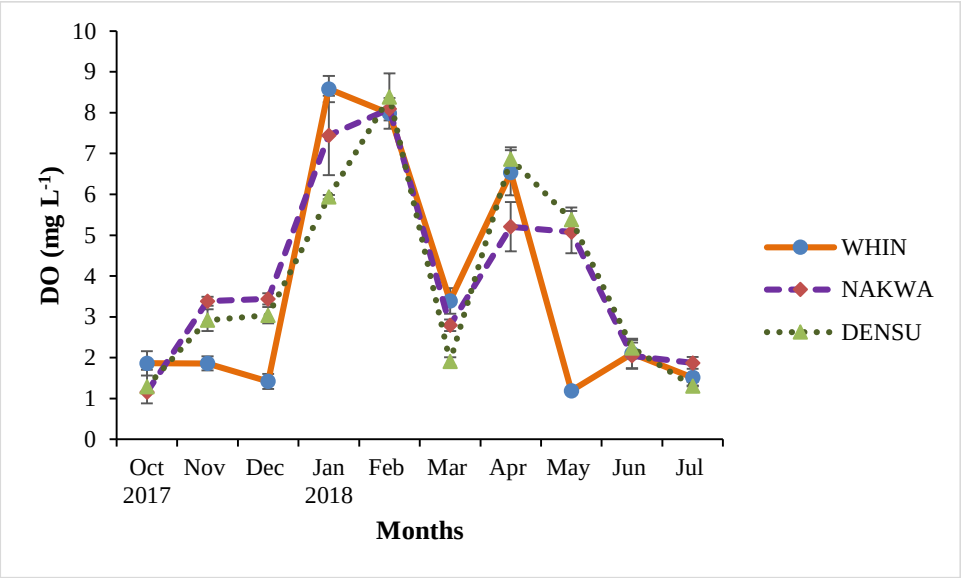

Figure 4.4: Monthly variations in dissolved oxygen in Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

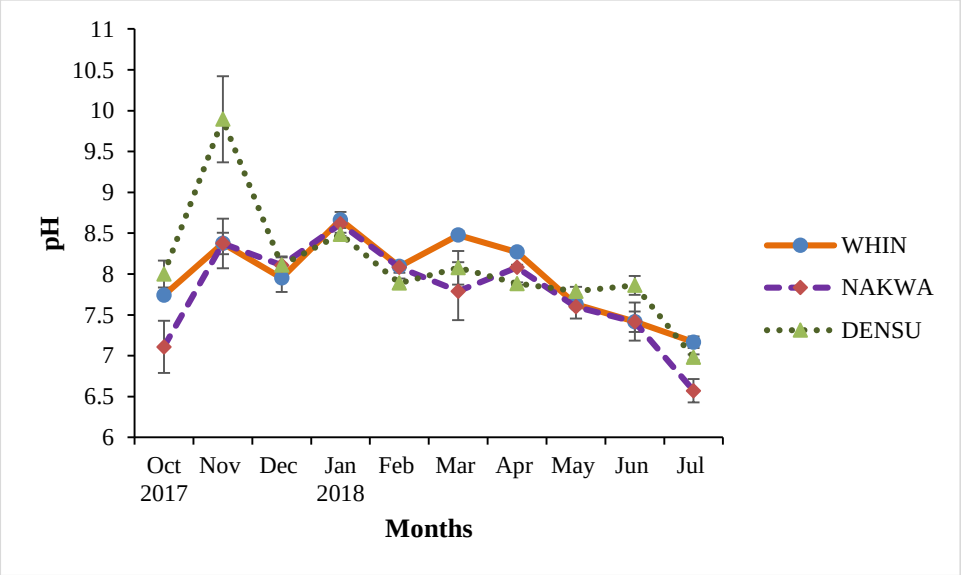

Figure 4.5: Monthly variations in pH in Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

The mean pH values were 8.0 ± 0.1, 7.88 ± 0.1 and 8.1 ± 0.1 for Whin, Nakwa and Densu respectively. For November 2017 and April and July 2018, there were significant statistical differences ($p \geq 0.05$) in the pH measurements from the water bodies (Appendix I).

4.1.5 Turbidity

The trends in turbidity recordings were similar in the three water bodies during the period of study except in October 2017 when a very high value (78.1 ± 18.0 NTU) was recorded in Nakwa Lagoon with low values in Densu Delta and Whin Estuary (21.0 ± 1.6 and 11.8 ± 3.2 NTU respectively), (Figure 4.5). Also in May 2018, whereas Nakwa and Densu had lower values (14.5 ± 1.5 and 8.1 ± 1.0 NTU respectively), Whin had a high turbidity value of 67.5 ± 1.3 NTU. Whin had the lowest mean turbidity of 26.2 ± 2.2 NTU with the highest value of 67.5 ± 1.3 NTU in May, 2018 and lowest of 11.1 ± 0.9 in April, 2018. Nakwa followed with the mean turbidity of 22.8 ± 3.1 NTU with the highest and lowest values of 78.2 ± 18.0 NTU and 6.3 ± 0.3 NTU respectively in October, 2017 and April, 2018. Densu was the water body with the least mean turbidity of 15.0 ± 0.8 NTU with the highest (21.3 ± 1.9 NTU) and lowest (6.3 ± 0.1 NTU) values recorded in November, 2017 and March, 2018 respectively. There were statistically significant differences ($p < 0.05$) between the turbidity measurements from the water bodies in April, May and July 2018 (Appendix I).

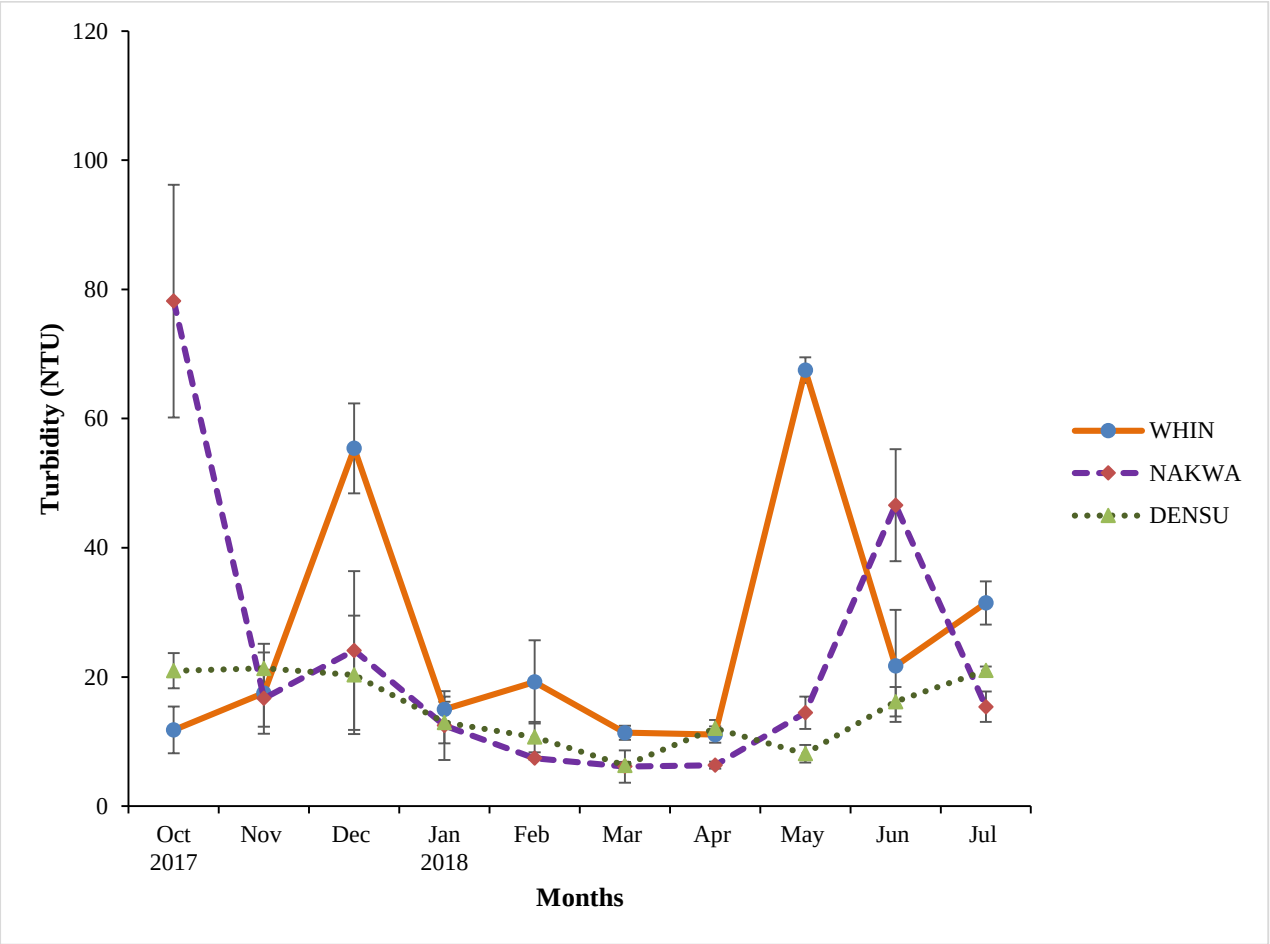

Figure 4.6: Monthly variations in turbidity in Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

4.1.6 Principal Components Analysis (PCA)

The principal components analysis conducted revealed that the aforementioed physicochemical parameters had variable influences on the dynamics of the water body (Appendix K). From the analysis, parameters that loaded highly in component one were presented as those with higher influence on the water body, as well as the organisms found in the water. The parameters with higher eigen values in the subsequent components had lesser contributions to the variations in the data. Parameters with higher negative eigen values were considered to have an inverse relationship with the parameters than had higher positive eigen values within a given component. However, the cumulative percentage of variance of the two components gives the total variance in the data.

In Whin, two components were generated. pH, temperature and DO loaded highly in component one whereas salinity and turbidity loaded highly in component two (Appendix K1). pH, temperature and DO had greater influence on the variability of the data from the estuary. The contributions of salinity and turbidity were smaller and there was an inverse relationship between the two, such that as salinity increased, turbidity decreased. In general, the physicochemical parameters accounted for 78% of the total variance in data from the estuary (Appendix K2).

Salinity and turbidity loaded highly in component one and therefore contributed more to the variability of the data from Nakwa Lagoon than temperature, DO and pH which loaded highly in component two (Appendix K3). Similar to Whin, only two components contributed to the variations in the data and there was an inverse relationship between salinity and turbidity. The

parameters accounted for about 62.0% of the total variance in the data from the lagoon (Appendix K4).

The situation at Densu was similar to that of Nakwa, with salinity, turbidity and DO having higher eigen values in component one and more influence on the delta, than temperature and pH in component two (Appendix K5). The parameters accounted for about 61.0% of the total variance in the data from the delta (Appendix K6). A summary of the influence of the five parameters on the dynamics in the three water bodies are presented in Table 4.1.

The rainfall trends (Appendix L) were quite similar to the trends in turbidity and microbial load (coliform and TVB) graphs. Again, Takoradi, Saltpond and Accra represent Whin, Nakwa and Densu respectively.

Table 4.1- *Summary of the influence of the physicochemical parameters on the dynamics in Whin, Nakwa and Densu*

Physicochemical parameters	Whin	Nakwa	Densu
Temperature	High positive influence	Low positive influence	Low positive influence
Salinity	Low positive influence	High positive influence	High positive influence
DO	High positive influence	Low positive influence	High positive influence
pH	High positive influence	Low positive influence	Low positive influence
Turbidity	Low negative influence	High negative influence	High negative influence

4.2 Bacterial Load

4.2.1 Total coliform (TC) and total viable bacterial (TVB) loads of water samples

The TC loads of water samples from the three water bodies during the study period are presented in Figure 4.6. Generally, the three water bodies recorded very similar trends except for a few months when some variations were observed. Between December 2017 and January 2018, the mean coliform loads for Whin Estuary and Nakwa Lagoon decreased from $0.37 \pm 0.06 \times 10^3$ to $0.05 \pm 0.01 \times 10^3$ cfu ml^{-1} and $0.33 \pm 0.08 \times 10^3$ to $0.04 \pm 0.02 \times 10^3$ cfu ml^{-1} respectively, while that of Densu Delta increased from $0.09 \pm 0.08 \times 10^3$ to $0.36 \pm 0.02 \times 10^3$ cfu ml^{-1}. There were no statistically significant differences ($p > 0.05$) between the TC loads of the water bodies for the entire study period. However, there were significant statistical differences ($p < 0.05$) between them for January, June and July 2018 (Appendix I). Among the water bodies, the highest TC load of $0.80 \pm 0.00 \times 10^3$ cfu ml^{-1} was recorded in Densu in May 2018 and the least was recorded in Nakwa (in April and July 2018) and Densu (October 2017), as $0.01 \pm 0.00 \times 10^3$ cfu ml^{-1}.

The highest TC load in Whin was recorded in July 2018 as $0.60 \pm 0.00 \times 10^3$ cfu ml^{-1} and the lowest was $0.01 \pm 0.00 \times 10^3$ cfu ml^{-1} in October 2017. In Nakwa, the highest load of $0.42 \pm 0.09 \times 10^3$ cfu ml^{-1} was recorded in March, whereas the lowest load of $0.03 \pm 0.00 \times 10^3$ cfu ml^{-1} was recorded in July. The highest TC load recorded in Densu Delta was $0.80 \pm 0.00 \times 10^3$ cfu ml^{-1} in May 2018, whereas the lowest was $0.01 \pm 0.00 \times 10^3$ cfu ml^{-1} in October 2017.

The TVB loads recorded for water samples from all the water bodies had a similar trend with the observed coliform load (Figure 4.7). However, Whin showed some variations from the trend in December 2017 and in March 2018. Densu also deviated from the trend in February 2018. There were no statistically significant differences in the total bacteria load of the water samples for the study period ($p > 0.05$). Nevertheless, the record for October and December 2017 and January and July 2018 differed significantly ($p < 0.05$) (Appendix I).

In Whin, the highest total bacteria load of $2.19 \pm 0.01 \times 10^3$ cfu ml^{-1} was observed in July 2018, with the lowest of $0.04 \pm 0.21 \times 10^3$ cfu ml^{-1} in October 2017. The highest and lowest loads in Nakwa were $1.70 \pm 0.13 \times 10^3$ cfu ml^{-1} and $0.08 \pm 0.03 \times 10^3$ cfu ml^{-1} in November 2017 and in April 2018 respectively. For Densu, $3.00 \pm 0.00 \times 10^3$ cfu ml^{-1} was the highest load recorded in May 2018 while $0.07 \pm 0.02 \times 10^3$ cfu ml^{-1} was the lowest in October 2017. For the entire study, the highest TVB load of $3.00 \pm 0.00 \times 10^3$ cfu ml^{-1} was recorded at Densu with the lowest of $0.04 \pm 0.21 \times 10^3$ cfu ml^{-1} recorded at Whin.

4.2.2 Total coliform and total viable bacterial loads of oyster samples

The observed TC load in oyster samples from all three water bodies were similar in trend, except for Densu between December 2017 and March 2018 and in July 2018. Whin also deviated from the general trend in November 2017. There was one major peak observed in Whin in October 2017 and two minor

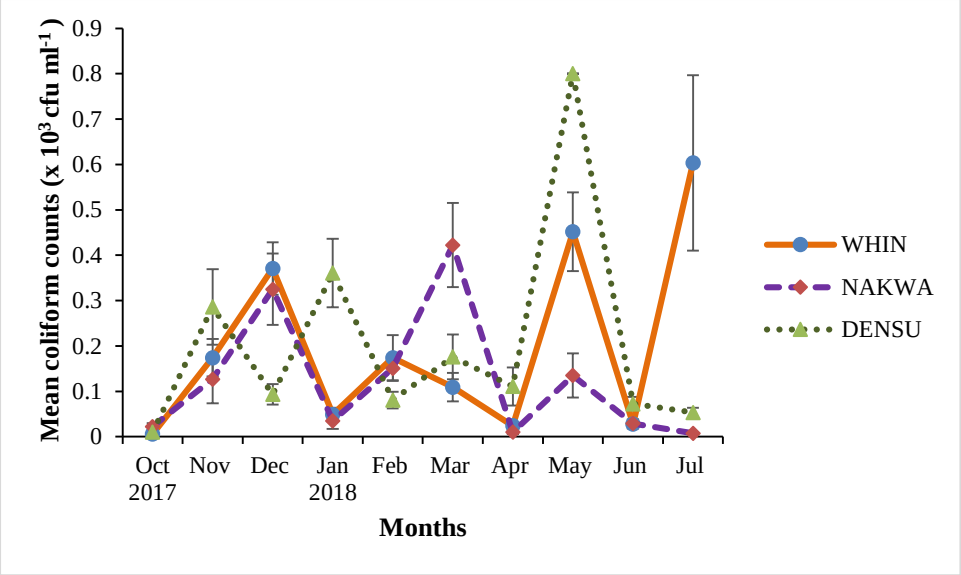

Figure 4.7: Mean coliform loads of water samples from Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

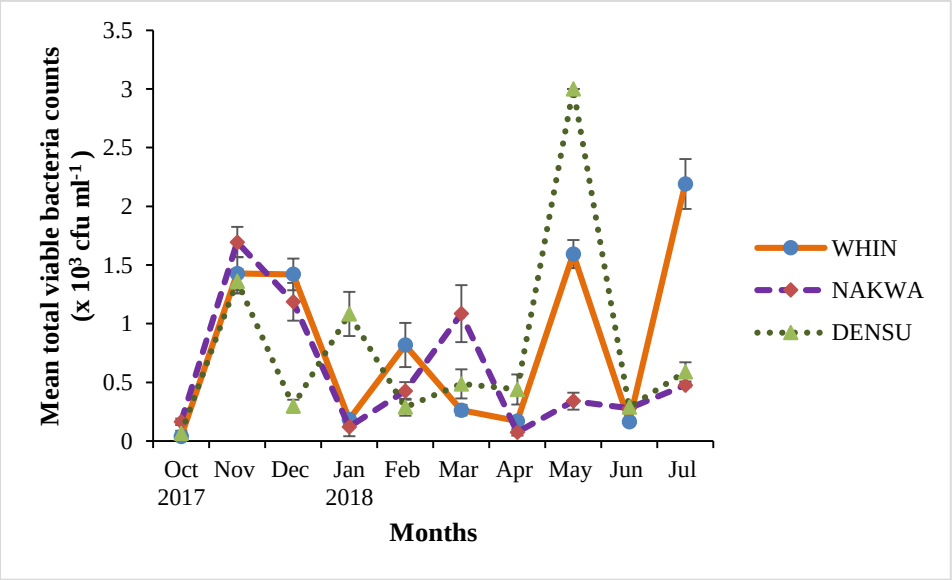

Figure 4.8: Mean total viable bacterial loads of water samples from Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

ones in March and in May 2018; one minor peak between in December 2017 and one major peak between in May 2018 for Nakwa and one major peak between in May 2018 and two minor in November 2017 and in January 2018 for Densu (Figure 4.8). Generally, the TC loads of the oysters were not significantly different ($p > 0.05$). However, there was significant difference ($p < 0.05$) between them in January 2018 only (Appendix I).

The highest load in Whin was recorded in October 2017 as 1.20 ± 0.00 x 10^8 cfu g^{-1}, whereas the lowest load was recorded in November 2017, as 0.01 ± 0.00 x 10^8 cfu g^{-1}. In Nakwa, the highest load of 1.20 ± 0.00 x 10^8 cfu g^{-1} was recorded between April and May 2018 and the lowest as 0.003 ± 0.00 x 10^8 cfu g^{-1} in June 2018. In Densu, 0.80 ± 0.02 x 10^8 cfu g^{-1} was the highest recorded load in May 2018, whiles 0.002 ± 0.00 x 10^8 cfu g^{-1} was the lowest in October 2017. For this study, the highest TC load in oysters was 1.2 ± 0.00 x 10^8 cfu g^{-1} for Whin and Nakwa and the lowest was 0.002 ± 0.00 x 10^8 cfu g^{-1} for Densu.

The general trends for the TVB loads in oysters from all the water bodies were similar (Figure 4.9). Only Densu had a different trend in October 2017 and in July 2018. Four major peaks (highest loads) were observed in all the water bodies. The observed peaks were similar for most months in all three water bodies. There were statistically significant differences ($p < 0.05$) between the water bodies in January and February only (Appendix I).

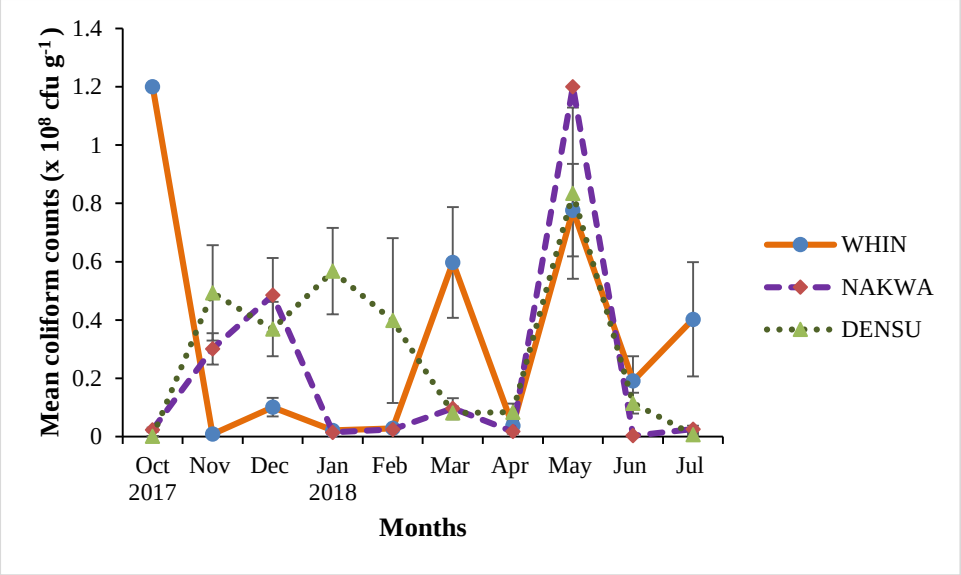

Figure 4.9: Mean coliform loads of oyster samples from Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

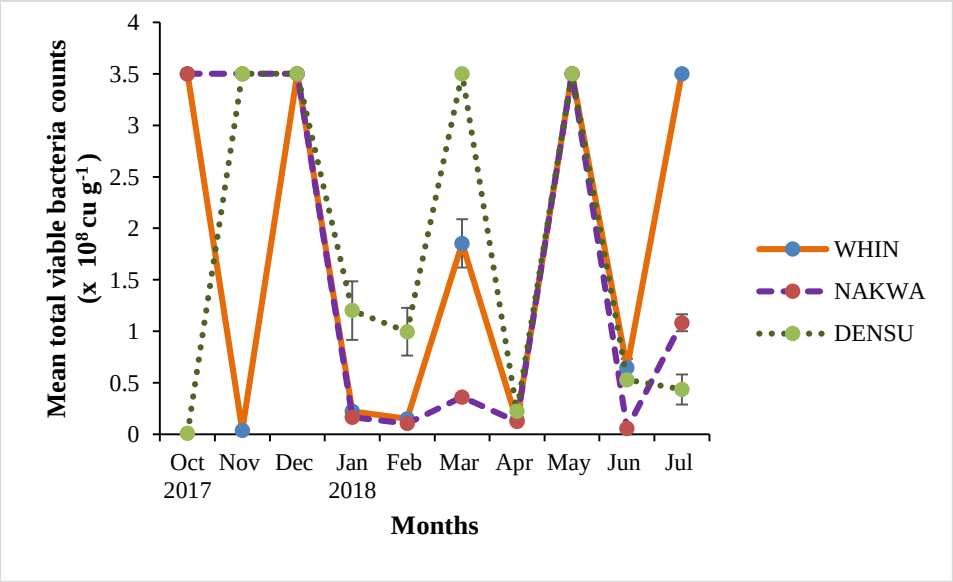

Figure 4.10: Mean total viable bacterial loads of oyster samples from Whin Estuary, Nakwa Lagoon and Densu Delta from October 2017 to July 2018

4.2.3 Identified bacterial isolates

The summary of bacterial isolates identified through biochemical tests are presented in Table 4.2. In all, 101 bacterial isolates were recovered from the three water bodies and were identified as belonging to 11 species. Some species of bacteria only occurred in one water body. For example, *Yersinia enterocolitica, Morganella morganii and Salmonella paratyphi A* occurred only in Nakwa Lagoon. Likewise, *Enterobacter sp* occurred only in Whin Estuary. In all 34, 35 and 31 bacterial isolates were recorded for Whin, Nakwa and Densu respectively (Table 4.2). The raw data are presented in Appendices N1 to N3. *Escherichia coli* was found in the highest number of isolates with a frequency of 30, followed by *Shigella sp* and *Klebsiella sp* with frequencies of 30 and 10 respectively, while *Morganella morganii* and *Salmonella paratyphi A* were found in the least number of isolates of 1 each. Pictures of some bacterial cultures obtained in this study are shown in Figure 4.10.

4.3 Heavy Metal Load

The heavy metals analysed in this study were Cadmium, Lead, Zinc and Copper. Metal concentration in oysters in October 2017 could not be measured because, the samples were destroyed by rodents in the laboratory. Also, the oyster samples collected from the stations were analysed as composite for this analysis, as mentioned in the previous chapter.

Table 4.2- *Distribution of bacterial isolates identified in Whin, Nakwa and Densu*

Bacteria species	**Number of bacterial isolates recovered from three water bodies**			
	Whin	**Nakwa**	**Densu**	**Total**
Escherichia coli	9	15	6	30
Klebsiella pneumonia	1	4	5	10
Yersenia enterocolitica	0	2	0	2
Shigella spp	12	5	7	26
Enterobacter spp	4	0	0	4
Citrobacter spp	3	2	4	9
Providencia spp	3	2	3	8
Serratia marcescens	1	1	4	6
Vibro cholera	1	2	2	5
Morganella morganii	0	1	0	1
Salmonella paratyphi A	0	1	0	1
Total	**34**	**35**	**31**	**101**

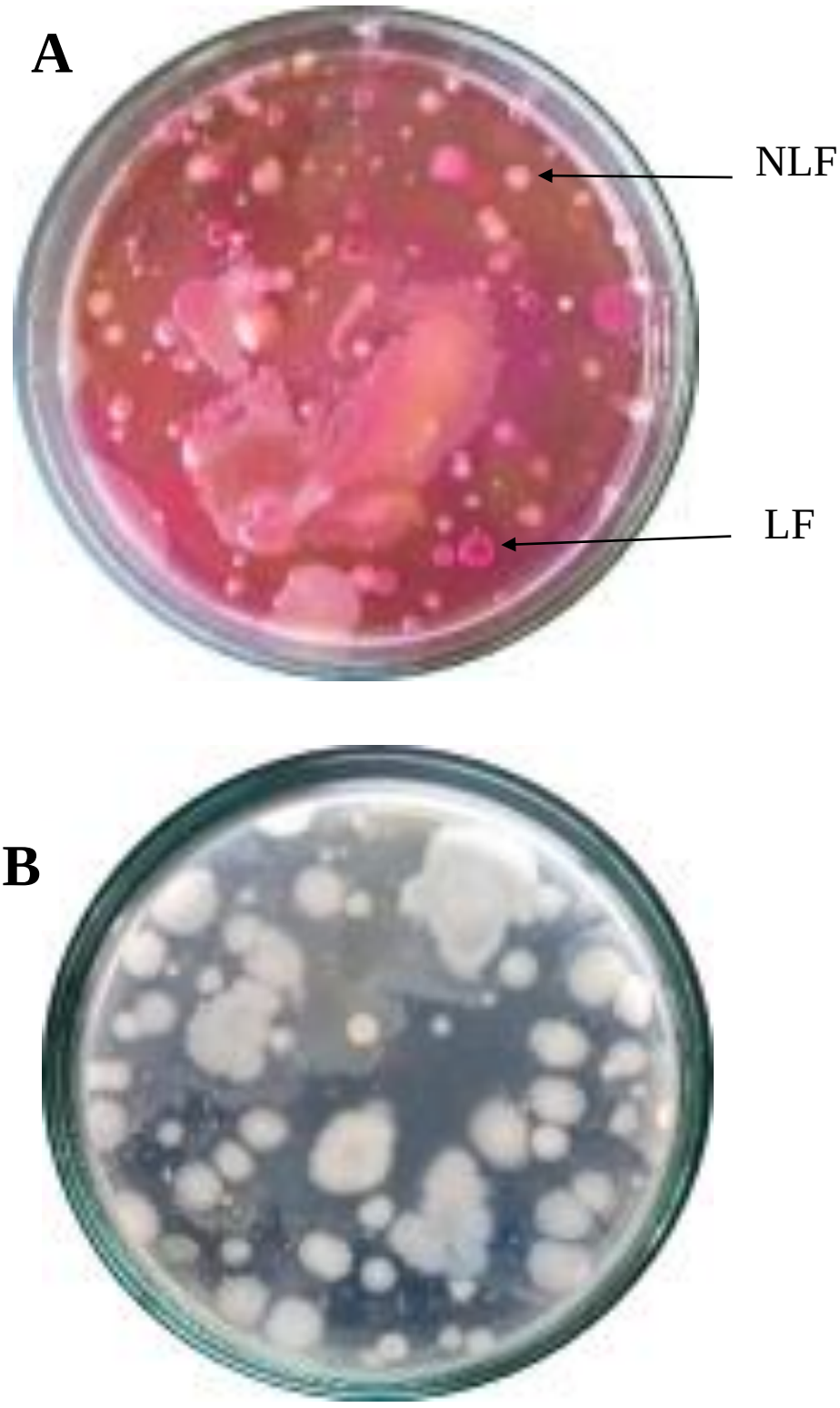

Figure 4.11: Mixed culture of coliform bacteria growth on MacConkey agar (A) and mixed culture of bacteria growth on Plate Count agar (B). *LF= Lactose fermenter and NLF= Non-Lactose fermenter

4.3.1 Cadmium in water

Figure 4.11 shows Cd levels in water samples from the three water bodies. Cd levels were below instrument detection limit (IDL) of 0.002 mg L^{-1} in all the water bodies for the first two quarters of the sampling period. In the last two quarters, lower Cadmium levels of 0.014 mg L^{-1} and 0.006 mg L^{-1} were recorded in Whin and Nakwa respectively in March 2018, compared to the relatively higher levels of 0.0224 mg L^{-1} and 0.065 mg L^{-1} in June, 2018. On the other hand, lower value of 0.0132 in Densu was recorded in June 2018 while the higher value of 0.0241 was in March. The Cd load for all the water bodies did not exceed the United States Environmental Protection Agency (USEPA) (2016) permissible limit of 0.03 mg L^{-1} (Appendix M). The recorded Cd levels did not differ ($p > 0.05$) in all the water bodies (Appendix I).

4.3.2 Cadmium in oyster

The highest Cadmium levels in oysters were recorded in June (2018) for Whin and Densu (0.24 mg kg^{-1} and 0.32 mg/kg respectively) and the lowest levels (0.2 mg kg^{-1} and 0.27 mg kg^{-1} respectively) in March for both water bodies. Cadmium in oysters from Nakwa were below detection limit throughout the sampling period. The Cd levels in oysters from Whin and Densu did not exceed the permissible Cd limit in oysters, as is seen in Figure 4.12. The Cadmium levels in oysters from Whin and Densu did not differ significantly as indicated by the standard error bars and did not also exceed the permissible limit (1 mg kg^{-1}) recommended by WHO/USEPA (Mortuza & Al-Misned, 2017) (Appendix M).

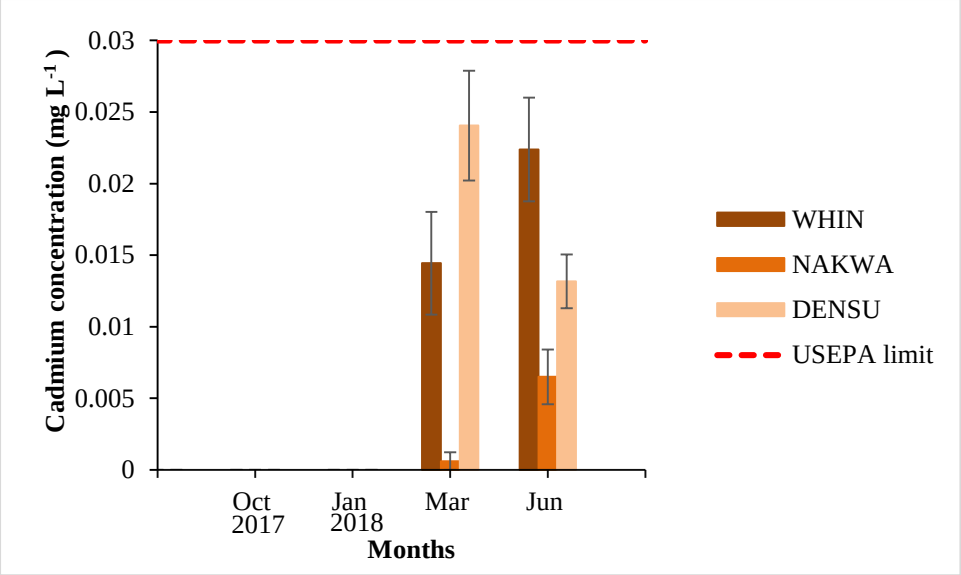

Figure 4.12: Mean concentration of cadmium in water samples from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

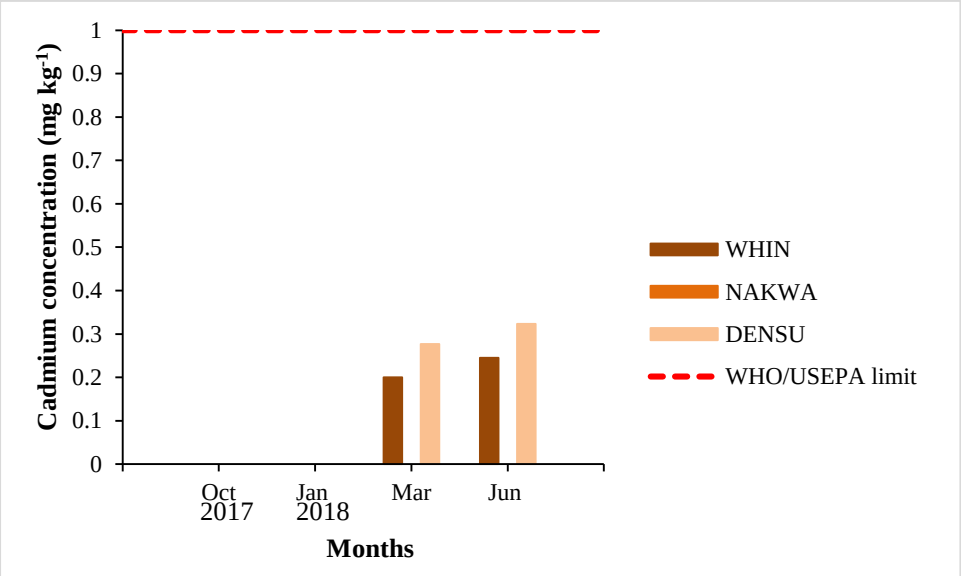

Figure 4.13: Mean concentration of cadmium in oyster samples from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

4.3.3 Lead in water

Detectable levels of Lead were recorded in water samples from all three water bodies in March and June, 2018, as levels in the previous quarters were below detection (Figure 4.13). The highest levels (0.02, 0.01 and 0.01 mg L^{-1}) were recorded in March for Whin, Nakwa and Densu respectively, whereas the lowest levels (0.005, 0.003 and 0.007 respectively) were recorded in June 2018. Lead levels were far below the USEPA (2016) acceptable limit of 0.2 mg L^{-1} (Appendix M) in all water bodies. The detectable levels of Pb in the three water bodies did not differ significantly ($p > 0.05$) a as shown in Appendix I.

4.3.4 Lead in oyster

Lead was detected in oysters from all three water bodies in January and March and in Whin and Nakwa in June, 2018 (Figure 4.14). The highest level of Lead contaminations in oysters from Whin and Nakwa (0.58 mg kg^{-1} and 0.61 mg kg^{-1} respectively) were recorded in January. The lowest levels (0.07 and 0.07 mg/kg) were recorded in March and June for Whin and Nakwa respectively. The highest Pb level for Densu (0.92 mg kg^{-1}) was recorded in March and the lowest (0.59 mg kg^{-1}) in January. The measured levels of Lead in oysters from all the water bodies were similar and below the WHO/USEPA (Mortuza & Al-Misned, 2017) permissible limit of 2 mg kg^{-1} (Appendix M) except in Densu where some individual measurements exceeded the permissible limit although the mean was well below it.

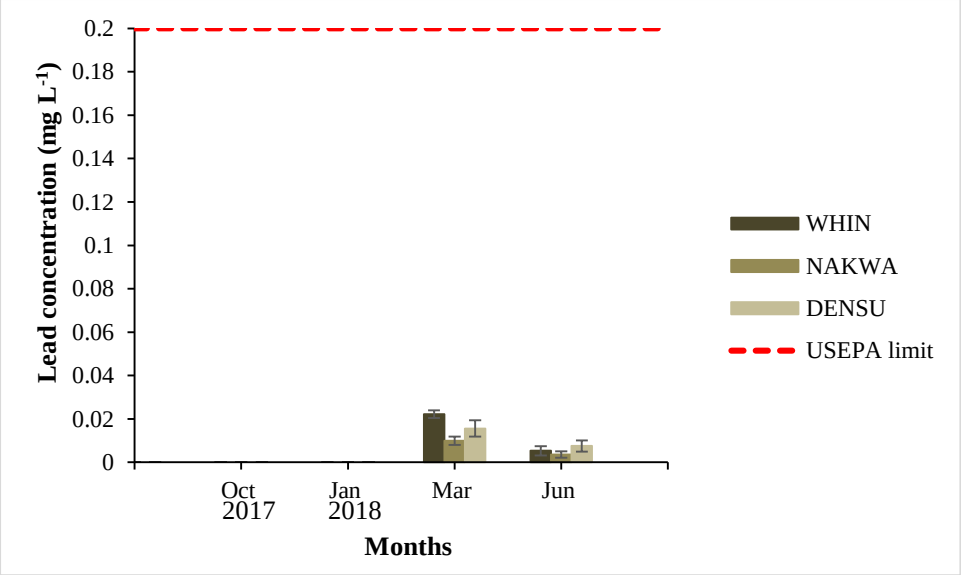

Figure 4.14: Mean concentration of lead in water samples from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

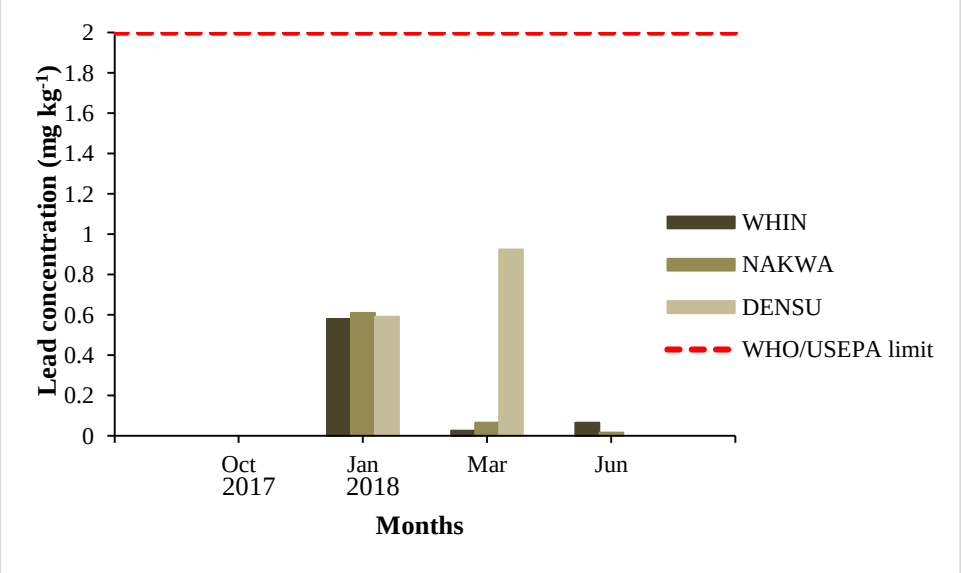

Figure 4.15: Mean concentration of lead in oyster samples from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

4.3.5 Zinc in water

From Figure 4.15, Zn was detected in Whin in the last two quarters and in Nakwa in March only. In Whin, the highest recorded value (0.01 mg L^{-1}) was in March 2018. In Densu, Zn was measured only in March. The record of Zn in Nakwa was below detection limit throughout the study. There was no significant difference ($p > 0.05$) in the concentration of Zn between the water bodies (Appendix I). Overall, Zn concentration in water samples from the three water bodies did not exceeded the USEPA (2016) permissible limit of 0.09 mg L^{-1} (Appendix M).

4.3.6 Zinc in oyster

It is seen from Figure 4.16 that Zinc was found in oysters from all water bodies in January. In March and June, Zinc only occurred in oysters from Whin and Densu. The highest recorded Zinc value (8.76 mg kg^{-1}) in Densu was in June with the least (5.36 mg kg^{-1}) in March. Overall, oysters from Whin had the highest recorded Zinc value (9.76 mg kg^{-1}) in January. The concentration of Zn in oysters from the three water bodies did not exceed the permissible limit (100 mg kg^{-1}) recommended by WHO/USEPA (Appendix M).

4.3.7 Copper in water

Copper levels were below detection limit in water samples from all the three water bodies during the entire study period. Concentrations of Copper were only recorded in oysters.

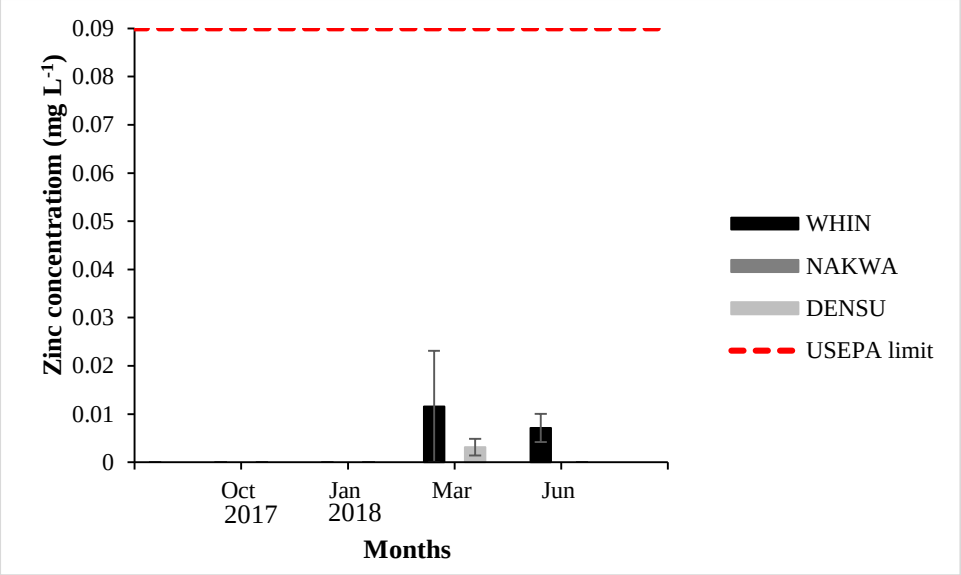

Figure 4.16: Mean concentration of zinc in water samples from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

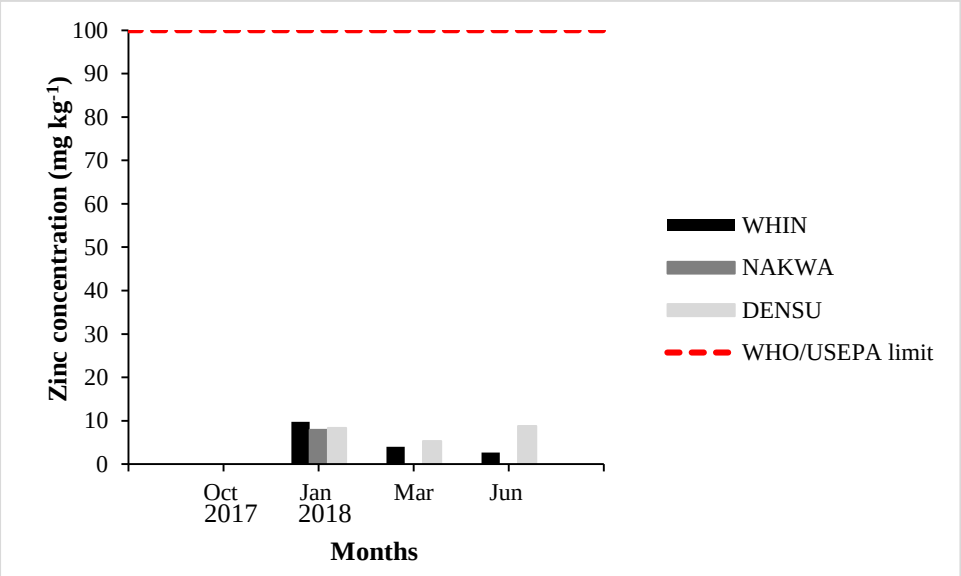

Figure 4.17: Mean concentration of zinc in oyster samples from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

4.3.8 Copper in oyster

Copper occurred only once in Nakwa (January, 2018), twice in Whin (January and March, 2018) and thrice in Densu (January, March and June 2018) as shown in Figure 4.17. The highest recorded Cu concentration (1.73 mg kg^{-1}) for Whin was in January, with the least value (0.94 mg kg^{-1}) in March. Copper level was highest in Densu in January (3.09 mg kg^{-1}) and lowest in June (2.10 mg kg^{-1}). Nakwa had the highest recorded Copper level (3.43 mg kg^{-1}) for the entire sampling period but, even this was far below the WHO/USEPA permissible limit of 30 mg kg^{-1} (Mortuza & Al-Misned, 2017) (Appendix M).

4.4 Condition Index

The Condition index (CI) of oysters in the three water bodies during the period of study are shown in Figure 4.18. CI did not show much variation among the water bodies except in February and May 2018 when oysters in Whin Estuary had significantly higher CI than those in Nakwa Lagoon and Densu Delta, based on the standard error bars. The trends in the CI variations in Nakwa and Densu were similar. It should however be noted that there was no record for Nakwa in June 2018 due to consistent harvesting of the oysters by the people of Nakwa community, and as a consequence, no live oysters were found. The highest CI of 64.0% was recorded in May 2018 for Whin, 53.3% in April 2018 for Nakwa and 53.9% in June 2018, for Densu. The lowest records were 33.3% and 33.2% in July, for Whin and Densu respectively and 32.9% in January for Nakwa. The mean CI for Whin, Nakwa and Densu were 45.7%, 39.5% and 41.0% respectively. The CI of oysters from all the three water bodies did not differ significantly ($p > 0.05$) in most of the sampling months (Appendix I).

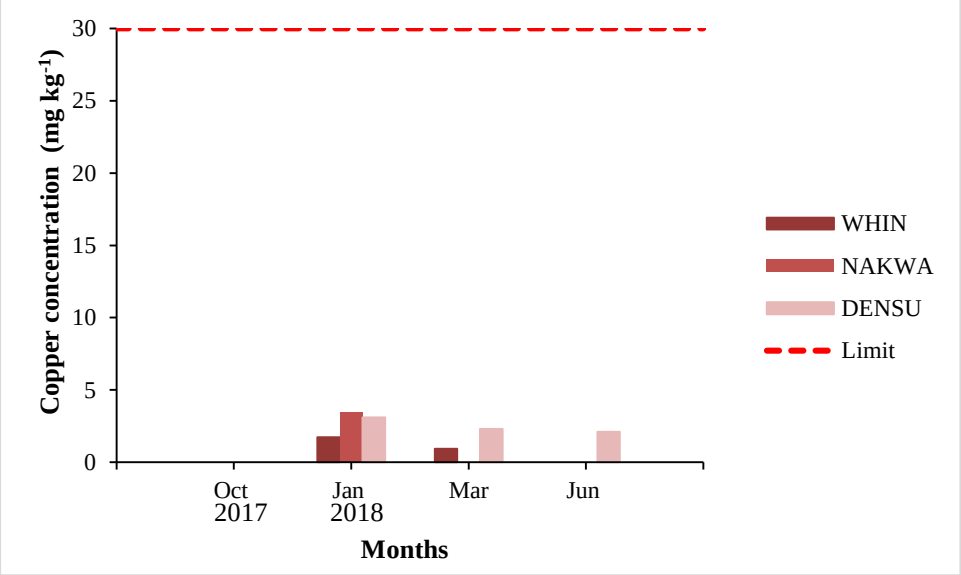

Figure 4.18: Mean concentration of copper in oyster samples from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

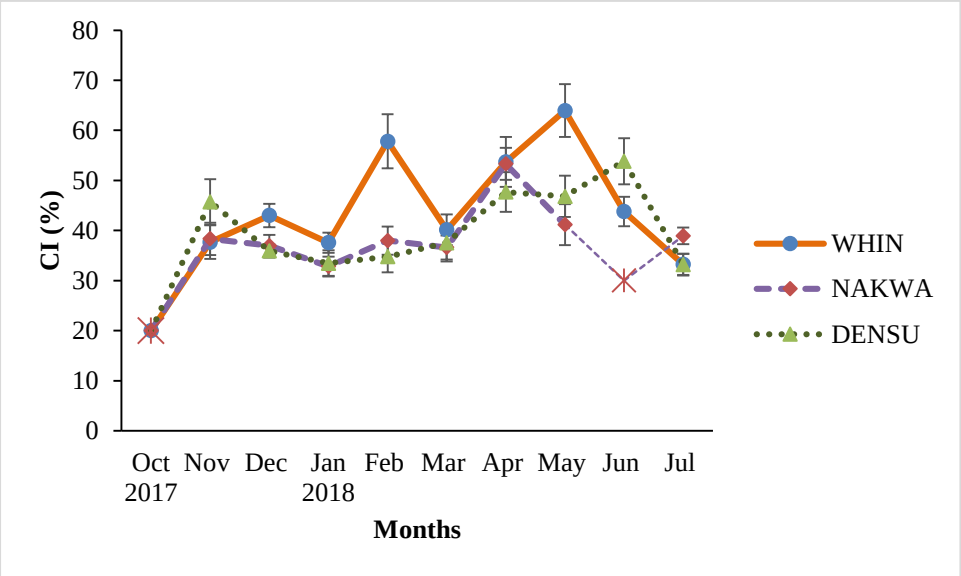

Figure 4.19: Monthly variations in condition index of oysters from Whin Estuary, Nakwa Lagoon and Densu Delta, from October 2017 to July 2018

4.5 Relationships

4.5.1 Physicochemical parameters and bacterial load in the three water bodies

Multiple Regression Analysis was conducted to determine the possible effect of physicochemical parameters on bacterial load of water samples from the three water bodies, to ascertain their status with respect to bacterial contamination. The detailed analyses are shown in Appendices O1 to O3, and the outcomes are summarised in Table 4.3. The results show that temperature had a negative significant relationship (p = 0.00) with total viable bacteria (TVB) in samples from Densu Delta only. The relationships in the other two water bodies were insignificant. The relationship between salinity and bacterial load in the water samples was negative and significant (p = 0.00) in Whin but insignificant in Nakwa (p > 0.05) and Densu (p > 0.05). DO showed positive significant relationship (p = 0.04) with bacterial load in the samples from Whin Estuary only. pH was negatively and significantly (p = 0.00) related to the bacterial load in the samples from Nakwa but positively and significantly (p = 0.00) related to bacterial load in the samples from Densu. However, the relationship in Whin was insignificant. Turbidity related negatively and significantly with bacterial load in samples from Whin (p = 0.04), but had insignificant (p > 0.05) relationship in Nakwa and Densu samples.

Table 4.3- *Summary of the effects of physicochemical parameters on microbial load (Total viable bacteria) in the water of the three water bodies*

Parameter	Whin Estuary	Nakwa Lagoon	Densu Delta
Temperature	Negative, not significant	Positive, not significant	Negative, significant
Salinity	Negative, significant	Negative, not significant	Positive, not significant
DO	Positive, significant	Negative, not significant	Negative, not significant
pH	Negative, significant	Positive, not significant	Positive, significant
Turbidity	Positive, not significant	Negative, significant	Positive, not significant

4.5.2 Physicochemical parameters and Condition Index (CI) of oysters from the three water bodies

From the multiple regression analysis conducted to determine the possible effect of physicochemical parameters on the CI of oysters from the three water bodies, the detailed results obtained have been presented in Appendices P1 to P3 and the outcomes are summarised in Table 4.4. The results show that temperature had a positive significant relationship (p = 0.00, 0.00 and 0.02 for Whin, Nakwa and Densu respectively) with the CI of oysters from the three water bodies. Likewise, salinity showed a positive relationship with CI of oysters from all the water bodies. However, the relationships were not significant ($p > 0.05$). The relationship between DO and CI was negatively significant at Whin (p = 0.04); positive for both Nakwa and Densu. However, it was significant (p = 0.00) in Densu only. pH was positively and significantly (p = 0.00) related to the CI of oysters from Whin, but not significantly related to CI of oysters from Nakwa and Densu. The relationship between turbidity and CI was positively significant at Densu (p = 0.00), negatively significant at Whin (p = 0.04) and negative but insignificant at Nakwa (p = 0.24).

Table 4.4- *Summary of the effects of physicochemical parameters on Condition Index of oysters from the three water bodies*

Parameter	Whin Estuary	Nakwa Lagoon	Densu Delta
Temperature	Positive, significant	Positive, significant	Positive, significant
Salinity	Positive, not significant	Positive, not significant	Positive, not significant
DO	Negative, significant	Positive, not significant	Positive, significant
pH	Positive, significant	Negative, not significant	Positive, not significant
Turbidity	Negative, significant	Negative, not significant	Positive, significant

4.5.3 Bacterial load and condition index from the three water bodies

The possible effects of bacterial load (total coliform (TC) and total viable bacteria (TVB) in the water and oyster samples on the condition index (CI) of the oysters were also determined by multiple regression analysis, as is indicated in Appendices Q1 to Q3. The outcomes are summarised in Table 4.5.

TC load in water had a negative significant relationship with the CI of oysters from Whin (p = 0.03) and Densu (p = 0.03). In Nakwa, the relationship was insignificant (p > 0.05). Conversely, the relationship between TC load in oysters and CI was positive and significant (p < 0.05) in all the three water bodies. TVB load in water did not have a significant relationship with CI in the water bodies. TVB load in oysters however, regressed negatively with CI of oysters from all the water bodies. The relationship was only significant in Whin (p = 0.02) and Densu (p = 0.00) but not in Nakwa (p > 0.05).

4.5.4 Heavy metals and condition (CI) of oysters from the three water bodies

The possible effect of heavy metal contamination (of water samples) on the condition of oysters from the three water bodies was conducted using multiple regression analysis. The detailed results are presented in Appendices R1 to R3, whereas the summary of the results are presented in Table 4.6.

From the analysis, it was discovered that the heavy metals had variable effects on the CI of oysters from the three water bodies. Cadmium showed a positively significant relationship with the CI of oysters from Whin Estuary and

Table 4.5- *Summary of effects of microbial load on Condition Index of oysters from the three water bodies*

Parameter	Whin Estuary	Nakwa Lagoon	Densu Delta
TC Water	Negative, not significant	Negative, significant	Negative, significant
TC Oyster	Positive, significant	Positive, significant	Positive, significant
TVB Water	Positive, not significant	Negative, not significant	Positive, not significant
TVB Oyster	Negative, significant	Negative, not significant	Negative, significant

TC= Total coliforms; TVB= Total viable bacteria

Table 4.6- *Summary of effects of microbial load on Condition Index of oysters from the three water bodies*

Heavy metals	Whin Estuary	Nakwa Lagoon	Densu Delta
Cadmium	Positive, significant	Negative, significant	Positive, significant
Lead	Positive, not significant	Positive, significant	Positive, not significant
Zinc	Negative, not significant	-	Negative, significant

Densu Delta, however the relationship was negatively significant in Nakwa Lagoon.The relationship between Lead and CI was positive in the three water bodies but significant only in Nakwa. Zinc showed a negatively significant relationship with oyster CI in Densu, but the relationship was negatively insignificant at Whin. The concentrations of Zinc were below detectable limit in water samples from Nakwa during the study.

4.6 Chapter Summary

The detailed results from the study have been presented in this chapter. Physicochemical parameters fluctuated in similar patterns and some variations were observed. Also, it was observed that physicochemical parameters, bacterial and heavy metal loads have variable influence on the condition index of oysters from the three water bodies.

CHAPTER FIVE:

DISCUSSION

The results of the study were presented in the previous chapter. This chapter presents interpretation and discussion of the results with reference to previous related works. As indicated in Chapter 1, this study aims at investigating the suitability of the three water bodies for oyster culture using physicochemical factors, microbial load, heavy metal pollution and condition index of the resident oysters as indicators. This chapter has therefore been structured to first discuss the influence of physicochemical parameters on bacterial load in the water bodies to emphasise their crucial roles in commercial production of oysters. This is then followed by discussion on the effects of physicochemical parameters, bacterial load and heavy metal contamination on the condition index of the oysters as a means of assessing whether any of these prevailing aspects of the water bodies could be detrimental to the culturing of oysters in them.

5.1 Effect of Physicochemical Parameters on the Bacterial Load of Water Samples

The effect of physicochemical parameters on the bacterial load of oyster was not determined, because oyster samples were treated as composite samples, as indicated in Section 3.2. It is evident from the observed results (Figure 4.7) that the bacterial load of the water bodies did not show distinct variation with seasons. Although higher loads were observed in months of high rainfall, the same is true for the dry months (with little or no rainfall). Similar results were

reported in the works of Ogburn and White (2009) and Edun, Akinrotimi and Makinde (2016). The bacterial loads recorded in the three water bodies exceeded the recommend threshold for bacteria in water in most months. The acceptable limit for total coliform in coastal water bodies is 70 MPN 100 ml^{-1} (Perkins, 1995; Grabowski et al., 2012), which may be equivalent to 0.07 cfu ml^{-1}, for this study.

The bacterial load of water samples from the Whin Estuary mirrored the rainfall and turbidity trends in December 2017 and February and May 2018. This suggests that the bacterial load of the estuary could have resulted from rainfall-induced turbidity, caused by organic matter which was brought in through run-offs. This observation supports the report of other authors (Huey & Meyer 2010; Walters, et al., 2011; Mignani et al., 2013). Suspended organic matter and silt in turbid waters, resulting from rainfall, may not be the main contributors of high bacteria loads in Nakwa and Densu, as may be the case for Whin. However, per the reports of other authors (Biancani et al., 2011; Mignani, 2013; Jana et al., 2014; Liu et al., 2018; Sorio & Peralta, 2018), other factors like nutrient availability, domestic sewage, urban waste (such as was the case in Densu), faecal matter from both humans and animals (especially at Nakwa) may be the major contributors to the bacterial load of the water bodies.

According to Hong, Qiu and Liang, (2010), physicochemical parameters influence the survival and growth of bacteria, especially coliforms. Several studies have reported the influence of temperature on bacteria populations in water bodies (Geldreich, 1983; Cook, 1991; Adams, Crump & Kling, 2010; Suh et al., 2015). The results from the present study showed that, among the three

water bodies, the observed patterns of temperature and bacterial loads were not similar (Figures 4.1 & 4.7).

In addition, the influence of temperature on the bacteria load in Whin and Nakwa water bodies was not significant, suggesting that the density of bacteria may be sustained by other factors or a combination of other factors. On the contrary, temperature had a negative significant relationship with bacteria in the Densu Delta (Table 4.3). This outcome corroborates the findings of Mill et al. (2006), who reported an inverse relationship between temperature and TVB load of water samples from an estuarine creek in Australia. Cherry, Guthrie and Harvey (1974) and Scofield, Jacques, Guimaraes and Farjalla (2015) also reported that increase in temperature reduced bacteria density in water.

High salinity tends to induce osmotic shock on bacteria and, thus, reduces their load in brackish water bodies, especially, in areas around the mouth of the estuaries or lagoons (Rozen & Belkin, 2001; Mill et al., 2006). Therefore, the negative significant relationship between salinity and bacteria load in Whin Estuary was not surprising. The insignificant relationship between salinity and bacterial load of Nakwa Lagoon and Densu Delta could be due to the dynamics of the water bodies, which tends to affect salinity. For instance, Nakwa Lagoon closes at some point in the year and Densu Delta is subjected to massive annual freshwater intrusion whenever the Weija dam is opened.

Dissolved oxygen is among other factors such as temperature, salinity and pH influence bacteria density in water bodies (Cavallo, Rizzi, Vozza & Stabili, 1999). This is because bacteria, among other microorganisms require

DO for decomposition of organic matter (Waksman & Carey, 1935). In the present study, a positively significant relationship was observed between DO and microbial load in Whin Estuary. A similar finding was reported by Xu et al. (2018) that DO, among other water parameters, had a significant influence on bacterial communities. A negative relationship between DO and bacterial richness has been reported by Spietz, Williams, Rocap and Horner-Devine, (2015), supporting the negatively significant relationship between DO and bacterial load in Nakwa and Densu water bodies. However, the variability in results suggests that the effect of DO on bacteria could be better explained when analysed with other environmental factors as suggested by Spietz et al. (2015) and Xu et al. (2018)

The variable influence of pH on bacterial loads of the three water bodies supports the fact that microbes are sensitive to pH shifts (Das & Mangwani, 2015). Also, according to Krause et al. (2012) the responses of bacterial groups to pH is possibly influenced by different environmental factors. Therefore, the variability in the influence of pH on bacterial load of the water bodies in this study (Table 4.3), could be attributed to the spatial and temporal variations in the environmental parameters. For instance, although Xu et al. (2018) found pH to have the least effect on bacteria load they indicated that other authors reported contrary results.

A major source of bacterial load in water is suspended organic matter which is noticeable in turbid waters (Mill et al., 2006; Badgley et al., 2010). As such, a significantly positive correlation between turbidity and bacterial loads of the three water bodies was expected. However, the turbidity levels in Densu

were relatively low and did not tally with months of higher bacterial loads; also the turbidity trend in Nakwa did not match that of bacterial load. But in Whin, the trend in turbidity marched that of bacterial load although the relationship was not significant. For Nakwa, a possible contributor to bacteria load in the water could be the faecal matter of both humans and domestic animals around the lagoon. Although the positive relationship between turbidity and bacterial loads in Densu and Whin suggests that although turbidity could contribute to the presence of bacteria, the contribution was not significant as evident in the regression analysis (Section 4.5.1).

In general, the results depict that there could be other sources of bacteria into the three water bodies. Probably, a more detailed and prolonged study on the contributions of turbidity and other factors, to bacterial populations in the three water bodies may give a clearer picture.

Although the relationships between the physicochemical parameters and microbial loads were not consistent in the three water bodies, the former were all within limits suitable for oyster production. On the other hand, the latter which were generally beyond permissible limits could be addressed through simple sanitation measures and depuration.

5.2 Effect of Physicochemical Parameters on Condition Index of Oysters

Condition index (CI) of oysters is indicative of the commercial quality of the species and is also important in predicting suitable harvest time in the year (Davenport & Chen, 1987; Yankson, 2004). The CI is influenced by the environment, therefore, monitoring key environmental factors and relating them

to the CI of wild oysters is very important in determining the suitability of the water body for commercial production of oysters.

CI varied considerably within the three sites during the study (Figure 4.18). The observed variations in CI could have been due to some physicochemical or biological factors- temperature, salinity, DO, pH, turbidity, food and light, genotype and gonadal development, among others (Shpigel, Barber & Mann, 1992; Rivonkar, Sreepada & Parulckar, 1993; Karayucel & Karayucel, 1997; Ellis et al., 2002; Gosling, 2003; Hemachandra & Thippeswamy, 2008; Range et al., 2013; Sreedevi, Uthayakumar, Jayakumar & Ramasubramanian, 2014). The influence of the aforementioned factors on CI of oyster may also vary with respect to geographical locations (Rainer & Mann, 1992), as is evident in this study.

CI ranged between 33 and 64% in Whin estuary; 33 and 53% in Nakwa Lagoon and 33 and 54% in the Densu Delta. These values are similar to the CI values of *Perna perna* (32 – 55%) from Iture rocky beach in Ghana, reported by Krampah, Yankson and Blay (2016). From the observations made in this study, oysters from Whin had the best condition index, followed by those from Densu and Nakwa in that order. Good CI of oysters have been reported in periods of their reproductive cycles (Gosling, 2003; Hemachandra & Thippeswamy, 2008; Freites et al., 2010). According to Krampah, Yankson and Blay (2016), *Perna perna* showed better CI in months of gonadal development. Therefore, the months in which condition index dropped within the three sites could be indicative of post spawning or unfavourable environmental or biological factors. This assertion requires further studies.

With the exception of salinity and transparency, Obodai, Yankson and Blay (1994) could not link the influence of temperature, DO and pH on the gonadal development of oysters from Benya Lagoon and Pra Estuary. Hence the application of multiple linear regression to determine the relationship between physicochemical parameters and CI in this study.

Linear regression analysis performed on CI and physicochemical parameters showed that physicochemical parameters influenced CI at varying degrees within the three sites. These parameters, interacting with other factors, could have accounted for the variations observed in CI (Hemachandra & Thippeswamy, 2008).

Temperature was the parameter that had most significant influence on CI of oysters from all water bodies (Table 4.4). This was also reported by Lorio and Malone (1994) and Gosling (2003).

Although temperature is species-specific, most bivalves have been found to survive within a wide temperature range of -3 to 44 °C (Gosling, 2003). However, according to Ajana (1979) and Yankson (1990) the optimal temperature range for settlement and growth, as well as larval development of *Crassostrea tulipa* (=*gasar*), a tropical oyster, is 20 to 30 °C. This is similar to the temperature ranges recorded in this study (i.e 25.2 - 32.0 °C, 24.9 – 32 °C and 26.8 - 31.6 °C in Whin, Nakwa and Densu respectively) suggesting that these water bodies could be utilised for the culture of the species.

Salinity is also another important factor that influences condition index of bivalves (Gosling, 2003). In the present study, it was the second most

influential factor on CI after temperature. This outcome, although not significant in the three water bodies, supports the reports of Quayle and Newkirk (1989) and Gosling (2003) that salinity may be the most important limiting factor for oyster populations in coastal aquatic ecosystems; and specifically, for tropical bivalve culture, it is the main variable of influence. Obodai, Yankson and Blay (1994) reported salinity ranges of 30 – 40 ppt and 0 – 29 ppt in Benya Lagoon and Pra Estuary respectively, where there were thriving *C. tulipa* populations at that time. The recorded salinity ranges in this study, 4.1- 27.8 ppt in Whin, 8.1 - 29.0 ppt in Nakwa and 2.7 - 32.0 ppt in Densu, indicate that *C. tulipa*, like other oysters, is euryhaline (Gosling, 2003); and hence the three water bodies, in terms of salinity, could be suitable for its culture.

Although DO, pH and turbidity are also important physicochemical parameters that influence the wellbeing of bivalves, these parameters exhibited variable influences on the CI of the oysters in the present study. For example, whereas DO had a significant positive influence on CI in Densu, it had a positive, but not significant influence in Nakwa and a significantly negative influence in Whin (Table 4.4). The influence of pH was significantly positive in Whin only. On the other hand, turbidity was significantly positive in Densu but significantly negative in Whin, whereas in Nakwa it was negative but not significant.

5.3 Effect of Microbial Load on Condition Index of Oysters

As already indicated, microorganisms are inimical to oyster culture as an industry due to incidences of microbial related diseases associated with the consumption of oysters (Perkins, 1995; Pillay & Kutty, 2005). Unfortunately,

all the 11 species of bacteria identified in this study, using Cheesbrough (2006), are pathogenic.

The bacterial load in oysters did not vary with respect to seasons suggesting that once the bacteria were accumulated in the oysters they did get released into the water over time, emphasising the cleansing role of bivalves, and for that matter, oysters in aquatic environments (Perkins, 1995; Grabowski et al., 2012). The microbial loads however, showed variability in the water bodies and also exceeded the permissible limits for bacterial load in shellfishes in most of the sampling months. The variations in the water bodies could be attributed to differences in the anthropogenic activities and other natural sources responsible for introduction of microbes into the various water bodies. According to the United States Food and Drug Administration (US FDA) (1991), as cited by Ekanem and Otti (1997), coliform loads in shellfishes should not exceed 100 g^{-1} and the total viable bacteria load should not also exceed 100,000 g^{-1}; but the corresponding values obtained in the present study were generally higher (Figures 4.8 and 4.9).

The results from the multiple regression analysis (Table 4.5) revealed that an increase in TC in water generally reduced the CI of the oysters from Nakwa and Densu. Perhaps the factors that caused increase in the TC load in the two water bodies were rather detrimental to the physiological wellbeing of the oysters. The significantly positive relationship between TC load in oysters and the CI of the oysters suggests that the presence of TC in oysters was not detrimental to their physiological condition. This observation contradicts what was reported by Lawrence and Scott (1982). In their report, higher loads of

coliform in water distinctively reduced the condition index of oysters. Also, in the report by Pridmore, Roper and Hewitt (1990), reduction in condition index of oysters were partly attributed to the concentration of coliforms in the bivalves. It therefore becomes imperative to further investigate the impact of coliforms on the physiology of oysters in Ghanaian water bodies.

In general, the negative relationship between TVB load in oysters and CI of oysters agrees with the report by Jana et al. (2014). According to their report, high microbial load coincided with low CI of oysters in India. Similarly, Farcy et al. (2011) reported that bacterial load in freshwater mussels resulted in reduction in weight and an increase in mortality of the bivalves. It is evident therefore, that bacterial load may affect the physiology of the oysters in the three water bodies. This requires measures to be put in place to limit the sources of organic pollution as a means of reducing the microbial load in the water bodies. This could be supplemented with depuration mechanisms, as done in many oyster production ventures, to render oysters produced in these water bodies completely wholesome.

5.4 Effect of Heavy Metals on Condition Index of Oysters

Oysters also assimilate heavy metals via feeding, as they do bacteria. Therefore, an observation of the level of heavy metal contamination in both water and oyster samples from the three water bodies is necessary for determining their suitability for commercial oyster culture. This section discusses the influence of heavy metals on the physiological condition of oysters.

The lumping of oysters for the heavy metal analysis, as indicated in chapters three and four, did not permit multiple regression analysis on heavy metals in oysters and CI. Nevertheless, the levels of heavy metals in the oysters were within acceptable limits provided by WHO/USEPA (Mortuza & Al-Misned, 2017). From the results of the multiple regression analysis conducted on heavy metals in water and CI of oysters, the former had variable influence on the latter as was presented in the previous chapter (Table 4.6).

Although Cadmium (Cd) concentration was below the thresholds for both water and oyster samples, it still had significant influence on the CI of oysters from all three water bodies. The influence of Cd was positively significant at Whin and Densu, but negatively significant at Nakwa. The relationship between Cd and CI of oysters from Whin and Densu agrees with the report by other authors (Lin & Hsieh, 1999; Soto-Jimenez, Páez-Osuna & Morales-Hernández, 2001) that, the accumulation of heavy metals in bivalves may not be detrimental to the physiology of oysters. In contrast, the inverse relationship between CI and Cd load of oysters from Nakwa was similar to the finding of Lares and Orians (1997). In addition, Guzman-Garzia et al. (2009), also reported that Cd loads in oysters had a deleterious effect on the physiology of the organisms. Furthermore, Sokolova, Ringwood and Johnson (2005) reported physiological damages on the gills and hepatopancreas of cadmium contaminated oysters. It appears the effect of Cd on oyster CI from Nakwa may be detrimental even at low concentrations.

The observed Cd contamination from Whin and Densu could have originated from either domestic, agricultural or industrial sources, which are situated around urban settlements (Farrell et al., 2018). No intense commercial

farming activities were observed around the water bodies, though some small farms were seen, especially at Nakwa. However, there are farming and industrial activities around the upstream areas of all the three water bodies.

Lead (Pb) had a positive relationship with CI of oysters from all the water bodies. However, the relationship was only significant at Nakwa. This may suggest that the observed Pb load in oysters may not affect the physiology of the oysters in the lagoon. Nevertheless, there could be a negative effect if the loads exceed recommended thresholds (Guzman-Garzia et al., 2009). Lead contamination in the water bodies and in oysters could have originated from the burning of fossil fuel during fishing, as well as leisure activities (Edward et al., 2009).

Zinc (Zn) concentration was highest in Whin throughout the sampling period. Paradoxically, Zn had a significant influence on oysters from Densu, but not Whin. The result from Whin corroborates the findings of Kumar and Weerasooriyagedara (2018), who reported that Zn loads in water bodies were higher in months of higher salinity.

Zn is used as an anticorrosion agent, and can get into water bodies via antifouling paints and incidental release of fuel and oil from boats (Lias et al., 2013). Zn contamination in Whin and Densu could have originated from these activities, as well as other natural sources.

Copper (Cu) concentration in water was below detectable limits in all the water bodies throughout the study. Similarly, Adokoh et al. (2011) also recorded minimal Cu concentrations in water samples from six coastal water bodies in Ghana, including Nakwa Lagoon. However, some concentrations of

Cu were recorded in oysters, probably because oysters are strong accumulators of copper (George et al., 1978; Frias-Espericueta et al., 1999). The concentration of Cu in oysters were however, below the WHO/USEPA permissible limits. Higher Cu contamination in oysters have been attributed to their closeness to anthropogenic activities, as well as other factors which could augment the assimilation and retention of such metals (Eisler, 1981).

All the observed metals were within tolerable ranges in both water and oysters, except for water samples from Whin which had Zn load exceeding the limit in March 2018. Although the metals did not exceed allowable limits, continuous consumption of contaminated oysters may result in the accumulation of these metals, with the associated health risks, such as damage to the skeletal and peripheral nervous systems and memory loss (Habte et al., 2015).

Depuration as a means of decontaminating oysters of microbial contaminants (Barile et al., 2009; Obodai et al., 2010) and heavy metal contaminants (El-Gamal, 2011), has been experimentally demonstrated. This procedure can therefore be adopted as a useful practice in a future oyster culture industry in Ghana.

5.5 Chapter Summary

In this chapter, the probable explanations and interpretations of the results have been provided using relevant literature. The results from this study agreed with some previous works but contrasted the findings of others. Further studies have been suggested for more information where necessary.

CHAPTER SIX:

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This chapter is a summary of the entire thesis, specifically pointing out the findings of the study, with respect to the stated hypotheses. Also, conclusions have been made based on the results obtained and finally, recommendations suggested for consideration in future studies towards the commencement of commercial oyster culture in Ghana.

6.1 Summary

The three coastal water bodies studied (Whin Estuary, Nakwa Lagoon and Densu Delta), were monitored monthly for ten months, with the aim of determining their suitability for commercial production of oysters. The aim of this research was achieved through the monitoring of key environmental factors like temperature, salinity, DO, pH turbidity, bacterial load and heavy metal contamination of the water. The oysters were also examined for their bacterial load, heavy metal contamination and condition index. The possible influence of the above physicochemical parameters, bacterial and heavy metal loads on the physiological condition of the oysters were also investigated.

The outcome of the research revealed that, the observed physicochemical parameters varied monthly but generally showed similar trends in the three water bodies. Additionally, the physicochemical parameters had variable influences on the dynamics of the three water bodies, as well as the physiological condition of the oysters therein. Also, bacterial loads of the water bodies and the oysters, were higher than the recommended limits in most

months, but did not show seasonal variations. The bacterial load of the water bodies had variable influence on the condition index of the oysters. Furthermore, heavy metal concentrations were within acceptable limits. The concentrations of the metals in the water bodies, although generally very low, had variable influence on the condition index of the oysters.

6.2 Conclusions

The physicochemical parameters had variable influence on the dynamics in the three water bodies, but exhibited similar trends in their monthly variations.

Condition indices of oysters from the three water bodies were influenced variably by the physicochemical parameters. For instance, temperature and salinity had positive influence on the CI of oysters from the three water bodies, although the influence was only significant for temperature, whereas, DO and turbidity influenced CI negatively in Whin Estuary.

Temperature had the strongest influence on the CI of the oysters from the three water bodies, followed by salinity.

The physicochemical parameters in the three water bodies were generally within suitable limits for oyster culture as indicated in the literature. In most of the sampling months, bacterial loads of the water bodies and the resident oysters exceeded acceptable limits for water bodies and oyster consumption.

Generally, bacterial load had negative influence on the condition index of oysters from the three water bodies. Although it appeared that total coliforms did not have deleterious effect when accumulated in the oysters.

Heavy metal loads of the water bodies were within acceptable limits, indicating good ecological health, which was also reflected by the low levels in the oyster meat.

Based on the above conclusions, all the alternative hypotheses postulated for this research should be accepted. Thus, the three water bodies were found to be suitable for commercial oyster culture provided efforts are made to limit activities responsible for the high microbial load.

6.3 Recommendations

Based on the outcome of this study, the following recommendations are made:

1. Other coastal water bodies in Ghana with thriving oyster populations should also be assessed for their suitability for the culture of oysters. This would help promote oyster culture as a viable alternative/supplementary livelihood for coastal communities at a time when marine fisheries are on the decline.

2. Future studies should include the assessment of other water quality parameters like chlorophyll 'a' concentration, nutrient load, bacterial and heavy metal concentrations in sediments, the presence of other microbial contaminants like fungi and viruses, and the presence of other toxic metals. In addition, studies on the reproductive biology of oysters from Whin Estuary, Nakwa Lagoon and Densu Delta should be undertaken, so as to better understand the observed fluctuations in CI.

3. The predictions from the statistical analyses conducted in this study should be further investigated in other water bodies facilitate general applications,

4. Good sanitation measures should be implemented around coastal water bodies in Ghana through;

i. promoting the maintenance of good water quality in water bodies, especially in areas where shellfishes and other edible aquatic organisms are harvested.

ii. the prohibition of open defecation around coastal water bodies, especially around the Nakwa Lagoon.

iii. the effective treatment of sewage before discharge into nearby water bodies

5. Ghana should develop its own permissible limits for substances which are toxic to human health.

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APPENDICES

Appendix A: Some photographs from Whin Estuary

Oysters submerged under water

Oysters attached to mangrove roots

The harvest of some oyster collectors

Empty shells of oysters which were harvested from the estuary

Appendix B: Some photographs from Nakwa Lagoon

Ongoing oyster collection by women from the community

Basins full of oysters harvested from

Cockle harvest from the lagoon

Exposed oyster bed at low tide

Appendix C: Some photographs from Densu Delta

Oyster harvesters from riparian communities

Canoe full of harvested oysters

Exposed oyster bed during low tide

Oyster collection for research analysis

Appendix D: Outline of the preparation of culture media

Normal saline

7.2 g of sodium chloride (g) was weighed and poured into a beaker, into which 800 ml of distilled water was added and thoroughly mixed by swirling until the salt was completely dissolved in the solution. For the serial dilution blanks, 9 ml aliquots of the solution were pipetted into 10 ml test tubes, capped an autoclaved at 121 ^{o}C for 15 minutes. Four ml aliquots of the normal saline solution were pipetted into test tubes for the biochemical test procedure.

MacConkey Agar

MacConkey agar (15.6 g) was dissolved in 300 ml of distilled water and autoclaved at 121 °C for 15 minutes. The molten medium was allowed to cool before use.

Plate Count Agar

300 ml of distilled water was added to 5.25 g of the PCA agar. The mixture was dissolved in a microwave and thereafter autoclaved at 121 °C for 15 minutes.

Nutrient Agar

0.56 g of nutrient agar was dissolved in 20 ml distilled water. 1 ml aliquots were pipetted into eppendoff tubes and autoclaved at 121 °C for 15 minutes. The media was allowed to cool and solidify before use.

Triple Sugar Iron (TSI) Agar

6.6 g of TSI agar was dissolved in 100 ml of distilled water and autoclaved at 121 °C for 15 minutes. Four ml aliquots of the molten media were transferred into test tubes with a pipette and allowed to solidify before use.

Simmons' Citrate Agar

100 ml of distilled water was added to 2.3 g of Simmons' Citrate agar. The mixture was dissolved and autoclaved 121 °C for 15 minutes. Afterwards, using a pipette, 4 ml aliquots of the medium were added into test tubes and allowed to solidify for use later.

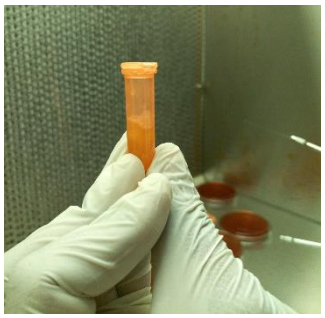
Urea Agar

2.4 g of Urea Agar Base media was dissolved in 100 ml of distilled water and autoclaved 121 °C for 15 minutes and allowed to cool. Subsequently, 10 ml of sterile urea was added to the solution and swirled to mix thoroughly. Four ml aliquots of the medium were pipetted into test tubes and allowed to solidify before use in the biochemical test procedure.

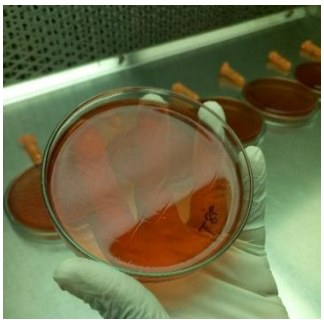
Peptone water

1.5 g of peptone water agar was dissolved in distilled water, after, 4 ml aliquots were transferred into test tubes with a pipette, capped and autoclaved 121 °C for 15 minutes. The media was allowed to cool before use.

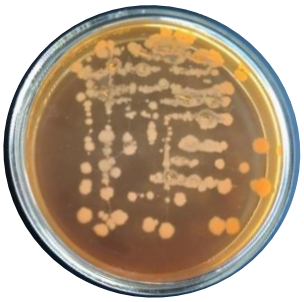
Appendix E: Some photographs of pure cultures of bacterial isolates



I



II



III

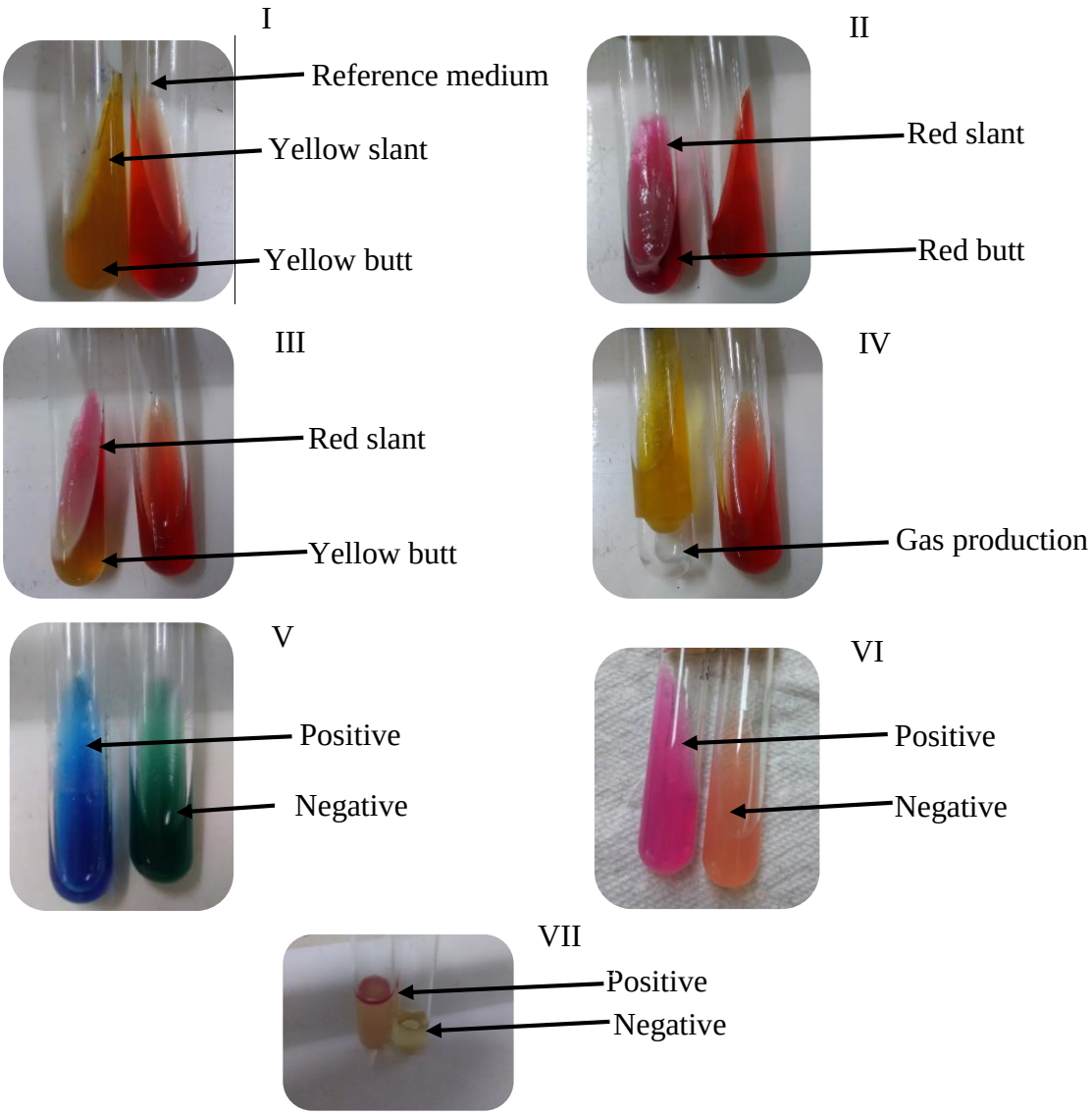
IV

(I) : Bacterial isolate growing on nutrient agar in an Eppendoff tube post incubation

(II): Freshly streaked bacterial isolate on MacConkey agar (before incubation)

(III & IV): Pure cultures of bacteria colonies, growing on MacConkey agar medium, 18 hours post incubation

Appendix F: Biochemical test results



(I): Triple Sugar Iron (TSI)- Yellow slant + Yellow butt

(II): TSI- Red slant + Red butt

(III): TSI- Red slant + Yellow butt

(IV): TSI- Yellow slant + Yellow butt + Gas production

(V): Simmons Citrate agar- Positive (blue) and Negative (green) reactions

(VI): Urea test- Positive (pink) + Negative reactions

(VII): Inoculated peptone water + Kovac's reagent (Positve = red ring and Negative = neutral)

Appendix G: Biochemical characteristics of Bacteria isolates obtained from the study

Biochemical characteristics of microbial isolates from water and oyster samples from Whin, Nakwa and Densu water bodies (adopted from Cheesbrough, 2006)

Triple Sugar Iron				Citrate	Urea	Indole	Probable Organism
Slant	Butt	Gas	H_2S				
Y	Y	+	-	-	-	+	*Escherichia coli*
Y	Y	+	-	+	+	-	*Klebsiella spp*
R	Y	-	-	-	+	-	*Yersenia spp*
R	Y	-	-	-	-	-	*Shigella spp*
Y	Y	+	-	+	-	-	*Enterobacter spp*
R	Y	+	-	+	+	-	*Citrobacter spp*
R	Y	-	-	+	-	+	*Providencia spp*
R	Y	+	-	+	-	-	*Serratia spp*
R	Y	-	-	+	-	+	*Vibro spp*

Appendix H: Reference Standards for Heavy metals of interest in the study

Reference standards for metals of interest

ELEMENT	WAVELENGTH nm	LAMP CURRENT mA	SLIT WIDTH nm	FUEL	SUPPORT
Cd	228.8	4	0.5	ACETYLENE	AIR
Pb	217.0	5	1.0	ACETYLENE	AIR
Zn	213.9	5	1.0	ACETYLENE	AIR
Cu	324.7	4	0.5	ACETYLENE	AIR

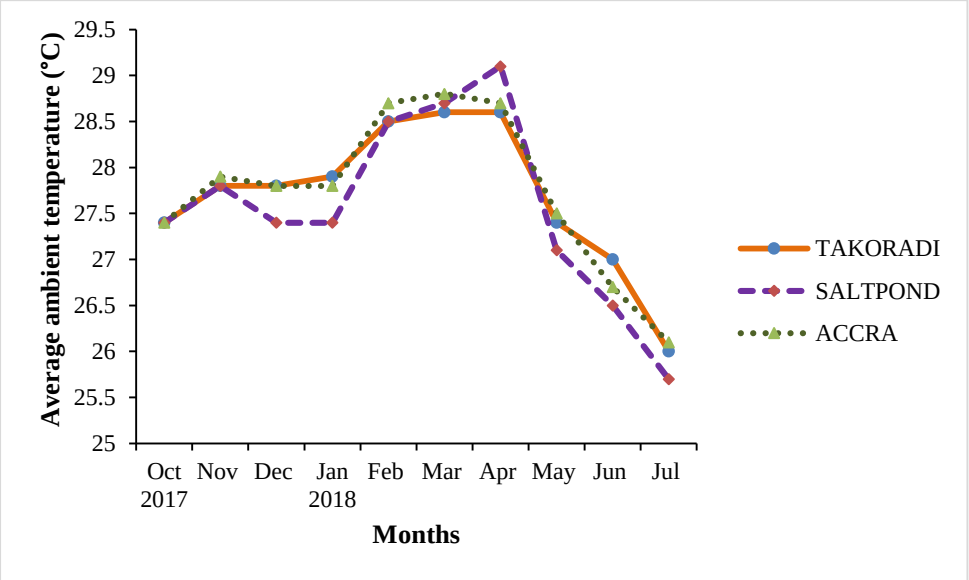
Ref: Varian Publication No 85- 100009-00, Revised March 1989.

Appendix I: Significant differences between monthly recorded parameters from the three water bodies for the entire study period

Parameter	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Study period
Temperature	0.020*	0.298	0.459	0.492	0.203	0.138	0.331	0.000**	0.021*	0.020*	0.766
Salinity	0.007*	0.358	0.290	0.008*	0.174	0.075	0.129	0.002*	0.793	0.012*	0.187
DO	0.308	0.207	0.000**	0.053	0.771	0.007*	0.120	0.000**	0.901	0.021*	0.661
pH	0.054	0.012*	0.648	0.197	0.054	0.204	0.000**	0.347	0.175	0.011*	0.348
Turbidity	0.096	0.645	0.081	0.885	0.175	0.104	0.021*	0.000**	0.187	0.008*	0.001**
Col. in water	0.281	0.538	0.384	0.000**	0.167	0.102	0.267	0.344	0.045*	0.000**	0.391
Col. in oyster	0.453	0.378	0.492	0.044*	0.288	0.285	0.059	0.784	0.171	0.297	0.334
TVB in water	0.049*	0.190	0.009*	0.001**	0.102	0.116	0.135	0.063	0.149	0.001**	0.361
TVB oyster	0.543	0.233	1.000	0.035*	0.036*	0.179	0.282	0.573	0.101	0.825	0.069
Cadmium	****			****		0.056			0.111		0.112
Lead	****			****		0.114			0.615		0.515
Copper	****			****		****			****		****
Zinc	****			****		0.486			0.128		0.152
Condition index	***	0.249	0.027*	0.258	0.000**	0.710	0.516	0.000**	0.000**	0.069	0.000**

* = significant; ** = Highly significant; *** = No available oysters; **** = Below detection limit

Appendix J: Monthly variations in ambient air temperature at Takoradi, Saltpond and Accra



Average monthly ambient temperature at Takoradi, Saltpond and Accra, from November 2017 to July 2018 adopted from Climate data: Ghana (https://en.tutiempo.net/climate/ghana.html)

Appendix K: Principal Component Analysis Tables for the three water bodies

K1: PCA Eigen values for Whin Estuary

	Component	
	1	2
TEMP	.835	.351
SAL	.151	.911
DO	.800	.304
pH	.894	.122
TURB	-.250	-.874
MB	-.407	-.702

K2: PCA Eigen values explained for Whin Estuary

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.641	60.686	60.686	3.641	60.686	60.686
2	1.065	17.744	78.430	1.065	17.744	78.430
3	.519	8.652	87.083			
4	.310	5.171	92.254			
5	.242	4.041	96.295			
6	.222	3.705	100.000			

K3: PCA Eigen values for Nakwa Lagoon

	Component		
	1	2	3
TEMP	-.289	.844	-.021
SAL	.863	-.017	-.068
DO	.468	.722	-.153
pH	.510	.636	.213
TURB	-.842	-.100	-.131
MB	.039	-.021	.984

K4: PCA Eigen values explained for Nakwa Lagoon

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.363	39.387	39.387	2.363	39.387	39.387
2	1.331	22.189	61.576	1.331	22.189	61.576
3	1.031	17.183	78.759	1.031	17.183	78.759
4	.504	8.397	87.156			
5	.430	7.163	94.319			
6	.341	5.681	100.000			

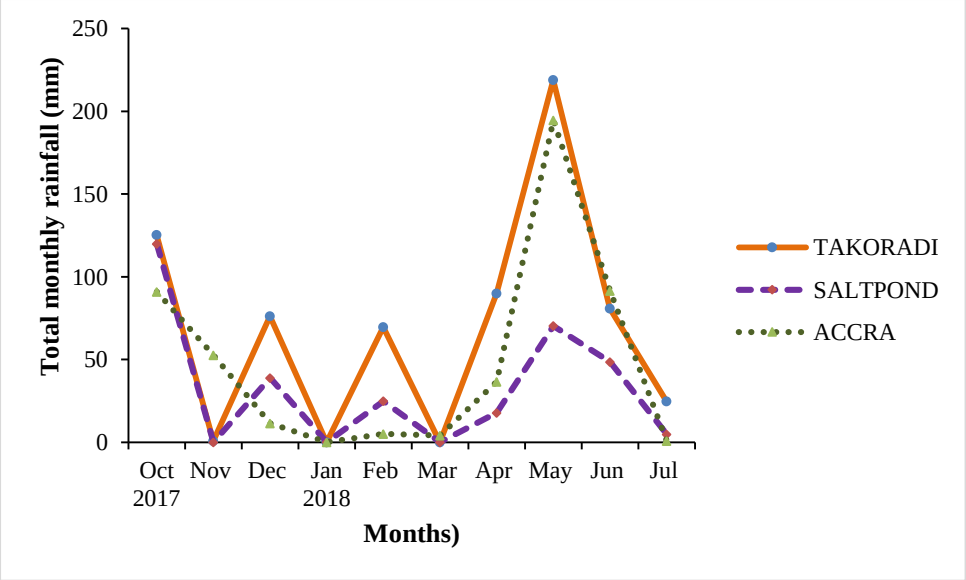
K5: PCA Eigen values for Densu Delta

	Component		
	1	2	3
TEMP	.035	-.120	.910
SAL	.937	.168	-.162
DO	.852	.104	.223
pH	-.026	.880	.065
TURB	-.687	.052	.557
MB	.263	.725	-.254

K6: PCA Eigen values explained for Densu Delta

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.454	40.907	40.907	2.454	40.907	40.907
2	1.232	20.540	61.447	1.232	20.540	61.447
3	1.100	18.326	79.773	1.100	18.326	79.773
4	.592	9.867	89.640			
5	.514	8.565	98.205			
6	.108	1.795	100.000			

Appendix L: Monthly variations in rainfall at Takoradi, Saltpond and Accra



Total monthly rainfall at Takoradi, Saltpond and Accra, from October 2017 to July 2018 adopted from Climate data: Ghana (https://en.tutiempo.net/climate/ghana.html)

Appendix M: Heavy metals recorded in water and oysters and USEPA and WHO/USEPA acceptable limits

Heavy metal	USEPA permissible limit in water (ml L^{-1})	WHO/USEPA permissible limit in oyster (mg kg^{-1})
Cadmium	0.03	1
Lead	0.2	2
Zinc	0.09	100
Copper	0.05	30

***** Extracted from USEPA (2016) and Mortuza and Al-Misned (2017)

Appendix N: Distribution of bacterial isolates identified and obtained from the three water bodies

N1: Whin Estuary

Bacteria species	**Number of bacterial isolates recovered from Whin Estuary**										
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	**Total**
Escherichia coli	1	1	2	1	3	0	0	0	0	1	**9**
Klebsiella spp	0	1	0	0	0	0	0	0	0	0	**1**
Shigella spp	0	0	0	0	1	2	2	4	3	0	**12**
Enterobacter spp	0	2	2	0	0	0	0	0	0	0	**4**
Citrobacter spp	1	0	2	0	0	0	0	0	0	0	**3**
Providencia spp	0	0	0	1	1	0	1	0	0	0	**3**
Serratia spp	0	0	1	0	0	0	0	0	0	0	**1**
Vibro spp	0	0	0	0	0	1	0	0	0	0	**1**
Total	**2**	**4**	**7**	**2**	**5**	**3**	**3**	**4**	**3**	**1**	**34**

N2: Nakwa Lagoon

Bacteria species	**Number of bacterial isolates recovered from Nakwa Lagoon**										
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	**Total**
Escherichia coli	3	1	1	1	0	0	2	2	2	1	**13**
Klebsiella pneumoniae	1	0	1	0	0	1	0	0	0	0	**3**
Yersenia enterocolitica	1	0	0	0	0	0	0	0	0	0	**1**
Shigella spp	0	0	0	2	0	0	1	0	0	1	**4**
Citrobacter spp	0	2	0	0	0	0	0	0	0	0	**2**
Providencia spp	0	0	0	1	0	1	0	0	0	0	**2**
Serratia mercescens	0	0	0	0	1	0	0	0	0	0	**1**
Vibro cholerae	0	0	1	0	0	0	0	0	1	0	**2**
Morganella morganii	1	0	0	0	0	0	0	0	3	2	**6**
Salmonella paratyphi A	0	0	0	1	0	0	0	0	0	0	**1**
Total	**6**	**3**	**3**	**5**	**1**	**2**	**3**	**2**	**6**	**4**	**35**

N3: Densu Delta

Bacterial species	Number of bacterial isolates recovered from Densu Delta										
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	**Total**
Escherichia coli	0	1	0	0	0	0	1	2	1	1	**6**
Klebsiella pnuemoniae	1	0	3	0	0	1	0	0	1	0	**5**
Shigella spp	1	0	0	1	1	0	2	1	1	0	**7**
Citrobacter spp	2	0	0	0	0	0	1	0	1	0	**4**
Providencia spp	0	0	0	1	0	2	0	0	0	0	**3**
Serratia mercenscens	3	0	0	0	1	0	0	0	0	0	**4**
Vibro cholerae	0	1	0	1	0	0	0	0	0	0	**2**
Total	**7**	**2**	**3**	**3**	**2**	**3**	**4**	**3**	**4**	**1**	**31**

Appendix O: Negative Binomial Regression analysis on the effect of physicochemical parameters on microbial load in water from the three water bodies

O1: Whin Estuary

MB	Coef.	Robust Std. Err.	z	P>z	[95% Conf.	Interval]
TEMP	-0.022	0.051	-0.43	0.667	-0.122	0.078
SAL	-0.063	0.019	-3.34	0.001	-0.101	-0.026
DO	0.106	0.050	2.11	0.035	0.007	0.204
pH	-0.709	0.215	-3.29	0.001	-1.131	-0.287
TURB	0.011	0.006	1.8	0.071	-0.001	0.022
_cons	13.050	1.369	9.53	0	10.367	15.733

O2: Nakwa Lagoon

MB	Coef.	Robust Std. Err.	Z	P>z	[95% Conf.	Interval]
TEMP	0.005	0.045	0.11	0.914	-0.083	0.093
SAL	-0.005	0.012	-0.42	0.676	-0.028	0.018
DO	-0.071	0.055	-1.3	0.194	-0.178	0.036
pH	0.011	0.140	0.08	0.939	-0.264	0.285
TURB	-0.011	0.005	-2.06	0.039	-0.021	-0.001
_cons	6.852	1.349	5.08	0	4.208	9.495

O3: Densu Delta

MB	Coef.	Robust Std. Err.	z	P>z	[95% Conf.	Interval]
TEMP	-0.220	0.062	-3.54	0	-0.342	-0.098
SAL	0.028	0.017	1.67	0.096	-0.005	0.061
DO	-0.008	0.058	-0.15	0.884	-0.122	0.105
pH	0.478	0.086	5.55	0	0.309	0.647
TURB	0.017	0.022	0.74	0.458	-0.027	0.061
_cons	8.204	1.379	5.95	0	5.502	10.906

Appendix P: Multiple Regression Analysis on the influence of physicochemical parameters on CI of oysters from the three water bodies

P1: Whin Estuary

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
TEMP	2.540616	0.2976027	8.54	0	1.948801	3.132432
SAL	0.0903875	0.1322722	0.68	0.496	-0.1726502	0.3534252
DO	-0.844531	0.4099292	-2.06	0.042	-1.65972	-0.0293419
pH	0.0141719	0.0020062	7.06	0	0.0101824	0.0181613
TURB	-0.2811573	0.1312109	-2.14	0.035	-0.5420845	-0.02023
_cons	-28.96492	8.514136	-3.4	0.001	-45.89621	-12.03363

P2: Nakwa Lagoon

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
TEMP	1.275535	0.4090568	3.12	0.002	0.4620806	2.088989
SAL	0.2715029	0.1405343	1.93	0.057	-0.007965	0.5509707
DO	0.7134598	0.6627508	1.08	0.285	-0.6044929	2.031412
pH	-1.397145	1.373742	-1.02	0.312	-4.128981	1.334691
TURB	-0.0790538	0.0673768	-1.17	0.244	-0.21304	0.0549323
_cons	2.074571	17.0656	0.12	0.904	-31.86224	36.01138

P3: Densu Delta

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
TEMP	0.8746828	0.3609845	2.42	0.018	0.1568256	1.59254
SAL	0.107808	0.1022945	1.05	0.295	-0.0956158	0.3112319
DO	2.239379	0.2455532	9.12	0	1.751069	2.727688
pH	1.590076	1.438112	1.11	0.272	-1.269767	4.44992
TURB	0.3292111	0.0789794	4.17	0	0.1721518	0.4862703
_cons	-10.38345	13.34149	-0.78	0.439	-36.91446	16.14756

Appendix Q: Multiple Regression Analysis on the influence of microbial load on CI of oysters from the three water bodies

Q1: Whin Estuary

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
TC in water	-0.0175666	0.076354	-0.23	0.819	-0.1710057	0.1358725
TC in oyster	0.0799715	0.0329428	2.43	0.019	0.0137704	0.1461726
TVB in water	0.012232	0.0932444	0.13	0.896	-0.1751496	0.1996135
TVB in oyster	-0.0608317	0.0250512	-2.43	0.019	-0.111174	-0.0104894
_cons	3.517851	0.6384302	5.51	0	2.234877	4.800824

Q2: Nakwa Lagoon

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
TC in water	-0.0324319	0.0146376	-2.22	0.032	-0.0620156	-0.0028482
TC in oyster	0.050633	0.0195489	2.59	0.013	0.0111232	0.0901429
TVB in water	-0.0043282	0.0227829	-0.19	0.85	-0.0503742	0.0417178
TVB in oyster	-0.0448358	0.0254703	-1.76	0.086	-0.0963132	0.0066415
_cons	3.797145	0.2044736	18.57	0	3.383889	4.210402

Q3: Densu Delta

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
TC in water	-0.0522669	0.0230326	-2.27	0.026	-0.0982402	-0.0062936
TC in oyster	0.0753938	0.0163972	4.6	0	0.0426649	0.1081227
TVB in water	0.0421404	0.0231391	1.82	0.073	-0.0040454	0.0883262
TVB in oyster	-0.0875556	0.0162685	-5.38	0	-0.1200276	-0.0550836
_cons	4.034824	0.280551	14.38	0	3.474842	4.594806

Appendix R: Multiple Regression Analysis on the influence of heavy metal load on CI of oysters from the three water bodies

R1: Whin Estuary

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
Cd	6.690515	1.376139	4.86	0	3.887412	9.493617
Pb	1.27511	0.78401	1.63	0.114	-0.32187	2.872085
Zn	-9.939764	10.44511	-0.95	0.348	-31.2158	11.33623
_cons	70.18318	6.666088	10.53	0	56.6048	83.76156

R2: Nakwa Lagoon

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
Cd	-9.90663	1.790275	-5.53	0	-13.54897	-6.264287
Pb	5.538097	1.499693	3.69	0.001	2.486948	8.589246
_cons	-7.38381	13.8108	-0.53	0.596	-35.48209	20.71447

R1: Densu Delta

CI	Coef.	Robust Std. Err.	t	P>t	[95% Conf.	Interval]
Cd	8.924866	2.525579	3.53	0.001	3.780429	14.0693
Pb	0.8917305	2.065116	0.43	0.669	-3.314774	5.098235
Zn	-500.9105	184.4797	-2.72	0.011	-876.6833	-125.1376
_cons	80.58819	8.862426	9.09	0	62.53602	98.64036