UNIVERSITY OF CAPE COAST

STUDIES ON PLANKTON ASSEMBLAGE, DIET SELECTIVITY AND LARVAL REARING OF MANGROVE OYSTER *CRASSOSTREA TULIPA* TOWARDS ITS SUSTAINABLE PRODUCTION IN GHANA

ERIC APPIAH KRAMPAH

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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 Doctor of Philosophy degree in Fisheries Science

MAY 2023

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: Eric Appiah Krampah

Supervisors' Declaration

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

Principal Supervisor's Signature ……. Date……...

Name: Professor Edward Adzesiwor Obodai

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

Name: Dr. Paul Kojo Mensah

ABSTRACT

Studies on plankton assemblage, diet selectivity and larval rearing of *Crassostrea tulipa* were carried out. Physicochemical parameters, plankton and oyster specimen were sampled from Narkwa and Benya lagoons, both located in the Central Region of Ghana, for one hydrological cycle. Three local microalgae isolates were tested as feed for oyster larvae in laboratory-rearing experiment. Physicochemical factors of both lagoons were generally within the acceptable range, except phosphate and nitrate which were above optimum limits. Annual mean DO, pH and turbidity were significantly higher in Narkwa, while salinity, nutrients and chlorophyll-*a* were notably higher in Benya. Higher number of plankton genera were recorded in Narkwa, but Benya recorded a higher annual mean density than Narkwa. Diatoms constituted ≈ 61 % of the plankton recorded in Benya, while dinoflagellates (majority of which were potentially toxic) were ≈70 % in Narkwa, with observed temporal variabilities in plankton compositions. Nutrients and pH were the significant predictors of diatoms and dinoflagellates, and these two plankton groups predominated oyster diet compositions, but diet selectivity analysis indicated a preferential selection for the less abundant groups. *Prorocentrum* spp were the dominant potentially toxic phytoplankton, with all year-round occurrence in the water and diet of oysters. Comparatively, Narkwa oysters were in better ecophysiological conditions. The individual local microalgal isolates supported growth and survival of oyster larvae at different scales, but a combination of all three promoted superior growth and survival of *C. tulipa* larvae. This scientific information is essential for the sustainable management of wild exploitation and aquaculture development of *C. tulipa* in Ghana.

KEY WORDS

Assemblage

Diet selectivity

Larval rearing

Mangrove oyster

Microalgal isolates

Plankton

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DEDICATION

To the memory of Professor Emeritus Kobina Yankson

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LIST OF ACRONYMS

APHA	American Public Health Association
ANOVA	Analysis of variance
ASP	Amnesic Shellfish Poisoning
AZP	Azaspiracid Poisoning
BV	Biovolume
CFP	Ciguatera Food Poisoning
CI	Condition index
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
DSP	Diarrhetic Shellfish Poisoning
DTX2	Dinophysistoxins
ESD	Equivalent Spherical Diameter
FAO	Food and Agriculture Organisation
GF/F	Glass fibre filter
HABs	Harmful algal blooms
MOFWRNA	Ministry of Fisheries, Water Resources and National Assembly Matters (Republic of the Gambia)
MoFAD	Ministry of Fisheries and Aquaculture Development (Republic of Ghana)

MY	Meat yield
NSP	Neurotoxic Shellfish Poisoning
OA	Okadaic acids
PCR	Polymerase chain reaction
POMS	Pacific oyster mortality syndrome
PSP	Paralytic Shellfish Poisoning
SH	Shell Height
SL	Shell Length
SW	Shell Width
UNESCO	United Nations Educational, Scientific and Cultural Organization
USEPA	United State Environmental Protection Agency
UV	Utraviolet

CHAPTER ONE

INTRODUCTION

The West African mangrove oyster *Crassostrea tulipa* (L.1819) is the most exploited estuarine shellfish in West Africa (Chuku et al., 2022a; Yankson, 2004). The mangrove oyster provides livelihoods and nutritional support to coastal dwellers in the sub-region (Chuku et al., 2022a; Mahu et al., 2022). The shells of the oyster also have many uses in the agricultural, building industries, cosmetics, as well as traditional medicine (Mahu et al., 2022; Obodai, 1997; Yankson, 2004). The mangrove oyster is also reported to have great potential for coastal aquaculture in West Africa (Ansa & Bashir, 2007; Asare, Acheampong & Obodai, 2019; Mahu et al., 2022; Obodai, 1997; Yankson, 2004). Nonetheless, the oyster fishery is currently dependent on unregulated wild exploitations leading to overfishing and stock decline (Asare et al, 2019; Obodai, 1997; Osei, 2020). To ensure sustainability of *C. tulipa* production, there is the need for sustainable management and conservation efforts. There is also the need to increase production through aquaculture (Asare et al., 2019; Chuku, 2019; Chuku et al., 2022a; Ministry of Fisheries and Aquaculture Development (MoFAD) & Fisheries Commission, 2018; Osei, 2019; Yankson, 2004). This thesis therefore sought to contribute scientific data on the plankton assemblage and utilisation as food by *C. tulipa* and larval rearing of the species to complement the broader scope of research on the species towards its sustainable aquaculture and exploitation in Ghana.

1.1. Background to the Study

The issue of feeding and providing livelihoods for an increasingly prosperous world population is being confronted by societies, while at the same

time identifying ways to mitigate the effects of changing climate patterns and rising occurrences of degradation of resource base (FAO, 2018a). Stagnating capture fisheries production coupled with overexploitation of some fisheries resources in the era climate change impact further exacerbate the challenges related to global food and nutrient security (Jiang, 2010). Fish and fishery resources are of paramount importance for good nutrition and health throughout the world, while also contributing to coastal communities' socioeconomic development (Chuku, 2019; MoFAD, 2015; Yankson, 2004). Ghana is a high fish consuming country, with fish constituting about 60% of animal protein intake and the country's per capita fish consumption is estimated at 25 kg, which is higher than the world average (20 kg) and far above African average of 10.5 kg (Onumah, Quaye, Ahwireng & Campion, 2020). The country's fish demand is estimated above one million metric tonnes, of which less than half the current demand is met by capture fisheries and aquaculture productions (Berchie, Nunoo & Iddrisu, 2017). Small coastal fishing communities are reported to have adverse effects on their livelihood and food security due to decreasing marine capture fisheries (Asare et al., 2019).

Bivalves (oysters, mussels, cockles, scallops and clams) have long been recognised as a valuable shellfish resource that are widely exploited at a subsistence or as small-scale fishery in many parts of the world (Vakily, 1989; Yankson, 2004). They are widely promoted as healthy and sustainable food resources with rising demand in recent years (FAO, 2018b). However, unregulated exploitation, pollution and habitat destruction are the major challenges that face the global shellfish industry (Wootton, Nursey-Bray, Reis-Santos & Gillanders, 2022). A number of community-based coastal resource

management projects have focused on sustainable management of bivalve exploitation as an alternative livelihood for small-scale fishers in order to enhance the socio-economic status in West Africa (Development Action Association (DAA), 2017; Gambia Ministry of Fisheries Water Resources and National Assembly Matters (MFWRNAM), 2012). This is due to the fact that bivalve exploitation management interventions are seen as a sustainable alternative livelihood option that can meet ecosystem-based conservation goals and demand for cheap animal protein in many coastal communities around the world (Asare et al., 2019; Chuku et al., 2022a; MoFAD, 2018). The increasing demand for fish in addition to decreasing landings of traditional finfish fisheries has necessitated sustainable management interventions of small-scale shellfisheries and development of commercial bivalve shellfish farming (Chuku et al., 2022a; MFWRNAM, 2012; MoFAD & Fisheries Commission, 2018; Vakily, 1989). The commonly exploited or cultivated bivalve species globally are oysters (*Crassostrea* spp.), cockles (*Anadara* spp.), and mussels (*Mytilus* spp. and *Perna* spp.) (Vakily, 1989).

The mangrove oyster, *Crassostrea tulipa*, is one of the most important commercial shellfish in West Africa, with distribution extending from Senegal to Angola (Yankson, 2004). The species is actively exploited, serving as a cheaper source of animal protein in many coastal countries in West Africa from Senegal to Nigeria (Adite, Abou, Sossoukpê & Fiogbé., 2013a; Ansa & Bashir, 2007; Chuku et al., 2022a; Obodai, 1997). The mangrove oyster fishery is solely based on wild exploitations, with recent intense commercial exploitation reported to have contributed to declining stocks of some coastal water bodies in Ghana (Asare et al., 2019; MoFAD, 2018; Osei et al., 2020). The species is

reported to have considerable economic potential in West Africa, and serious effort at sustainable management of wild exploitations and aquaculture development of the species would not only afford cheap source of animal protein, but will provide livelihood, nutritional support to coastal inhabitants, and help enhance the health of the ocean (Yankson, 2004).

1.2. Statement of the Problem

The West African mangrove oyster *C. tulipa* is reported to have considerable economic potential, with active wild exploitations reported in many coastal West African communities (Adite et al., 2013a; Ajana 1979; Ansa & Bashir, 2004; Asare et al., 2019; Carney, 2017; Chuku et al., 2022a; Obodai, 1997; Osei et al., 2019; Yankson, 2004). Proper regulation and sustainable management of mangrove oyster exploitation and aquaculture development in West Africa could help reduce overexploitation, maximise oyster production and enhance the livelihood and well-being of coastal inhabitants (Asare et al., 2019; Chuku, 2019; Chuku et al., 2022b; Mahu et al., 2022; Osei, 2019). However, sustainable management of oyster fishery and aquaculture development requires relevant scientific information on the biology and ecology of the species (Quayle & Newkirk, 1989). To this end, a number of studies and some management interventions have been carried out over the years on *C. tulipa*, towards ensuring increase production and sustainable exploitation of the wild stocks along the coast of Ghana (Asare et al., 2019; Chuku, 2019; MoFAD & Fisheries Commission, 2018; Obodai, 1997; Obodai & Yankson, 2000; Obodai & Yankson, 2002; Obodai, 2007; Obodai et al., 2010; Yankson, 1974; Yankson, 1990; Yankson & Moyse, 1991). Nonetheless, there is dearth of information on the trophic ecology of *C. tulipa*. Such information is essential in

evaluating energy sources for biological functions such as reproduction, growth and for oyster aquaculture development planning (Adite, Stanislas & Ghelus, 2013b). In the case of *C. tulipa*, the only available literature on its feeding ecology is that of Adite et al. (2013b), who emphasized the need for more studies on nutritional needs of *C. tulipa* and toxic phytoplankon compositions at mangrove oyster harvesting grounds towards an integrated oyster production management.

Understanding bivalve feeding behaviour and natural cycles of food supply is essential to maximise their productivity (van Ruth & Patten, 2018). Therefore, information on plankton assemblage and utilisation as food by C. *tulipa* can be useful in assessing how extensive oyster farming could affect plankton assemblage and planktonic food web structure of their environment (Jiang et al., 2022). Furthermore, abundance and quality food supply is considered limiting factor for commercial bivalve production (Grant 1996; Rheault & Rice 1996 as cited in Loret, Pastoureaud, Bacher & Delesalle Loretl, 2000). Data on diet compositions (food resource available to oysters) and supply cycles (period of availability and abundance) will therefore provide useful information on the carrying capacity of their environment for sustainable production. It is widely known that phytoplankton are the main source of nourishment for oysters. Thus, high phytoplankton productivity is crucial for the commercial production of oyster (Swan & Davidson, 2011). A wide range of phytoplankton of different genera and species abound in marine and estuarine ecosystems, and whilst larger number of these organisms are harmless, a few of the species produce toxins, which accumulate in the tissue and organs of filter-feeding shellfish, and can possess health challenges to humans or other

mammals that utilise the shellfish as food (Hinder et al., 2011; Shepard et al., 2012; Swan & Davidson, 2012). Occurrence of toxic phytoplankton have also been associated with shellfish mortalities and the sub-lethal effects which includes paralysis, a lack of byssus thread production, and reduced growth on bivalve shellfish (Rolton et al., 2022). In the wake of global warming, there is growing concern that the changing global climate will cause changes in phytoplankton assemblage, including a greater prevalence and widespread of HABs (Wells et al, 2015). In many parts of the world, periodic monitoring of HABs occurrence and toxicity levels in bivalve shellfish is well incorporated into the bivalve shellfishery regulation regimes (Silva et al., 2016; Swan & Davidson, 2011). However, data on the occurrence of toxic phytoplankton occurrence in Sub-Saharan Africa is generally lacking (Intergovernmental Oceanographic Commission-UNESCO, 2021). In the case of Ghana, the only available literature that reports on toxic phytoplankton or Harmful Algal Blooms (HABs) occurrence in the coastal waters of Ghana and toxic levels in bivalve shellfish is a study by Denutsui (2019). Scientific studies on plankton assemblage in coastal waters, particularly at oyster fishing grounds is therefore important for the fledgeling oyster fishery in Ghana and the rest of sub-region. This can provide insight into distribution and occurrence periodicity of harmful/toxic microalgae species, which is necessary to ensure sustainable oyster production, and safety of oysters for human consumption. This could contribute vital information oyster exploitation management and aquaculture development planning.

Commercial oyster aquaculture thrives on a regular supply of seeds (juveniles) for growing on to market size (Obodai, 1997; Yankson, 1990). The

seeds can be obtained primarily in one of two ways: (i) by wild collection or (ii) through hatchery rearing. The former is cheaper because one needs only to invest in cultches (seed collectors), which can be made from cheap local materials (Chuku, 2019; Obodai, 1997; Yankson, 2004). That not withstanding, the wild seed collection is limited by seasonality in seed abundance (i.e. seed collection can only be carried out within short period of the year) and unpredictability due to variations in recruitment from year to year. (Obodai, 1997; Ponis, Probert, Véron, Mathieu & Robert, 2005). Therefore, for a commercial oyster aquaculture venture to be sustainably viable, wild seed collection needs to be supplemented with hatchery reared seeds. One advantage of hatchery seed production is that breeding programmes can be undertaken to select for desirable characteristics (i.e. fast growth rate, disease resistance, etc.) for stock improvement (Yankson, 1990). Some earlier studies have been conducted on laboratory/hatchery propagation of *C. tulipa* seed. For instance, Yankson (1990) reported on successful rearing of the larvae till settlement in the laboratory. Yankson and Moyse (1991), on the other hand, reported on the influence of cryopreservation on the viability of the spermatozoa of *C. tulipa* and three other oyster species. It is worth noting that the above studies were carried out in the United Kingdom, where development of techniques for oyster larval rearing are far advanced. Obodai et al., (2010), on the other hand reported on the embryonic development of *C. tulipa* from *in-vitro* fertilisation. All these studies point to the fact that *C. tulipa* is amenable to laboratory/hatchery production. Nevertheless, the progress on the development towards laboratory rearing of *C. tulipa* in Ghana was stalled due to the lack of technical know-how

on isolation and culture of local microalgae strains to serve as food source as well as unavailability of commercial microalgae stock cultures in the country.

Bivalve seed production in the hatchery relies on live microalgae to provide the nutritional elements that meet the larvae's metabolic needs, as there is no artificial feed that has been shown to be suitable for the replacement of live microalgae (Hemaiswarya, Raja, Ravi-Kumar, Ganesan & Anbazhagan, 2011; Mamat & Alfaro, 2014). The culture of microalgae is necessary because the water treatment processes remove nearly all natural phytoplankton that needs replacing in cultures of selected, highly valued food species (Shoji & Ajithkumar, 2015). There are many naturally occurring microalgae, but few are of food value for shellfish aquaculture and are adapted to large scale laboratory culture. These types of microalgae strains can best be selected based on research (Parvin, Zannat & Habib, 2007). In this sense, isolation and culturing of local microalgal strains have an edge over their temperate strains because they are well suited to the local climatic conditions (Larkum, Ross, Kruse & Hankamer, 2012). From the foregoing, there is no doubt that scientific studies such as this on isolation and culture of local microalgal strains as feed for the rearing *C. tulipa* larvae is crucial for mangrove oyster aquaculture development in Ghana and the sub-region.

1.3. Purpose of the Study

To contribute to considerable ongoing interest and research towards sustainable management of oyster fishery and aquaculture development along the coast of West Africa (Asare et al., 2019; Chuku et al., 2022a; Chuku et al., 2023; Mahu et al., 2022; Osei et al., 2021), this study aimed to provide scientific information on the natural cycles of plankton assemblages and abundance, diet

compositions and selectivity of *C. tulipa* in two tropical lagoons (with various forms of anthropogenic influences) in the central coast of Ghana. The study also investigated the potential use of local microalgae isolates for the larval rearing of *C. tulipa*. This is an attempt to fill in the knowledge gap on the oyster's trophic ecology and hatchery seed production. Data from this study will be important for evaluating the energy sources and natural cycles of food supply of *C. tulipa* populations. It will also give indication of the occurrence of toxic phytoplankton and the potential human health risks from oyster consumption (Adite et al., 2013b; Wolny et al., 2020). This can help inform oyster exploitation management policies and aquaculture development in Ghana and across the sub-region.

1.4. Research Objectives

The primary aim of this study was to investigate plankton assemblage, ecophysiology, diet compositions and selectivity of *C. tulipa* in two tropical coastal lagoons, and rearing performance of the oyster larvae on local microalgal isolates.

The specific objectives were to:

1. Monitor some selected hydrographic conditions of Benya and Narkwa Lagoons.
2. (i) Characterise the plankton community compositions in the two lagoons.

   (ii) Determine the relationship between hydrographic conditions and the plankton community compositions

   (iii) Model environmental predictors of the abundance of the dominant plankton groups.

3. Assess the plankton diet compositions, diet selectivity (preference) and compositions of ingested potential toxic phytoplankton taxa in the diet of *C. tulipa* population in the two lagoons.
4. Investigate the ecophysiological conditions and determine the environmental predictors of meat yield and condition index of *C. tulipa* populations in the two water bodies.
5. Assess the rearing performance of the oyster larva on local microalgae isolates.

1.5. Significance of the Study

Along the coast of West Africa, *C. tulipa* fishery has been reported to have considerable economic potential. However, lack of scientific information on the ecology and biology of the species, among others threatens the sustainability of the oyster fishery in Ghana and the sub-region. This study, therefore, is deeply rooted in UN Sustainable Development Goals, particularly 1 (No poverty), 2 (Zero hunger), 3 (Good health and well-being) and 14 (Life under water). It also aligns with the values of sustainable oyster fishery and aquaculture development, in the context of Africa's blue economy space. The outcome of the study will impact on the sustainable oyster exploitation and oyster aquaculture in Ghana, which will ultimately enhance the nutrition and livelihood of the vulnerable coastal dwellers and ultimately socio-economic empowerment of coastal communities in Ghana and West Africa.

1.6. Delimitation

Mangrove oysters are reported to be found in a number of estuarine water bodies along the coast of Ghana. However, Benya and Narkwa lagoons were selected for field sampling for three reasons: First for close proximity to

the Fisheries and Coastal Research Laboratory located in the University of Cape Coast, where laboratory analyses which formed the bulk of the study were carried out. Secondly, initial field survey gave indication of the relative importance of the two water bodies with regard to oyster ecology and fishery. Narkwa lagoon, for instance, is considered as one of the hotspots of oyster exploitation along the coast of West Africa. Lastly, the level of anthropogenic influence on these two lagoons was worthy of studies to understand how they impact on the water conditions and the oyster fishery.

Measurement of hydrographic conditions, sampling of oysters and water samples were carried out at the designated sampling points in triplicates within the water column. However, the sampling stations in the two water bodies were incomparable, so mean values estimated from pooled data from the various sampling stations were compared for the water bodies instead of the sampling stations.

Several techniques for stomach content analysis to investigate the diet compositions and feeding preferences in oysters have been described in literature. However, microscopic analysis method, which is relatively inexpensive, easy to perform and can provide a detailed understanding of the diet and feeding behaviour of oysters, was employed in this study to investigate the diet compositions and feeding preference of *C. tulipa* populations in Benya and Narkwa. The method, albeit time-consuming, was chosen based on the availability of equipment and availability of materials and resources to aid in identification of plankton food items.

The microalgae used in the oyster larval rearing experiment were isolated from the relatively less turbid water samples collected from off the coast of Elmina, due to the relatively high turbidity and contaminants levels of the lagoons studied which could hamper the microalgae isolation processes.

Data normality and homogeneity tests were carried out and appropriate transformations applied where necessary to satisfy assumptions of before ANOVA and Student t-test analyses.

1.7. Limitation

Plankton identification in the water column and in the oyster stomach content were done to the generic level due to the limitation in the technical know-how required for species level identification.

Despite the fact that these factors should be pointed out as limiting factors on the study, it must be stressed that their effect is confined to its research level and does have no bearing on the conclusions of science drawn from this study.

1.8. Definition of Terms

Aquaculture -rearing of aquatic animals and plants, for food and other economic purposes.

Culture -maintain under conditions suitable for growth.

***In-vitro* fertilization**- is a form of assisted reproductive technology in which an egg is fertilized by sperm outside the body, in a laboratory dish.

Microalgae–Microalgae are microscopic, single-celled algae that are found in aquatic environments such as oceans, lakes, and rivers.

Oyster larvae- typically free-swimming and planktonic early developmental stage of an oyster.

Oyster seed- the juvenile stage of an oyster, typically measuring between 1-2 mm in size.

Plankton- a diverse group of microscopic organisms that live suspended in water and drift with ocean currents.

Rearing- the process of raising and caring for young animals until they reach maturity, including providing them with food, shelter, and veterinary care.

1.9. Organisation of Study

There are six chapters in this thesis. The overall concept of the study is set out in Chapter One, giving an overview and a description of the issue, purpose, objectives and importance. Chapter Two presents a thorough examination of the literature which has relevance to this study. The study areas are described, the methods used to investigate them and statistics instruments used for analysing data are explained under Chapter three. The results of this study are set out in Chapter Four, illustrated in figures and tables with statistical analyses as well as brief observations. The main findings are discussed in Chapter five, and the inferences made on the basis of appropriate literature are presented. A summary of research findings, as well as conclusions and recommendations from the study are presented in Chapter six.

CHAPTER TWO

LITERATURE REVIEW

A review of literature relevant for the study is presented in this chapter. The literature review covered the following thematic areas: a brief history and overview of global oyster fishery: current production trends, challenges and opportunity for growth, general oyster biology and ecology, oyster feeding biology and ecology, plankton dynamics and utilisation by oysters, oyster diet compositions and stomach content analysis, socioeconomic impact of toxic/harmful microalgae on oyster and other shellfish production: global overview, ecophysiology of oysters: the case of oyster growth and condition index, microalgae isolation and culture characteristics: *Rhodomonas*, *Nannochloropsis* and *Pseudanabaena* species and oyster larval rearing on microalgae isolates. Review on these thematic areas are structured by way of introduction/background, body and conclusion.

2.1. A Brief History and Overview of Global Oyster Fishery

Oysters have been exploited for food dating back several millenia, with evidence of oyster consumption dating back to ancient Rome and Greece (Bortone & Davis, 1994). In the centuries that followed, oyster fisheries developed throughout Europe, North America, and Asia, with oysters becoming an important commodity in the global economy. In North America, oyster fisheries were established in the 17th and 18th centuries, with oysters being harvested from both wild and cultivated beds. By the mid-19th century, oyster production in the United States had become a major industry, with millions of metric tonnes of oysters being harvested each year from the Chesapeake Bay, Gulf of Mexico, and other coastal areas (Mann, 2000). In Europe, oyster

fisheries have a long history dating back to Roman times, and were an important industry throughout the Middle Ages and Renaissance. In the 19th century, oyster production in Europe began to decline due to overfishing, disease outbreaks, and pollution (Bortone & Davis, 1994). Oyster fisheries in Asia have a similarly long history, with evidence of oyster cultivation in China dating back to the 5th century BC. Today, China is the largest producer of oysters in the world, with oyster farming being an important industry along the country's coasts (Botta, Asche, Borsum & Camp, 2020).

Oyster exploitation in Africa and sub-Saharan Africa has a long history, with evidence of oyster consumption dating back to prehistoric times (Haupt, Griffiths, Robinson, Tonin, & De Bruyn, 2010). Oyster farming is reported to have been implemented as a sustainable alternative to wild oyster harvesting, with several successful oyster farming projects in countries such as Senegal, the Gambia, Benin and Tanzania. These projects have provided new economic opportunities for coastal communities and helped to reduce pressure on wild oyster populations (FAO, 2010). In East Africa, oyster fisheries are also an important source of food and income, with oysters being harvested from wild and cultivated beds along the coast of Tanzania, Kenya and Mozambique (Mafambissa, Gimo, Andrade, Macia, 2023; Mavutil, Kimani & Mukiama, 2005). However, oyster production in the region is limited by lack of investment in the industry and limited access to markets (Olivier, Heinecken & Jackson, 2013). In West Africa, the mangrove oyster, *Crassostrea gasar* (*tulipa*) is an important fishery resource, providing a source of nutrition and income for many coastal communities in the sub-region. (Ajana, 1980; Ansa & Bashir, 2007; Chuku, 2022; Yankson, 2004). In Ghana, a number of coastal communities are

reported to be involved in wild oyster harvesting in large quantities from estuarine and mangrove at low tides (Asare et al., 2019; Chuku, 2019; Obodai, 1997; Osei, 2019; Osei, Chuku, Effah, Kent & Crawford, 2021; Yankson, 2004). The majority of oyster harvesters are women and children, and the exploitation and trade of oysters is reported to be the second most important economic activity in some coastal communities (Asare et al., 2019; Chuku et al., 2022a; Osei et al., 2020; Osei et al., 2021). Despite the potential benefits of oyster exploitation in Africa and sub-Saharan Africa, challenges such as overexploitation, habitat degradation, pollution and climate change impact on the oyster fishery in the region (Asare et al., 2019; Chuku et al., 2022; Manu et al., 2022). The oysters are harvested from the wild, either by hand or using various forms of traditional fishing gear, such as hand axe (Adite et al., 2013a; Ajana 1980; Ansa & Bashir, 2007; Asare et al., 2019; Carney, 2017; Chuku et al., 2022; Obodai, 1997; Osei et al., 2019; Yankson, 2004). The oyster fishery within the sub-region is largely unregulated, with little or no control, and increasing demand for oysters in local and international markets has raised concerns over sustainability and the need for better management practices (Asare et al., 2019; Chuku et al., 2022; FAO, 2017). However, community-based management initiatives have shown promise in improving sustainability and increasing the income of those who depend on this resource (Chuku et al., 2022; Salack et al., 2015). Such initiatives involve the establishment of community-led management committees, which are responsible for regulating the number and size of oysters harvested, and for enforcing rules on the use of fishing gear and the protection of breeding populations (DAA & Asare, 2017; MFWRNAM, 2012; MoFAD, 2020). In addition to community-based

management, aquaculture has also been identified as a potential means of reducing pressure on wild oyster populations in West Africa (Adite 2013b; Ajana, 1980; Ansa & Bashir, 2007; Asare et al., 2019; Mbaiwa, 2019; Yankson, 2004). Thus, oyster exploitations provide livelihoods and nutritional support in Sub-Saharan Africa. However, unsustainable exploitations, limited investment in the sector, pollution, among many other challenges continue to hinder the growth of the industry.

2.2. Current Oyster Production Trends

In recent decades, global oyster production has shifted from extensive wild exploitations to aquaculture production in many parts of the world. Oysters are typically produced using one of three main methods: traditional intertidal farming, subtidal farming, or land-based tank systems. Traditional intertidal farming involves oyster cultivation in shallow coastal waters, where oysters are exposed to the air at low tide. This culture method is common in many parts of the world, including Asia, Europe, and North America, and is often used for high-value oyster species like the Pacific oyster (*Crassostrea gigas*) and the European flat oyster (*Ostrea edulis*) (FAO, 2020b). Subtidal farming involves growing oysters in deeper waters, usually using long-lines or cages that are suspended from buoys or other structures. This method is often used in areas where intertidal farming is not feasible, such as in areas with high tidal ranges or strong currents. Land-based tank systems, on the other hand, involve cultivating oysters in tanks or ponds that are filled with seawater or recirculating aquaculture systems. This method is becoming increasingly popular in some parts of the world, particularly in areas where land and water resources are limited (FAO, 2020b). However, in West Africa, oyster exploitation is still

largely based on wild exploitations, even though small-scale and experimental aquaculture in some cases have been reported in Senegal, the Gambia, Sierra Leone, Ghana, Togo and Benin (Adite et al., 2013a). While the biological information and technology for mangrove oyster aquaculture is lacking in the region, strategies for optimising seed collection from the wild are well documented (Chuku 2020; Obodai, 1997). Lack of technical know-how and investment into microalgae production has stalled the development of hatchery rearing of oyster seed (Obodai, pers comm). Oyster aquaculture can provide a more sustainable and predictable source of oysters, while also generating income and employment opportunities for coastal communities (Asare et al, 2019). In conclusion, the history of global oyster production is a complex and dynamic story, reflecting oysters as important fisheries resources.

2.3. Challenges and Opportunity for Growth

Despite the many benefits of oyster production, the industry faces a number of challenges that are impacting negatively on its growth and sustainability. One of the main challenges is disease, which has had a devastating impact on oyster populations and production. For example, outbreaks of the Pacific oyster mortality syndrome (POMS) caused significant losses in oyster farms in Australia and New Zealand (Green, Montagnani, Benkendorff, Robinson & Speck, 2013). Other challenges include climate change impact and overfishing of wild stocks (Atindana, Fagbola, Ajani, Alhassan & Ampofo-Yeboah, 2020; Mahu et al., 2022). Efforts to restore and sustainably manage oyster populations have been implemented in many regions, including the United States, Europe, Asia and recently in West Africa (Chuku et al., 2022; Pogoda, 2018; Wasson et al., 2020). Despite these challenges, the

growing demand for sustainably produced seafood, advances in technology opening up new possibilities for oyster production, development of new genetic strains that are more resistant to disease and environmental stress, and the growing interest in the development of new value-added products from oysters are also emerging opportunities for growth in the global oyster industry (FAO 2022). The oyster industry globally is facing a number of challenges. However, significant opportunities for growth and innovation exist. By leveraging on research, emerging technologies, value addition and adopting conservation, sustainable and innovative production methods, *C. tulipa* production can continue to meet the growing demand for quality and eco-friendly seafood, while also contributing to the socioeconomic development of communities. In the case of *C. tulipa*, unregulated wild exploitations, pollution, habitat loss, lack of scientific information to inform management policies are some of the challenges facing its sustainable production. This study therefore feeds into a broader scope of research on the species towards its sustainable management and production Ghana.

2.4. Oyster Biology and Ecology

2.4.1. Classification

By way of taxonomic classification, oysters belong to Kingdom Animalia, Phylum Mollusca, Class Bivalvia and Order Ostreida. They were earlier grouped under the two Families; Ostreidae and Gryphaeidae. However, most members of Gryphaeidae are extinct, with the extant species reassigned to Family Ostreidae (Quayle, 1980). Oysters are probably among the most studied invertebrate organisms, and even though their distribution in the tropics and

subtropics is well documented, extensive study on their biology have been reported among the temperate species (Angell, 1986).

Tropical oysters support usually small-scale oyster fishery, which largely depends on wild exploitations, and advancement in oyster aquaculture has been hampered by lack of awareness of its potential among fishery development advocates and lack of information on culture technology, processing and marketing (Angell, 1986). Many genera of living oysters are documented in literature. However, most of the commercially important species belong to the genera *Crassostrea*, *Saccostrea*, *Ostrea* and *Magallana* (Angell, 1986; Salvi & Mariottini, 2021). The most well-known species is the Pacific oyster, *Crassostrea gigas*, with many other species of oysters found throughout the world's oceans and estuaries (Beck et al., 2011). The genus *Crassostrea* includes species such as the Pacific oyster (*Crassostrea gigas*) and the American oyster (*Crassostrea virginica*), which are two of the most widely cultured oyster species in the world (Miossec, Le Deuff & Goulletquer, 2009). *Crassostrea rhizophorae* is particularly abundant in Brazil, where it is one of the most important species of oyster in terms of aquaculture production (Varela et al., 2007). *Crassostrea tulipa* is also reported to support a vibrant women-led small-scale oyster fishery along the coast of West Africa (Chuku et al., 2022; Mahu et al., 2022). The genus Ostrea includes species such as the European flat oyster (*Ostrea edulis*) and the Olympia oyster (*Ostrea lurida*), both of which are also commercially important. The genus Saccostrea includes species such as the mangrove oyster (*Saccostrea commercialis*) and the rock oyster (*Saccostrea cucullata*) which are important in various regions of the world (Bayne, 2017). The genus *Magallana*, formerly classified with the *Crassostrea*

(Salvi & Mariottini, 2017; Salvi & Mariottini, 2021), includes species such as the Portuguese oyster (*Magallana angulata*) and the Kumamoto oyster (*Magallana sikamea*), which are commercially important in their respective regions (FAO, 2023).

2.4.2. Habitat and Distribution

In terms of habitat and distribution, oysters are generally considered to live in coastal areas which include intertidal or shallow subtidal zones including lagoons, marshes and bays with their range ranging from temperates to tropical latitudes throughout the world (Bayne, 2017; Gosling, 2015; Ruesink et al., 2005). Saccostrea oysters are reported to inhabit subtropical and tropical areas, whiles the *S. cucullata* (Born, 1778) is confined only to the Indo-Pacific waters (from the Red Sea to Australia) (Hamaguchi, Shimabukuro, Usuki & Hori, 2014), the genus *Crassostrea*, just like the *Ostrea* spp have subtropical and tropical distributions except in Polynesia and Melanesia (Angell, 1986). Through reintroduction of species for aquaculture purposes and shipping activities, changes in the native range of these commercially important oysters have been reported (Bergström, Thorngren & Lindegarth, 2022).

2.4.3. Ecological roles

Anatomy of oysters from the genus *Crassostrea* is illustrated in figure 1. Oysters are filter-feeders, whereby it filters tiny particles of organic matter from the water column (Dame, 1996). They are able to filter large volumes of water, which can help improve water quality by removing excess nutrients and particles, and increase light penetration to benefit seagrasses and other aquatic vegetation (Newell, 2004). Oysters play crucial role in estuarine and coastal ecosystem ecology as they provide vital habitat and sanctuaries for an extensive

diversity of marine organisms, including fish, crabs or more invertebrates. Additionally, oyster reefs have been shown to be beneficial in terms of physical structure and stability of their surrounding habitats, helping to reduce erosion and promote sediment deposition (Beck et al., 2011). However, oysters are also vulnerable to a number of threats, including habitat loss, overfishing, pollution, and disease (Beck et al., 2011). In recent years, many oyster populations have suffered declines as a result of these threats, leading to efforts to restore and conserve oyster habitats and populations through a variety of management strategies, such as habitat restoration, stock enhancement, disease control and exploitation management (The Nature Conservancy, 2016). From the forgoing, oysters are among the most extensively studied invertebrate organisms, with a complex and multifaceted biology and ecology. A lot more studies is said to have concentrated on temperate species, leaving a research gap on the tropical species. Much more research effort is required to fill in the information gap on the tropical species.

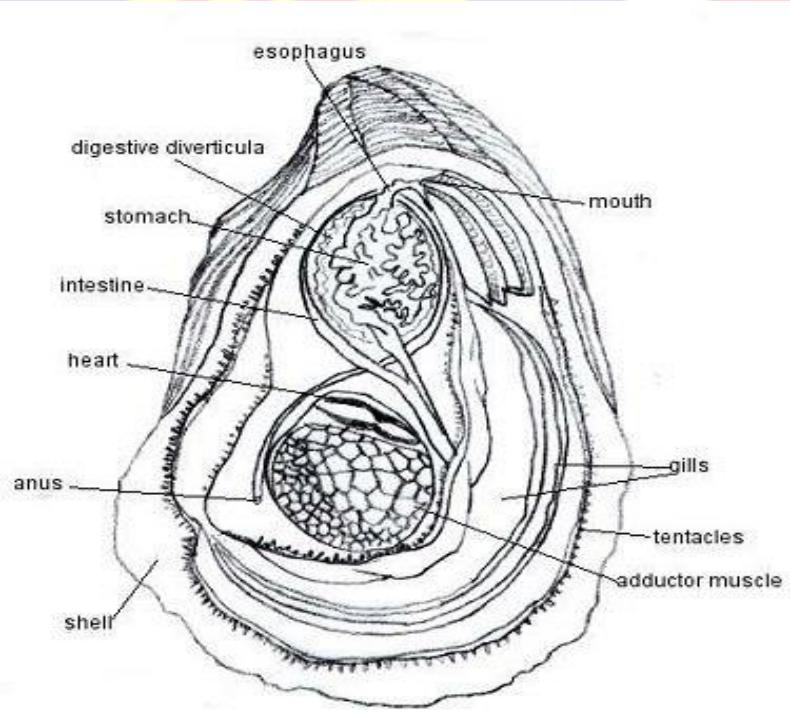

Figure 1: Anatomy of oyster (*Crassostrea* sp). Retrieved from https://www.bumblebee.org/invertebrates/BivalviaOysters.htm

2.5. Feeding Biology of Oysters

Oysters, like other filter-feeding bivalve shellfish, rely on the availability and quality of phytoplankton as a primary food source (Ramdani, et al., 2009). The rate of filtration depends on a number of factors such as oyster size, water temperature, and food concentration (Ramdani et al., 2009; Sytnik, 2020). Oysters can feed continuously and have been shown to have the ability to selectively choose the most nutritious phytoplankton species (Ward & Shumway, 2004). Additionally, oyster feeding behaviour may be changed by adjusting its filtration rates as well as selecting different food sources, in response to changes in food availability and quality (Smaal et al., 2013).

Research has been conducted extensively on the feeding and ecological situation of oysters, with research focusing on a number of topics including particle capture mechanisms (Bayne, 1976; Newell & Bayne, 1980; Riisgård & Larsen, 2010; Rosa, Ward & Shumway, 2018), effects of environmental factors on feeding (Cassis, Pearce & Maldonado, 2011; Dekshenieks, Hofmann, Klinck & Powell, 2000) and phytoplankton selectivity in oysters (Loret et al., 2000; Weissberger & Glibert, 2021). Depending on their species and the environment, oysters are said to be capable of filtering particles from a size as little as 1m all the way up to 5 mm (Gaspar, Chi, Parrow & Ringwood, 2018). The rate of particle capture determined by a variety of factors, including temperature, salinity, food availability, and water flow (Bayne, 1976). Through their feeding activities, oysters help in nutrient cycling in aquatic ecosystems, they help to reduce turbidity and improve water clarity through the filtration of

particles from the water column, which in turn can benefit seagrass beds and other benthic habitats (Lenihan & Peterson, 1998).

Oysters are also able to assimilate and store substantial amounts of nutrients, including nitrogen and phosphorus, which can be released back into the ecosystem through the elimination of faeces and pseudofaeces (Dame, 1996). However, oyster feeding can also be influenced by predation and competition. For example, the presence of predators, for example as crabs and gastropods can reduce oyster feeding rates, while competition with other filter-feeders such as barnacles and mussels can lead to reduced feeding efficiency (Peterson et al., 1999). From the forgoing, oyster foraging behaviour and mechanism are important aspect of their biology and ecology, and therefore studies on plankton foraging behaviour of *C. tulipa* will provide comprehensive understanding of their biology and ecology. Scanty scientific information exists on the plankton foraging behaviour of *C. tulipa* (Adite et al., 2013b), which make the current study imperative to fully understand of the biology and ecology of *C. tulipa*.

2.6. Phytoplankton Dynamics and Utilisation by Oysters

According to Kim et al. (2019), phytoplankton assemblage plays a critical role in oyster production, as oysters rely on phytoplankton as their primary source of nutrition, thus, the growth of active phytoplankton is essential for the commercial production of oysters. Although oysters mainly feed on phytoplankton, it is reported that they also consume detritus, fragments of seagrasses, microscopic and mesozooplankton as well as eggs and larvae from fin and shellfish (Bayne, 2017; Haines & Montague, 1979; Newell, 1988). Understanding the relationships between phytoplankton dynamics and oyster

production is therefore essential for effective oyster management and conservation. Phytoplankton communities are complex and dynamic, influenced by various physical and environmental factors (Cloern, 1996; Huang et al., 2022). Seasonal, spatial and annual variations in the composition and abundance of these phytoplankton species is well documented (Barth, Walter, Robbins & Pasulka, 2020). In particular, environmental conditions such as temperature, light availability, nutrient availability and water flow can affect phytoplankton growth and community structure (Cloern, 1996). According to Smaal et al. (2013), oyster feeding activities can have profound influence on phytoplankton populations dynamics, as selective feeding can lead to shifts in community composition.

A bloom of phytoplankton, characterised by an excessive increase in phytoplankton biomass, may have positive and adverse effects on the production of oysters. In some cases, phytoplankton blooms can lead to increased food availability for oysters and higher growth rates. However, in other cases, blooms could result in oxygen depletion and lead to oyster mortality. Environmental conditions such as temperature, salinity and nutrient availability can also impact phytoplankton dynamics and subsequently affect oyster production. For instance, elevated nutrient levels can lead to phytoplankton blooms and subsequent changes in water quality that can negatively impact the environment where oysters thrive (Livingston, 2007). Phytoplankton diversity and composition can also influence oyster production. The nutritional quality of phytoplankton is essential for oyster growth and health. Different phytoplankton species have varying nutritional value, with some providing essential fatty acids, amino acids, and vitamins that are

necessary for oyster growth and reproduction (Ward et al., 2000). However, excessive or inadequate phytoplankton availability can lead to negative impacts on oyster growth and health (Smaal et al., 2013). Lack of food, for example, can slow growth and increase the susceptibility to disease while excessive phytoplankton concentrations may result in oxygen depletion and subsequent mortality (Mann, 2000). Also, oysters may preferentially feed on certain types of phytoplankton, and changes in phytoplankton composition can affect oyster growth and survival (Weissberger & Gilbert, 2021). This is because when it comes to nutrition quality, not all types of phytoplankton are equal, and bivalves have been shown to preferentially utilise phytoplankton species based on their nutritional value and cell size (Cucci et al., 1985; Kiørboe & Mohlenberg, 1981; Møhlenberg & Riisgård, 1979; Rouillon et al, 2005).

Diverse collections of phytoplankton of different taxa occur in different aquatic ecosystems, and whilst the majority of the species are benign, some phytoplankton species are capable of producing toxins that can accumulate in oysters and cause health risks for human consumers (Swan & Davidson, 2011; Anderson et al., 2008). To understand the mechanism of feeding preference or differential utilisation of phytoplankton food resources in bivalve, and temporal changes in food supply, under field conditions, some authors have proposed comparing the contents of oysters' gut and aquatic column or superficial sediment in terms of phytoplankton species composition to be an effective approach. (Jiang et al., 2022; Kamermans, 1994; Konrad, 2014; Muñetón-Gómez, Villalejo-Fuerte, & Lizárraga, 2001; Rouillon et al., 2005; Shumway et al., 1987). In using these methods, Kamermans (1994) reported that species composition in the gut of epifaunal and infaunal bivalves living on mudflats

were similar to water column content compared with superficial sediment but no evidence of selective feeding could be derived from her data. Shumway et al. (1987) and Muñetón-Gómez et al. (2001), comparably, similar seasonal variations of species composition have been reported in seawater and stomach for *Placopecten magellanicus* and *Spondylus leucacanthus*. However, Ciocco and Gayoso (2002) observed that diet composition analysis of ribbed mussels (*Aulacomya atra*) did not reflect the occurrence of diatom blooms recorded in the area of study. From the forgoing, Plankton, particularly phytoplankton, are important food resources for oysters. Understanding the feeding preference of oysters and the seasonal changes in plankton assemblage is an important research subject, as it has impacts on understanding the effects of habitat change, oyster fishery management and the suitability of habitat for oyster aquaculture (Kasim & Mukai, 2009; Shumway et al., 1985). Phytoplankton compositions also give indications of the safety of oysters to human consumers. Therefore, scientific studies that provide data on phytoplankton community composition, their natural cycles of abundance and their utilisation by *C. tulipa* are essential to ensure sustainable production and for effective exploitation management species.

2.7. Oyster Diets Compositions and Stomach Content Analysis

Oysters are filter-feeding bivalve molluscs that obtain their nutrition by filtering small particles from the surrounding water. Depending upon the environment factors, e.g. temperature, salinity and availability of food resources, their diet may differ. Several studies have investigated the diet composition of oysters using stomach content analysis. A number of studies found that the diet of the oysters was dominated by diatoms and dinoflagellates,

with smaller contributions from bacteria, protozoa, and detritus. (Adite et al., 2013b; Rouillon, 2005; van Ruth & Patten, 2018; Weissberger & Glibert, 2021). Another study by Weissberger & Glibert, (2021) to examine the diet of the oyster, *Crassostrea virginica*, in the Chesapeake Bay, USA, also revealed that the oysters primarily consumed phytoplankton, with diatoms and dinoflagellates being the dominant groups, with detritus and bacteria consumed to a lesser extent. In a study by Arapov, Ezgeta-Balic, Peharda & Gladan (2010), to determine the diet of the oyster *Saccostrea cucullata* in a tropical estuary in Australia, showed that the oysters consumed a variety of food sources including phytoplankton, detritus and microzooplankton. The study also reported on seasonal variations in the diet of the oyster, with a higher proportion of phytoplankton observed in summer and a higher proportion of detritus in the winter. From the above studies, a wide range of foodstuffs, including phytoplankton, debris, bacteria and microzooplankton, is utilised as food by oysters. These food sources change depending on the seasons and prevailing environmental conditions. Continuous research is therefore needed to understand food source dynamics in the face of changing climate.

Stomach content analysis is a commonly used method to investigate the diet compositions of fishes, including oysters. According to Froelich, Gómez-Chiarri & Hedgecock (2018), there are different methods available for stomach content analysis, each with its own advantages and disadvantage. Microscopic analysis involves examining the stomach contents of oysters under a microscope to identify and quantify the different food items present. Such a method is relatively inexpensive and easy to carry out, which can provide detailed information on the oysters' diet. However, it can be time-consuming and

requires specialized equipment and expertise to accurately identify and quantify the different food items present (Froelich et al., 2018). According to Cordova, Simonsen & Gaston (2019), a DNA-based analysis involves using molecular techniques such as PCR to identify the DNA of different food items in the oyster stomach. This method can provide highly accurate and specific information about the diet of oysters and can also detect rare or hard-to-identify food items. Nonetheless, this method can be expensive and time-consuming, and requires specialized equipment and expertise to perform. The stable isotope analysis involves measuring the ratios of stable isotopes (e.g. carbon, nitrogen, and sulphur) in the oyster tissue and comparing them to the ratios in potential food sources. This method can provide information about the general composition of the oyster diet over time, as well as information about the source of the food items consumed. However, this method may not accurately reflect short-term changes in the diet, and interpretation of results can be complex (Rochette, Blanchet & Pouil, 2018). Another technique known as the biochemical analysis involves analysing the chemical composition of the oyster tissue and comparing it to potential food sources. This method can provide information about the general composition of the oyster diet and can also identify specific nutrients or compounds that may be important for oyster health. However, just like the stable isotope analysis, this method may not accurately reflect short-term changes in the diet, and interpretation of results can be difficult (Perez-Paralle, Guerra & Eirin-Lopez, 2017). The High-throughput sequencing technique as described by Liu, Wu & Xu (2018), involves sequencing the DNA of all the organisms present in the oyster stomach contents and using bioinformatics analysis to identify and quantify the different food items present. This method

can provide highly accurate and specific information about the diet of oysters, as well as information about the diversity and abundance of the different food items present. However, this method can be expensive and requires specialized equipment and expertise to perform (Liu et al., 2018). In summary, there are several different methods available for oyster stomach content and diet composition analysis, each with its upside and setback. Researchers should carefully consider the goals of their study, as well as the available resources and expertise, when selecting a method for stomach content analysis. This presents research opportunity to compare and validate the different methods available. In this study, microscopic analysis method, which is relatively inexpensive, easy to perform and can provide a detailed understanding of the diet and feeding behaviour of oysters, was employed to investigate the diet compositions and feeding preference of *C. tulipa* populations.

From the forgoing, oysters are filter feeders that depend on the availability and quality of phytoplankton for energy for biological functions. Understanding the diet composition of oysters and factors that influence their natural cycles of abundance is important for understanding their ecology and essential for oyster aquaculture planning. Various physical and environmental factors can influence phytoplankton growth and community structure: oyster feeding activities also have influence on phytoplankton populations. Different phytoplankton species have varying nutritional value, and excessive or inadequate phytoplankton availability can lead to negative impacts on oyster growth and health. Further, few species of phytoplankton are reported to produce toxins which can accumulate in the tissues of oysters and cause health risks to oyster consumers. Information on phytoplankton community

compositions therefore provide insight into the quality and safety of oyster populations for human consumption. In the case of *C. tulipa*, sustainable management and aquaculture development efforts will require good understanding of the natural cycles of ecosystem components, such as food supply, which are source energy for biological functions. Scientific studies on phytoplankton dynamics in relation to oyster production, as well as prediction and monitoring mechanisms to reduce the risk of harmful algal blooms, are important steps towards sustainable oyster production.

2.8. Socioeconomic Impact of Harmful/Toxic Microalgae on Bivalve Shellfish Production: Global Overview

Bivalve molluscs such as clams, mussels, oysters and scallops can rapidly accumulate algal toxins at levels that may be fatal to humans or other consumers when toxic phytoplankton are filtered from water as food (Silva et al., 2016; Swan & Davidson, 2011). The toxic syndromes associated with the toxic phytoplankton have been categorized as paralytic (PSP), diarrhetic (DSP), neurotoxic (NSP), amnesic (ASP), ciguatera (CFP) and azaspiracid (AZP) shellfish poisoning, and with the exception of ASP, all other biotoxins are synthesized by dinoflagellates (Anderson et al., 2001; Anderson et al., 2008; Anderson et al., 2021; Hinder et al., 2011; Silva et al., 2016). There may also be adverse effects on humans due to exposure to aerosolized algal toxins and dermatological contact. Hypoxic and anoxic conditions associated with harmful blooms, fish and wildlife mortalities, and other severe impacts as a result of the accumulation and decay of high biomass phytoplankton blooms can lead to ecosystem disruption (Anderson et al., 2021). The main species reported by HABs monitoring programs in parts of the world belong to the genera *Pseudo-*

nitzschia, *Alexandrium*, *Prorocentrum*, *Dinophysis*, *Gymnodinium*, *Ostreopsis*, *Karenia*, *Lingulodinium*, *Protoceratium*, *Gonyaulax*, *Pyrodinium*, *Protoperidinium* and *Azadinium* (Hinder et al., 2011; Silva et al., 2016; Swan & Davidson, 2011). The impact of HABs on global shellfisheries has been significant, with outbreaks resulting in mass mortality of shellfish and finfish. For example, in 2010, an outbreak of PSP caused by *Alexandrium* spp. in the Pacific Northwest of the United States resulted in the closure of oyster fisheries and an estimated loss of $9 million in revenue (Anderson et al., 2021). Similarly, in Europe, HABs caused by the dinoflagellate *Dinophysis* spp have resulted in the closure of oyster fisheries in several countries, including France, Spain, and Ireland (Karlson et al., 2021). In the Gulf of Mexico, *Karenia brevis* blooms have led to the closure of shellfish harvesting grounds and a loss of revenue for the oyster and clam industries (Hoagland, Anderson, Kaoru, Y., & White, 2002). The oyster industry in Apalachicola Bay, Florida, was particularly hard hit, with harvests reduced by up to 75% in some years (Florida Department of Agriculture and Consumer Services, 2015). The economic impact of these closures was estimated to be up to $22 million in lost revenue and 150 job losses in one year alone (Hoagland et al., 2002). In California, *Pseudo-nitzschia* blooms have led to the closure of the Dungeness crab fishery, resulting in a loss of $48 million in revenue and 330 jobs in one season (Heisler et al., 2007). The razor clam industry in Washington State has also been impacted, with closures due to *Pseudo-nitzschia* blooms leading to losses of up to $9 million in one season. In Chile, toxic HABs caused by *Alexandrium catenella* have led to the closure of the shellfish industry, resulting in a loss of $15 million in one season (Díaz & Figueroa, 2023). In the Philippines, for example, the closure of shellfish

harvesting grounds due to toxic HABs has led to significant economic losses for small-scale fishers (Adams, Larkin, Hoagland & Sancewich, 2018). Similarly, in Bangladesh, toxic HABs have had a negative impact on the livelihoods of shrimp and crab farmers, with losses estimated at $9 million in one year (Wongbusarakum, De Jesus-Ayson, Weimin & DeYoung, 2019). In South Africa, A*lexandrium* spp. blooms have led to the closure of the mussel and oyster fisheries, resulting in losses of up to $2.5 million in one year (Probyn, Pitcher, Pienaar & Nuzzi, 2001).

In response to the threat of toxic microalgae to oyster fisheries, a number of management strategies have been instituted to monitor and mitigate the impacts of HABs. These include regular monitoring of water quality and shellfish toxicity, the use of selective harvesting techniques to remove contaminated shellfish, and the development of early warning systems to detect the presence of HABs (Anderson et al., 2008). Additionally, research is ongoing to develop new methods for detecting and quantifying toxin levels in oysters and other shellfish, as well as to better understand the ecological and environmental factors that contribute to the occurrence of HABs (Karlson et al., 2021). In conclusion, toxic microalgae outbreaks have had severe economic impacts on oyster and shellfisheries worldwide. These impacts are expected to be aggravated in the future due to changing global climate and other environmental factors, making it essential to develop effective strategies to mitigate the effects of HABs on the bivalve shellfish industry. Unfortunately, data on HABs dynamics in the sub-Saharan Africa. Data on HABs dynamics Ghana is scanty. Only one study exists that reports on their occurrence and biotoxin contamination in bivalves along the coast of Ghana (Denutsui, 2019).

Further studies are necessary to mitigate the impacts of negative impact of HABs in Ghana. Studies such as this are essential to provide baseline information necessary for the monitoring of the proliferation of harmful/toxic phytoplankton species in oyster harvesting areas.

2.10. Ecophysiology of Oysters: Oyster Growth, Condition index and Meat yield

In the production of bivalve shellfish, growth is considered to be one of the most important factors. The reason for this is that consumers demand the right size in order to be able to achieve an optimal price, which is of particular interest to bivalve shellfish producers (Vakily 1989). Bivalve growth is generally estimated as an increase in shell length or weight or both (Rivonkar, Sreepada, & Parulekar, 1993). In estimating the growth rate bivalve shellfish, Quayle and Newkirk (1989) proposed four approaches, namely: measurements of shell dimension of randomly sampled animals, sequential measurements of tagged individuals, measurements of growth rings (upon validation) and acetate peels of cut shells. Many factors are thought to affect growth in bivalves (Gosling, 2003), and of these, food supplies are said to be of paramount importance, since it is not possible to keep growth going indefinitely if there is no such supply. (Gosling, 2003; Seed & Suchanek, 1992). In the case of oysters, growth has been linked to many environmental variables such as, food availability, salinity and temperature (Kraeuter, Ford & Cummings, 2007). These environmental modulators of growth are said to collectively interact, making it difficult to determine the exact effect of a single factor on growth in natural populations of bivalves (Gosling, 2003).

The condition index (CI) is a measure of the nutritional status of oysters and is commonly used as an indicator of the health of oysters and of the plumpness of meat (Yankson, 2004). This simple index provides an indication of the long run changes in bivalve nutrient status, storage energy and their metabolic response to environmental stress (Ismail & Kong, 1999). Barber and Blake (1991) and Gosling (2003) suggest that, CI can be used as an indirect approach for the assessment of the reproductive stage of bivalves. Several studies have reported on oyster ecophysiology in relation to the condition index (Yildiz, Berber, Acarli, Vural & 2011). Several studies have reported on the impact of different environmental stressors on oyster condition. One such studies has shown that changes in salinity can impact oyster growth and reproduction, which in turn can impact the condition index (Sühnel et al., 2023). Marco et al. (2023), on the other hand, found that higher water temperatures led to a decrease in oyster condition index due to increased metabolic rates and decreased food availability. Other studies have investigated the role of diet in oyster condition. One of such study has shown that the nutritional quality and quantity of food can have a significant impact on oyster condition. Specifically, oysters fed on diet rich in microalgae were reported to have a higher condition index compared to those fed a diet of macroalgae (Omont et al., 2021). The relationship between oyster disease and condition index has also been investigated. For instance, Paynter and Burreson (1991) found that oysters infected with *Perkinsus marinus*, a pathogenic protozoan, had a lower condition index than healthy oysters due to the parasite's impact on the oyster's digestive system. Furthermore, the impact of microbial contaminants and heavy metals on oyster condition index are well documented (Hood, Ness, Rodrick & Blake,

1984; Rebelo, Amaral & Pfeiffer, 2005). In conclusion, growth in bivalve shellfish, oysters included, has been linked to many environmental variables which interact together making it difficult to determine single factor effect on growth. Studies have indicated variety of environmental and anthropogenic factors can impact oyster condition index, including changes in water temperature and salinity, diet quality and quantity, disease, microbial contaminants and heavy metals. Nonetheless, further studies in these areas are needed, particularly as regards to *C. tulipa*, to better understand the complex interrelationships between growth, oyster condition index and environmental factors to aid in the development of effective management strategies.

2.11. Microalgae Isolation and Culture Characteristics: *Rhodomonas*, *Nannochloropsis* and *Pseudanabaena* species

In order to maintain an ongoing supply of very good quality feed for oysters larvae, it is necessary to isolate and cultivate microalgae. A number of studies have investigated the use of different microalgae species for oyster larval rearing, with a focus on optimizing growth, survival, and health of the larvae (Doroudi & Southgate, 2000; Romberger & Epifanio, 1981). Advancement in techniques for large scale microalgae culture has also facilitated a great deal of research interest of food value of microalgae to bivalves (Epifanio, 1979). This is because hatchery production of bivalve seed relies heavily on microalgae cultures to provide the nutritional elements that meet the metabolic needs of the reared larvae (Mamat & Alfaro, 2014; Hemaiswarya et al., 2011). According to Ponis et al. (2006), about 50 species of microalgae have been screened over the years as food for bivalves, with less than 10 species, belonging to the Bacillariophyceae (i.e. diatoms), the Haptophyta and the Prasinophyceae are

often cultured in shellfish hatcheries. The lack of limited number of microalgae available to molluscs rearing poses a major challenge for the development of shellfish aquaculture, in particular those areas where technological and infrastructure improvements are lacking that would enable them to be grown. However, ongoing research has been carried out in many experimental labs across the globe on isolation and culture of microalgal species with high nutritional value over recent years (Brown, Mc Causland & Kowalski, 1998; Ponis et al., 2006; Southgate, Beer, Duncan & Tamburri, 1998). Majority of bivalve aquaculture concentrated on temperate species, therefore majority of the selected microalgae species for used in bivalve hatchery were of temperate origin (Leonardos & Lucas, 2000; Utting & Millican, 1997). Due to growing aquaculture in the tropics, there has also been a rise in demand for isolation and culture of high value Tropical microalgae which are capable of tolerating climatic conditions (Martínez-Fernández, 2006; Renaud & Parry, 1994). According to Larkum, Ross, Kruse and Hankamer, (2012), Isolation and culturing of local microalgal strains is thought to have a competitive advantage as they are well suited to the local climatic conditions. According to Ponis et al. (2006), microalgae isolates to be used in bivalve shellfish hatcheries, must fulfil four main criteria: “they must be of an appropriate size to be efficiently ingested, they should not have a cell covering that inhibits digestion, they should have a high nutritional value and, lastly, they should be amenable to production in the different systems and scales operating in hatcheries”. Aspects of this study, therefore, assessed the potential of novel local microalgal isolates, specifically *Rhodomonas*, *Nannochloropsis* and *Pseudanabaena* spp, for use in the hatchery rearing of *C. tulipa*, taking into account the criteria outlined above

2.8.1. *Rhodomonas*

Rhodomonas is a genus of unicellular, freshwater and marine cryptophyte microalgae that is widely used as a feed source for zooplankton in aquaculture and as a model organism in biological research. It belongs to the class Cryptophyceae and family Pyrenomonadaceae. The successful isolation and culture of *Rhodomonas* species in the laboratory are essential for its commercial applications and scientific investigations. The species can be isolated from freshwater and marine environments using various techniques (Derbel et al., 2022). Once isolated, *Rhodomonas* species can be cultured in various media, including F/2 medium, BG-11 medium, and modified Bristol medium (Derbel et al., 2022; Lafarga-De la Cruz et al., 2006). Culture conditions, such as temperature, light intensity, and nutrient concentration, are reported to affect the growth of *Rhodomonas* in the laboratory, with the species said to exhibit a higher growth rate under high nutrient concentrations and moderate light intensities (Chaloub, Motta, de Araujo, de Aguiar & da Silva, 2015; Oostlander, van Houcke, Wijffels & Barbosa, 2020). Literature suggests that *Rhodomonas* species have a range of growth characteristics, with growth rates ranging from 0.32 to 0.53 dayand maximum cell densities ranging from 1.15 to 1.84 × 10^6 cells mL^{-1}. The equivalent spherical diameter of Rhodomonas species is reported to be 2-10 µm. Dry carbon weight and carbon energy content vary depending on the strain and growing conditions, but reported values for other microalgae suggest that *Rhodomonas* species could have a dry weight of up to 0.4 g L^{-1} and a carbon energy content of up to 21.8 kJ g^{-1} dry weight (Moheimani & Borowitzka, 2007; Guedes, Amaro, & Malcata 2011).

2.8.2. *Nannochloropsis*

Nannochloropsis is a genus of marine microalgae that has gained interest for its high lipid content and potential as a source of biofuels and other bioproducts. It belongs to the class Eustigmatophyceae and family Monodopsidaceae. Isolation of *Nannochloropsis* strains from natural samples can be challenging due to their small size and low abundance in the environment. Several methods have been reported for isolation of *Nannochloropsis* strains from marine environments, including filtration, centrifugation, and flow cytometry (Borowitzka & Moheimani, 2013; Liu, Mao, Zhou, Guarnieri, & Chen, 2020). Filtration has been reported as a simple and efficient method for isolating *Nannochloropsis* strains from seawater, with pore sizes ranging from 0.45 to 8 µm used to capture cells (Borowitzka & Moheimani, 2013). Centrifugation can also be used to concentrate and isolate *Nannochloropsis* cells from seawater samples, with various protocols reported in the literature (Liu et al., 2020). Once *Nannochloropsis* strains have been isolated, they can be cultured in the laboratory for further study and experimentation. *Nannochloropsis* is a photoautotrophic microalga, and light, carbon dioxide, and nutrients are vital for its growth. Culture conditions such as light intensity, temperature, pH, and nutrient concentrations can all affect *Nannochloropsis* growth and productivity (Wahidin, Idris & Shaleh, 2013). Also, they grow best under high light intensity (100-200 µmol photons/m^2/s), moderate temperatures (20-25°C), and slightly acidic to neutral pH (6.5-7.5). Furthermore, *Nannochloropsis* species have a short doubling time (ranging from 6 to 48 hours) and can achieve high cell densities (ranging from 0.1 to 2.0 g/L) under optimal culture conditions, with an ESD ranging from 2 to 5 µm.

Their biomass has a dry carbon weight ranging from 0.1 to 2.0 g/L, and a carbon energy content ranging from 16 to 28 MJ/kg (Borowitzka and Moheimani, 2013; Liu et al., 2020).

2.8.3. *Pseudanabaena*

Pseudanabaena is a genus of filamentous cyanobacteria commonly found in freshwater ecosystems (Ramos, Morón-López, Flores-Muñoz & Paniagua-Michel, 2020). However, some studies have reported the occurrence in marine environments (Gao, Li, Li, Li & Li, 2015; Rastogi, Bhattacharya, & Sen, 2019). It belongs to class Pseudanabaenales and family Pseudanabaenaceae. Gao et al., (2015) reported the first occurrence of *Pseudanabaena* sp. in the marine environment of the South China Sea using both morphological and molecular methods. They identified the strain as *Pseudanabaena limnetica* based on its morphological features, such as filament length and width, cell shape, and size. Additionally, they confirmed the identity of the strain using 16S rRNA gene sequencing. Similarly, Rastogi et al. (2019) reported the occurrence of *Pseudanabaena* sp. in the marine environment of the Arabian Sea using metagenomic sequencing. They identified the strain as *Pseudanabaena* sp. FII5 based on its 16S rRNA gene sequence and suggested that its presence in the Arabian Sea might be due to eutrophication. Isolation and culture of *Pseudanabaena* species from marine environments have been described in several studies. For instance, in a study by Lembke, Hense and Kremp (2018), indicated a *Pseudanabaena* sp. was isolated from seawater samples collected from the South Atlantic Ocean. The authors used a modified liquid BG-11 medium for the isolation and culture of the strain. Similarly, Mahadevi and Madhavan (2020) isolated *Pseudanabaena* sp. from seawater

samples collected from the Bay of Bengal using BG-11 medium. In another study, isolated *Pseudanabaena* sp. from coral mucus samples collected from the Great Barrier Reef in Australia (Gugger, Lyra & Sivonen, 2002). Other studies have reported the carbon weight of *Pseudanabaena* species isolated from marine environments. For instance, Kang et al. (2019) reported a carbon weight of 36.7 ± 0.6% for *Pseudanabaena* sp (JC001) isolated from the Yellow Sea. Similarly, Guedes, Passavante & Rocha, (2016) reported a carbon weight of 36.6 ± 1.1% for *Pseudanabaena biceps* isolated from a coastal lagoon in Brazil. Hameed, Shahina, Linh & Young, (2017) reported an ESD of 3.3 ± 0.2 µm for *Pseudanabaena catenata* isolated from the Arabian Gulf. Similarly, Kang et al. (2019) reported an ESD of 3.16 ± 0.04 µm for *Pseudanabaena* sp. JC001 isolated from the Yellow Sea. With respect to biovolume, Hameed et al. (2017) reported a biovolume of 80.7 μm^3 for *Pseudanabaena catenata* isolated from the Arabian Gulf. Similarly, Kang et al. (2019) reported a biovolume of 47.1 µm3 for *Pseudanabaena* sp. Carbon energy content, of *Pseudanabaena* sp. JC001 isolated from the Yellow Sea was reported to be 17.4 ± 0.4 kJ/g (Kang et al., 2019). Similarly, Guedes et al. (2016) reported a carbon energy content of 20.2 ± 0.7 kJ/g for *Pseudanabaena biceps* isolated from a coastal lagoon in Brazil.

2.12. Oyster Larval Rearing on Microalgae Isolates

Oyster larval rearing is a critical aspect of oyster aquaculture, and it involves the use of microalgae as a primary food source for the larvae. A number of trials aimed at optimising growth, survival and health in the larvae have been carried out on the use of various microalgae species for rearing larval oysters from different oyster species. One of such studies by Li, Li, Zhang & Li (2018),

investigated the use of three different microalgal diets, *Isochrysis galbana*, *Pavlova viridis*, and *Nannochloropsis oculata* for the larval rearing of Pacific oysters. In this study it has been found that *I. galbana* and *P. Viridis* are the best suited nutritional sources for optimum growth and survival of larvae. Kang, Kang & Lee (2014), reported on the effects of different microalgal diets, *Isochrysis galbana*, *Tetraselmis suecica*, *Chaetoceros calcitrans* and *Pavlova lutheri* on the survival and growth of Pacific oyster larvae. The study found that *T. suecica* and *C. calcitrans* were the most suitable diets for optimal growth and survival of the larvae. *Tetraselmis chuii* and *Isochrysis galbana* were reported to be the most suitable diets for optimal growth and survival of the mangrove oyster *Crassostrea rhizophorae* larvae (Moreira, Ribeiro & Martinez-Porchas, 2019). Guan, Li, Li, Zhang & Li (2019), also reported on the effects of some microalgae on larval growth, survival and biochemical composition of Pacific oysters, *Crassostrea gigas*. The study found that *I. galbana* and *P. viridis* were the most suitable diets for optimal growth and survival, while *C. muelleri* resulted in higher lipid content in the larvae. Another study investigated the effects of different microalgal (*Isochrysis galbana*, *Chaetoceros calcitrans*, and *Nannochloropsis oculate*) diets on survival, growth and fatty acid composition of Pacific oyster (*Crassostrea gigas*) larvae, and found that *C. calcitrans* was the most suitable diet for Pacific oyster larvae (Yuan, Dong, Shi, Wu, Wu, & Gao, 2016). In conclusion, this review has demonstrated the importance of microalgae as a food source for the rearing of oyster larvae, and highlight the need to identify optimal microalgae species for oyster larval rearing through research. Studies on the potential of local microalgae strains as food resources for the hatchery rearing of *C. tulipa* larvae are therefore critical towards the

optimisation of hatchery seed production of the species to support large-scale oyster aquaculture along the coast of West Africa.

2.13. Chapter Summary

This chapter has covered an extensive review of literature covering various themes pertinent to this study, which has provided theoretical justification for this study.

CHAPTER THREE

MATERIALS AND METHODS

This chapter presents a description of the study sites from where water and oyster samples were obtained for the study. Description of the sampling techniques, data collection procedures and statistical tools and analysis employed are also presented.

3.1. Study Area

The study was carried out in two coastal lagoons in Ghana namely, Benya and Narkwa lagoons, where there are established wild oyster populations. Benya's Lagoon (Figure 2A) is an artificially open lagoon, which maintains continuous contact with the Gulf of Guinea year round. The lagoon is located at the western end of Elmina, along the southern coast of the Central Region of Ghana. The lagoon lies within the perimeter 1°20′50″ W, 5°04′59″ N and 1°21′26″ W, 5°05′18″ N, and presently occupies an area of approximately 0.13 km2 along a length of about 2 km, with an average and maximum depth of 0.7m and 1.5 m respectively (Chuku, 2019). The lagoon serves as the landing and canoe/vessel docking site, and also as fishing (subsistence) ground for the Elmina community. The lagoon serves as a receptor of fish processing effluent and all kinds of household effluents. Refuse disposal/open defecation site and pig styles are also located along the banks of the lagoon. The lagoon does not have any obvious source of freshwater. Established wild *C. tulipa* population inhabits the roots of the red mangrove fringing the lagoon. Finfish, such as black chin tilapia (*Sarotherodon melanotheron*) and Mullets; shellfish, bloody cockle *(Anadara senilis*) are other important food fish exploited from the lagoon.

Narkwa lagoon (Figure 2B), is described as a complex and intermittent lagoon estuary (Chuku, Yankson, Obodai, Acheampong & Aheto, 2023). It is located within the perimeter 0°56′22″ W, 5°12′17″ N and 0°54′41″ W, 5°12′32″ N, and is about 50 km east of Cape Coast, Ghana. It is an open lagoon; however, the mouth seals off due to persistent sand accretion once in a while to form part of a continuous sand bar that separate the lagoon from the ocean (Chuku et al., 2023). The month of the lagoon, which can shift positions, is forced opened by water from the riverine source during the rains or mechanically opened by the community to avert resulting flooding during rains. The lagoon serves as fish landing and canoe docking ground for the Narkwa community (Ansa-Asare et al., 2008; Asare et al., 2019). The lagoon covers an area of about 0.6 km^2, with an average and maximum depths of 0.5 and 1.4 m respectively (Chuku, 2019). It is connected by the tributaries of Okye and Narkwa rivers, and has an established wild oyster population on the bottom sediment, which supports a vibrant small-scale oyster fishery (Asare et al., 2019; Chuku et al., 2022). Over the years, Narkwa lagoon has attracted the attention of oyster researchers due to its potential for commercial oyster culture (Asare 2019, Chuku, 2019; Obodai 1997). Just like the Benya Lagoon, finfish such as black chin tilapia (*Sarotherodon melanotheron*) and Mullet, and shellfish, bloody cockle (*Anadara senilis*) are also harvested from the lagoon. The lagoon also serves as a foraging ground for migratory birds which were observed at a certain period of this study

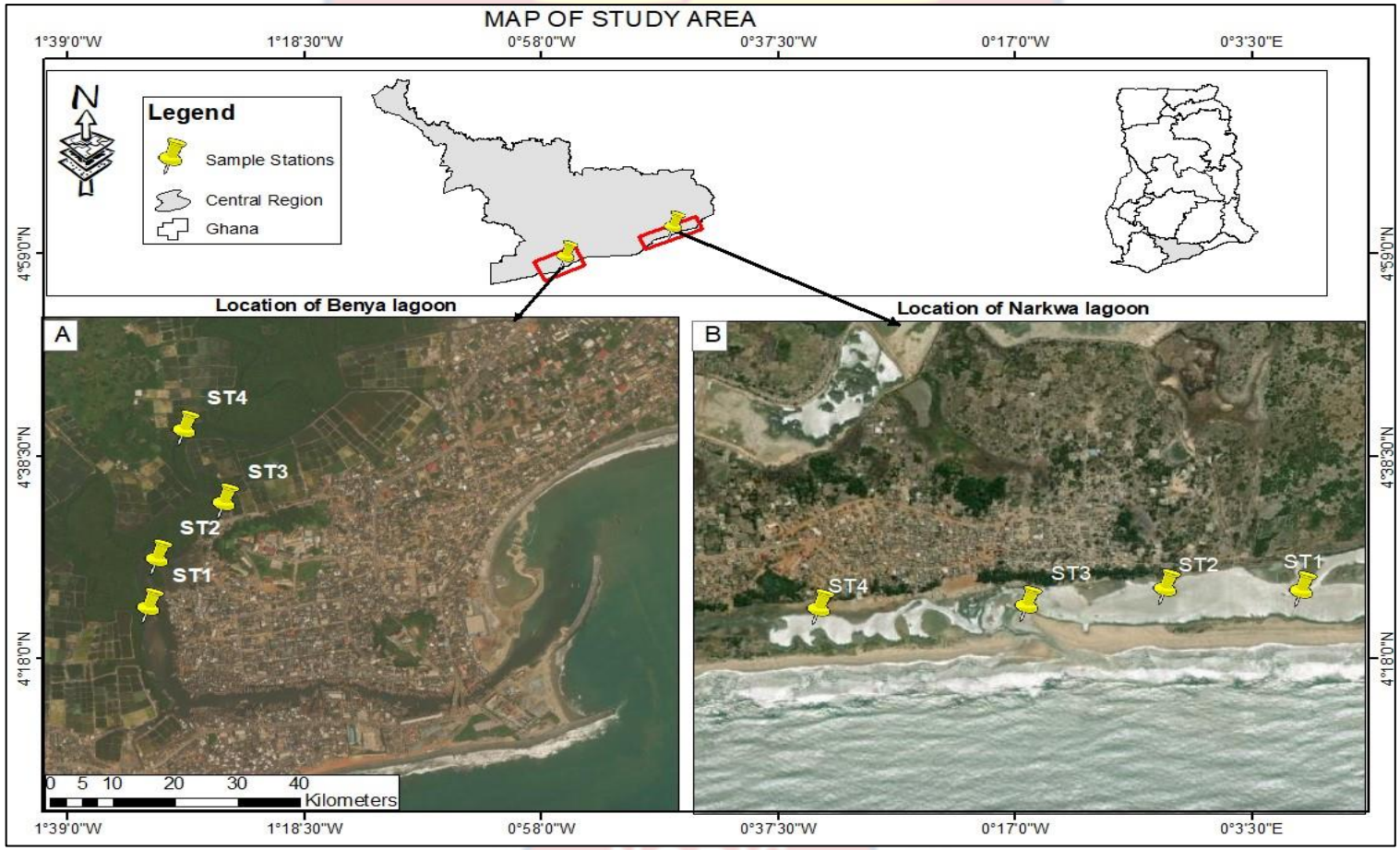

Figure 2: Map of Central Region showing (A) Benya and (B) Narkwa lagoons with the sampling stations

3.2. Study Design

Two study sites (Benya and Narkwa lagoons) were selected in this study. Each study site was divided into four sampling stations based on varying conditions that exist in different portions of the study environment. Sampling were triplicated at each of the four sampling stations. The selection of sampling stations were carried out by the use of systematic random sampling, where an initial sampling point had been selected at random and remaining sampling points set according to a regular pattern. This was done to ensure representative sampling. Water and oyster sampling were carried out on monthly bases for a period of 12 months. Sampling was carried out in the morning between 7 – 11 GMT.

To ensure that the feed treatment groups are comparable and that any differences in the outcomes were attributable to the treatment and not to confounding variables or experimental biases, a complete randomized design was employed for the larval rearing and feeding experiment.

3.3. Sampling, Data Collection and Analyses

3.3.1. Physicochemical parameters

Temperature, dissolved oxygen (DO), salinity, pH, turbidity, nutrients (nitrate and phosphate), chlorophyll-*a*, plankton abundance and compositions were monitored monthly at low tide from August, 2020 to August, 2021. Temperature (°C), Salinity (ppt), DO (mg/L) and pH were measured *in-situ* with a multi-parametric water quality checker (Eutech PCD 650) by immersing the probe into the water to a depth of about 30 cm from the surface. Turbidity (NTU) on the other hand, was measured with a turbidimeter (Oakton T-100).

Water samples for nutrients, chlorophyll a concentration, phytoplankton cell density and composition estimations were collected using a 2 Litre Van Dorn's water sampler, divided into two aliquot and stored in a labelled 500 ml polyethylene sample bottles. Water samples for nutrients and chlorophyll *a* analyses were immediately fixed on ice in cool box to help preserve the integrity of the water samples by slowing down biological and chemical reactions. On the other hand, water samples collected for phytoplankton cell density and composition estimations were immediately fixed with a modified Lugol's solution (Vollenweider, 1974) (3-5 ml of the Lugol's solution to 500 ml water sample) and transported in a dark box to the laboratory to help maintain the chemical integrity of the fixative and the water sample, ensuring that the preserved samples are suitable for later analysis. avoid iodine reaction with light. Nitrate and phosphate concentrations (mg/L) in water samples were measured by colorimetric procedures in the laboratory, using a multi-parametric hand-held colorimeter (Hach DR 900) and Reagent Powder Pillows (NITRAVER 5 & PHOSVER 3, respectively). Following the USEPA Powder Pillow procedure for determination of nitrate concentration (APHA, 1992), a sample vial was filled up to the 10 ml mark with a replicate water sample collected from the sites, and one reagent powder pillow (NitraVer®5) was emptied into it and shaken vigorously for one minute, and then placed down for a five-minute reaction time. The formation of an amber colour indicates the presence of nitrates, and after zeroing the instrument with a blank sample, the nitrate (NO_3-) concentration (in mg/L) was determined at a wavelength of 355 nm (355 N, Nitrate HR PP). The procedure was similar for determination of phosphates (PO_4^{3}-) using reagent powder pillow (PhosVer®3). The sample was

shaken vigorously for 30 seconds after adding the reagent and allowed a reaction time of two minutes. The occurrence of a blue coloration indicates the presence of phosphate, and the concentration (mg/l) was determined at a wavelength of 490 nm (490 P React. PP).

The chlorophyll-*a* concentrations in water samples were measured following the spectrophotometric method of Aminot and Rey (2001). Known volumes of water samples were gently filtered using a glass-fiber filter (GF/F) (Whatman type, 25 mm or 47 mm in diameter) at a residual pressure of 0.7 bar (maximum vacuum of 0.3 bar). Pigment extractions were carried out by grinding the filters in few milliliters of 90 % acetone for 1 minute, under subdued light. Filters were immediately frozen (at least -20 °C) in case extraction was not performed at the time of filtration. After grinding, the extracts were carefully transferred to a stoppered graduated centrifuge tube. The glass homogeniser and the pestle are rinsed properly with 90 % acetone and the rinsing volumes added to the centrifuge tube (making a total volume of about 5 ml). The extracts were subjected to thorough mixing andcentrifugation for ten minutes at 4000 rpm immediately before the measurement. Centrifuged sample extracts were carefully transferred by pipetting from the centrifuge tubes to cuvettes. Using a spectrophotometer (Jenway 7315), the absorbances of the sample extracts were measured at 750, 664, 647, and 630 nm against a 90 % acetone blank. Chlorophyll a concentration was calculated according to the equations of Jeffrey and Humphrey (1975):

$$\text{Chlorophyll a (mg/L)} = [(11.85\times (E664 - E750) - 1.54\times (E647 - E750) - 0.08\,(E630 - E750)] \times Ve/L \times Vf \quad (1)$$

Where:

L = Cuvette light-path in centimetre

Ve = Extraction volume in millilitre

Vf = Filtered volume in litre

Data for monthly rainfall or precipitation (mm) in the vicinity of the two study sites was extracted from the online global climate database (Tutiempo.net, 2019) for the nearest meteorological data station, which was Saltpond.

3.3.2. Plankton Enumeration and Identification

To determine plankton abundance compositions and distribution in the two study sites, plankton enumeration and identification were carried out following the UtemÖhl method (1958). Water samples were sedimented in 50 mL UtemÖhl counting chamber for 24 hours following which species identification was done using an Olympus CK-2 inverted microscope at ×100 to ×400 magnifications. Identification of plankton were carried out with the help using the following plankton guides; Cupp (1943), Tomas (1997), Van den Hoek et al. (1995) and other online repositories such as; https://planktonnet.awi.de/index.php#content and https://diatoms.org/species Cell density was calculated following an equation by Intergovernmental Oceanic Commission of UNESCO (2010);

$$\text{Cells mL}^{-1} = N \times (At/Ac) \times (1/V) \quad \ldots\ldots (2)$$

Where V: volume of counting chamber (mL)

At: total area of the counting chamber (mm^2)

Ac: counted area of the counting chamber (mm^2)

N: number of units (cells) of specific species counted

C: concentration (density of the specific species

3.3.3 Stomach content analysis and diet selectivity of *C. tulipa* population in Benya and Narkwa lagoons

Fresh oyster samples (three samples per sampling station) were randomly handpicked early in the morning at low tide from Benya and Narkwa lagoons, quickly opened, and a Pasteur pipette was used to suck the stomach content after carefully punching through the stomach wall. Sucked stomach content was stored in 5% formalin in 5ml Eppendorf tube and brought to the laboratory for examination. Prior to examination, the replicated samples were pooled together and diluted with a known volume of water, and then stained with Lugol's solution. Plankton enumeration and identification were carried out following the Utemohl methodology outlined in the preceding section. Identification was done to the genus level under Olympus CK-2 inverted microscope at ×100 to ×400 magnifications with the help of identification resources listed in the preceding section. Overall and monthly diet compositions from the stomach content analysis were computed and the plankton diet selectivity was calculated with a modified Ivlev's (1961) electivity index (Ei) (Rosa et al., 2013; Weissberger and Glibert, 2021). The values have been calculated to relate the composition of plankton in the gut with that found in the water column as:

$$Ei = (r-p)/((r+p)-2(r \times p)) \quad \ldots\ldots (3)$$

Where, r is the proportion of a particular plankton in the diet and p is the proportion of that taxa in the water column.

3.3.4 Ecophysiology of *C. tulipa* population in Benya and Narkwa lagoons

Oysters were hand-picked at low tide from the two study sites monthly from August 2020- August, 2021. Between 30-50 matured oysters (between 3 and 9 cm) were sampled and placed on ice and brought to the laboratory. Shells of oysters were washed and cleaned of fouling organisms and debris in the laboratory. They were then blotted dry with an absorbent paper before shell dimensions and weights were determined. The whole weight (harvested weight) of the individual oysters were determined with an electronic balance prior to determination of shell dimensions. Shell length, height and width were measured with a pair of Vernier callipers to the nearest 0.1 cm. Soft tissues were carefully removed from the shell, blotted for excess moisture removal and weighing at the nearest value of 0.01 g with an electronic balance. The shells were also blotted dry and weighed to the nearest 0.1 g. Oyster shell height data were sorted into classes of 1.0 cm intervals and plotted into size-frequency distribution histograms. Shell morphology (shape indices) of oyster populations from the two water bodies were determined following Caill-Milly et al. (2014), as elongation index (H/L), compactness index (W/L) and convexity index (W/H).

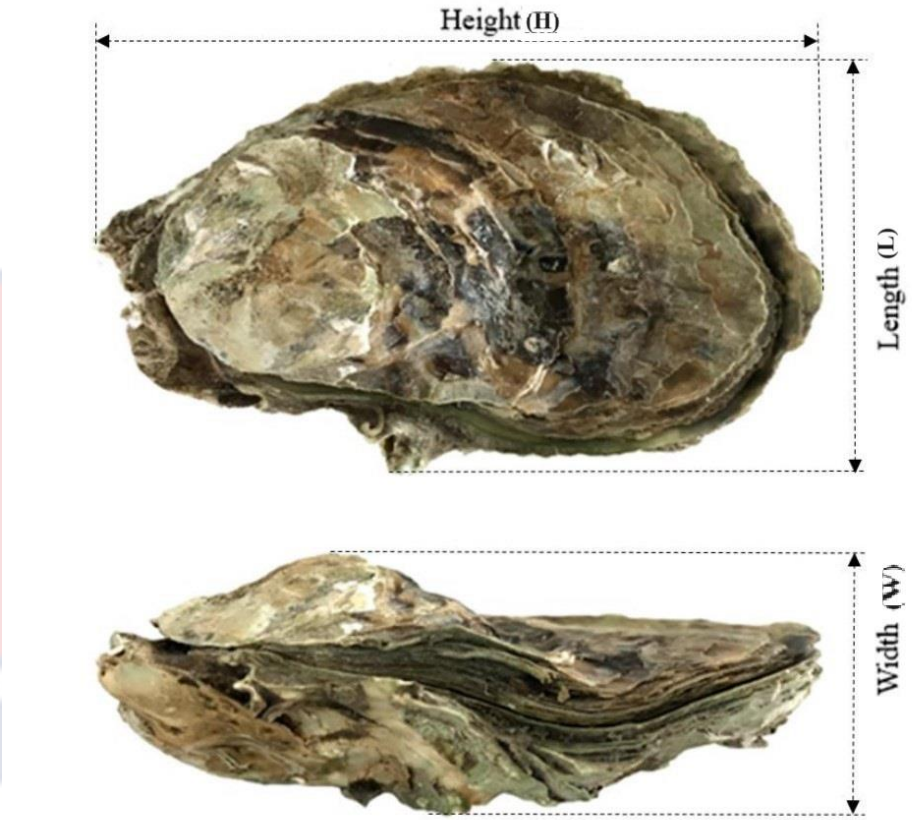

Figure 3: *C. tulipa* shell dimensions (adapted from Osei, 2019)

The meat yield of oysters was estimated from the equation (Yildiz, Berber, Acarli & Vural, 2011):

MY (%): [soft tissue weight (g)/Live weight (g)] X 100…….....(4)

For the purpose of determining condition Index CI, oyster tissues were oven dried at 75°C for 48 hours in order to obtain a constant dry weight (Walne, 1976; Crosby & Gale, 1990). Condition index (CI) of oysters were then determined by the following equation (Crosby & Gale, 1990):

CI= [dry tissue weight (g) / Internal shell cavity(g)] × 1000…… (5)

The inner shell volume was calculated as the difference between whole live

weight and empty shell weight of the oysters (Crosby and Gale, 1990; Obodai, 1997; Asare et al., 2019).

3.3.5 Microalgae isolation and culture

Microalgal isolation was carried out at the Wet laboratory of the Department of Fisheries and Aquatic Sciences (DFAS), University of Cape Coast. Water samples for microalgal isolations were collected from 1 km off the coast of Elmina (5° 04’07.6 "N 1°19'49.9"W). This because water from the lagoons studied were turbid and could hamper the isolation process. Prior to isolation, water samples were filtered first through 200 µm and then 30 µm sieve to eliminate zooplankton and other suspended particles. The water samples were then filtered through a Whatman GF/F filter (1.5µm) and the filtrate quickly rinsed into filtered and UV treated seawater enriched with f2 media, and Isolation of the microalgae was carried out via serial dilution of the enriched samples. Microscopic examination was carried out to check if isolates were made up of single species. Repeated serial dilution was carried out if the culture contained multi species. Culture of the local microalgae strains were carried out following the inoculation of unialgal isolates into 2-L media in transparent polyethylene bags under continuous dim light (4×20 watts of white fluorescent tube light) at 24 °C.

3.3.6 Microalgae growth performance and characterisation

Growth performance of the local microalgae strains were assessed through the daily estimation of cell density until the stationary phase was observed. Characterisation of the microalgae strains were carried out by estimation of their biovolume, carbon and energy content. Linear dimensions of cultured microalgae species were estimated with aid of a calibrated ocular scale

micrometer under inverted microscope at x 40 magnification with the. From the measured dimensions, biovolume of the microalgae were estimated according to the different geometric shapes and formulae proposed in Hillebrand, Dürselen, Kirschtel, Pollingher and Zohary (1999). Carbon content ($pgCCell^{-1}$) of the microalgae were estimated using the regression equation (Menden-Deuer & Lessard, 2000):

$$PgCCell^{-1}=0.216\times BV^{0.939} \quad (6)$$

where, BV is the biovolume of the microalgae

Carbon content was converted into energy content using the following conversion (Salonen, Sarvala, Hakala, & Viljanen, 1976):

$$1gC=46\times10^{3}J \quad (7)$$

Figure 4: Cultures of the microalgae isolates in the laboratory

3.3.7 Larval rearing of *C. tulipa* in the laboratory

Adult oysters (3-7 cm) used for the *in-vitro* fertilization were obtained from the Benya and Narkwa lagoons. Oysters were brought to Wet laboratory, cleaned of bio-fouls, and held in filtered and UV treated sea water for depuration. Gametes for the *in-vitro* fertilization were obtained by stripping method (Yankson, 1990; Obodai et al., 2007). Male gametes (spermatozoa) were flushed through 20 µm mesh to remove non-spermatid cells. Female gametes (eggs) were flushed through 100 µm mesh and collected on 30 µm mesh and the number of eggs estimated. The egg density was then adjusted to 200 eggs/200 mL treated seawater prior to fertilization. Fertilization was carried out at the ratio of 1 egg/mL to 0.001 sperm suspension (1 mL spermatozoa in 200 mL of treated seawater) (Yankson, 1990; Obodai 1997). The egg-sperm mixture was carefully mixed with a plastic plunger to ensure uniform fertilization and aerated. Fertilized eggs were decanted three (3) hours after fertilization and fresh filtered and UV treated seawater was added. Developmental stages of the fertilized eggs were monitored and D-stage larvae yield was estimated 24 hours post-fertilization.

Three (3) of the locally isolated microalgal strains (*Rhodomonas* sp., *Nannochloropsis* sp. and *Pseudanabaena* sp.) were used as feed for the oyster larval rearing. Five (5) experimental treatments, each consisting of three replicated cultures were randomly set up (as illustrated in figure 5). Treatments A, B and C were unicelled diet of the microalgae isolates: *Rhodomonas* sp., *Nannochloropsis* sp. and *Pseudanabaena* sp respectively. Treatment D was mixed-cell diet of the microalgae in equivalent biovolume proportions, whiles Treatment E was a control which contained untreated seawater filtered through

20 µm mesh to exclude larger suspended particles. Salinity was maintained at 30 ppt by diluting seawater (37 ppt) with distilled water. Rearing temperature was maintained at 28 °C in a water bath equipped with a with a thermostatic heating device (EHEIM Thermo control 200). The rearing salinity and temperature were based on the ambient conditions at the time of broodstock collections. Each culture consisted of 2,500 D-veliger larvae (averaged 65 µm) in 500 ml (5 larvae mL^{-1}) of filtered and UV treated seawater (Yankson, 1990; Rico-Villa, Bernard, Robert, Pouvreau, 2010). The rearing setups were gently aerated by bumbling air through the culture chamber to ensure mixing. The larvae were fed at 1200 $µm^{-3}$ mL^{-1} of microalgae daily, which was increased to 1400 $µm^{-3}$ mL^{-1} after the first week (based on the Dynamic Energy Budget growth model for *Crassotrea gigas* larvae (Rico-Villa et al., 2010). The rearing water was changed every other day during which larval sampling was done for growth (shell length) measurement and survival determination. Shell length of 25-30 larvae under each diet treatment were measured under Olympus CK-2 inverted microscope fitted with ocular micrometer at ×10 to ×40 magnification (as illustrated in figure 6). Oyster larvae were reared for 21 days, from the D-stage larvae to the pediveliger (competent) larvae stage. Specific growth rate (SGR) (%/day) was estimated using the formula:

$$SGR\ (\%/day) = (InL2 - InL1/\Delta t) \times 100 \quad (8)$$

Where; L2 is final shell length,

L1 is initial shell length

Δt is the number of days between culture period.

The survival percentage has been calculated with the formula given below:

Survival = (Number of survivors/Number stocked) ×100.....(9)

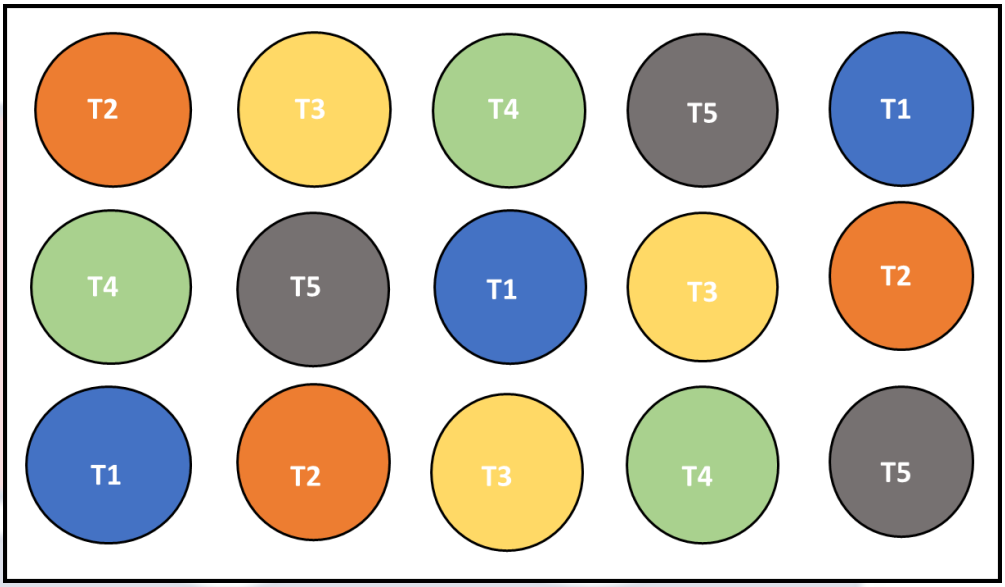

Figure 5: Experimental setup for the rearing of *C. tulipa* larvae on different microalgae diet treatments

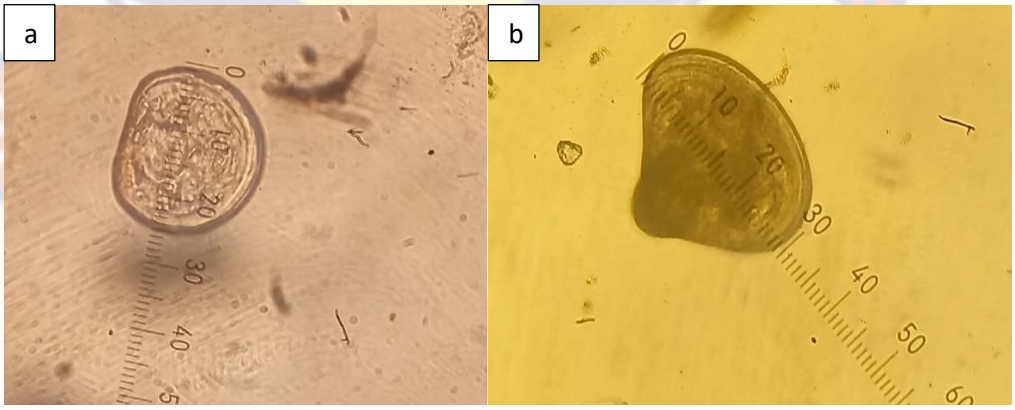

Figure 6: Measurement of shell length of different stages of *C. tulipa* larvae under microscope (a) D-stage veliger stage (×40 magnification) and (b) Pediveliger stage (×10 magnification)

3.4. Data Analyses

Minitab (version 20.3) and Microsoft Excel 2019 were employed for the analyses, and an alpha level of 0.05 was used for all inferential statistics. Spaciotemporal distribution patterns of the physicochemical parameters were constructed using the estimated means with its associated standard errors of the pooled triplicate samples from the sampling stations within the water bodies. Spatial (Water body) and temporal (months) distributions of the measured physicochemical parameters were constructed using means with its associated standard errors of the triplicates. Two-way ANOVA was used to deduce the spatio-temporal differences among the measure physicochemical parameters. A 2-sample t-test, assuming unequal variance was used to compare the annual mean estimates of the two water bodies.

Temporal distributions of the plankton cell density were constructed using means with their associated standard errors of the triplicate counts. A 2-sample t-test, assuming unequal variance was used to compare significance of estimates of the two water bodies. The data of plankton were cleaned up and all rare species which might have caused noise to be found in analysis were eliminated (Lamptey, 2015). In the 12 sampling months, species were considered rare if they were encountered only once (with < 5 individuals). For the two lagoons, the percentage composition of the taxonomic and functional groups of plankton was calculated. Temporal distributions of the abundance (percentage compositions) of the plankton groups in the two water bodies were constructed using bar graphs.

To examine the relationships between hydrodynamic parameters and plankton group composition, Pearson correlation coefficients was applied.

Multiple linear regression model was used to determine the hydrographic predictors of the dominant plankton group compositions. Multiple linear regression model was used to determine the hydrographic (biotic and abiotic) predictors of meat yield and condition index in oysters. One-way ANOVA was used to assess the oyster larval rearing performance under the different diet treatments. Identification of specific differences between diet treatments was carried out using Tukey's pairswise and simultaneous comparisons. Data normality was confirmed with Kolmogorov–Smirnov test and the Box-Cox transformation was applied to satisfy assumptions where necessary before ANOVA and multiple linear regression analysis were carried out.

To illustrate the data, line graphs, bar graphs, histograms, growth curves, and tables were used. For means in charts and tables, unless otherwise specified, standard error (± SE) bars and values were used.

CHAPTER FOUR

RESULTS

The results of this study are presented in this chapter. The results are based on 13 months of field sampling (physico-chemical paramters and oyster samples) from August, 2020 to August, 2021 from Benya and Narkwa lagoons, and laboratory analysis to determine plankton dynamics, oyster diet compositions and feeding preference, compositions of ingested potential toxic phytoplankon, growth characteristics, meat yield and condition index of oysters. This chapter also reports on culture of local microalgae isolates, rearing performance of mangrove oyster larvae on local microalgae isolates.

4.1. Physicochemical Parameters of Benya and Narkwa lagoons

4.1.1. Temperature

The water temperature of Benya and Narkwa lagoons is shown in Figure 7. The annual variation of water temperature in Benya was 3.4 ºC. Monthly mean temperatures ranged from 26.5 to 29.9 ºC with an annual average of 28.6 ± 0.10 ºC. The trend in temperature variations in Benya was characterised by general fluctuations from September, 2020 to May, 2021 and a decreasing trend from June to August, 2021. The lowest temperature (26. 5ºC) was recorded in August, 2021 (wet season) and the highest temperature (29.9 ºC) recorded in February, 2021 (dry season). For Narkwa, the annual variation of water temperature was 4.4 ºC. Monthly mean temperatures of the lagoon, ranged from 26.8 ºC to 31.2 ºC with an annual average of 28.7 ± 0.16ºC. The temperature of Narkwa was low, below annual average, from September to October of 2020, and from July to August of 2021. The lowest temperature (26.8 ºC) of the lagoon was recorded in August of 2020. The lagoon was warmest (31.2 ºC) in April,

2021. Significant temporal variations in temperatures were observed in the two lagoons (ANOVA; F-value=40.49; df=12; p=0.000), however, mean annual temperatures were not significantly different (t= -0.51; df= 133; p=0.610).

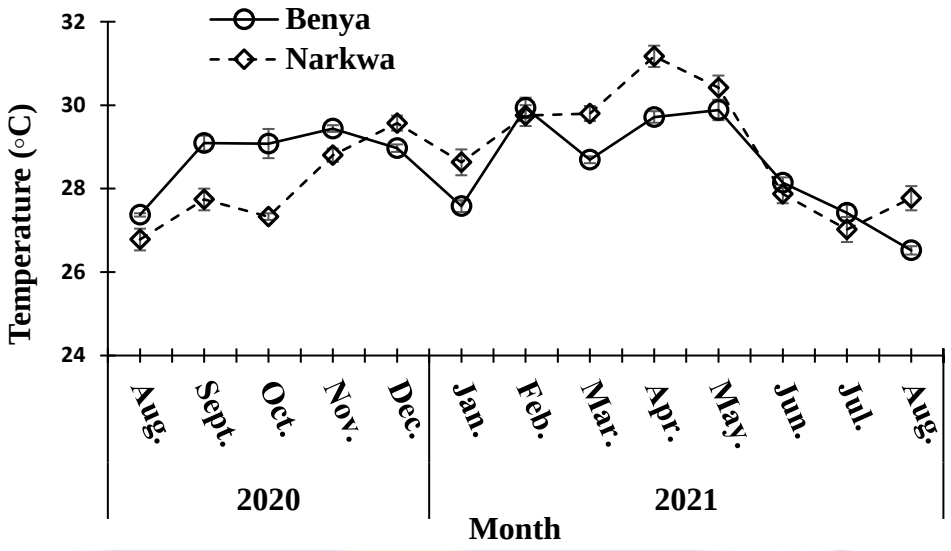

Figure 7: Temporal variations in water temperatures of Benya and Narkwa lagoons from August, 2020 to August, 2021. (Error bars indicate standard errors of means).

4.1.2. Salinity

Figure 8 shows the changes in the salinity of Benya and Narkwa lagoons during the period of this study. Generally, significantly higher salinity with relatively low variability (typical of marine) was observed in Benya. The salinity of Benya ranged from 30.0 to 38.5 ppt, with the annual average at 35.7 ± 0.23 ppt. The highest salinity in Benya was recorded in January, 2021 (dry season), and the lowest salinity recorded in October, 2020 (wet season). Salinity in Narkwa, on the other hand, exhibited a wider variability (typical estuarine gradient). Monthly mean salinity in Narkwa ranged from 3.81 ppt to 31.82 ppt with an annual average of 19.58 ± 1.00 ppt. The highest salinity, here again, was

recorded in January, 2021 (dry season), with the lowest salinity recorded in November, 2020 (wet season). There was a significant temporal variation in salinity in the two water bodies (ANOVA; F-value=17.39; df=12; p=0.000), with the mean annual salinity in Benya being significantly higher than was observed in Narkwa lagoon (t=15.76; df= 84; p=0.000).

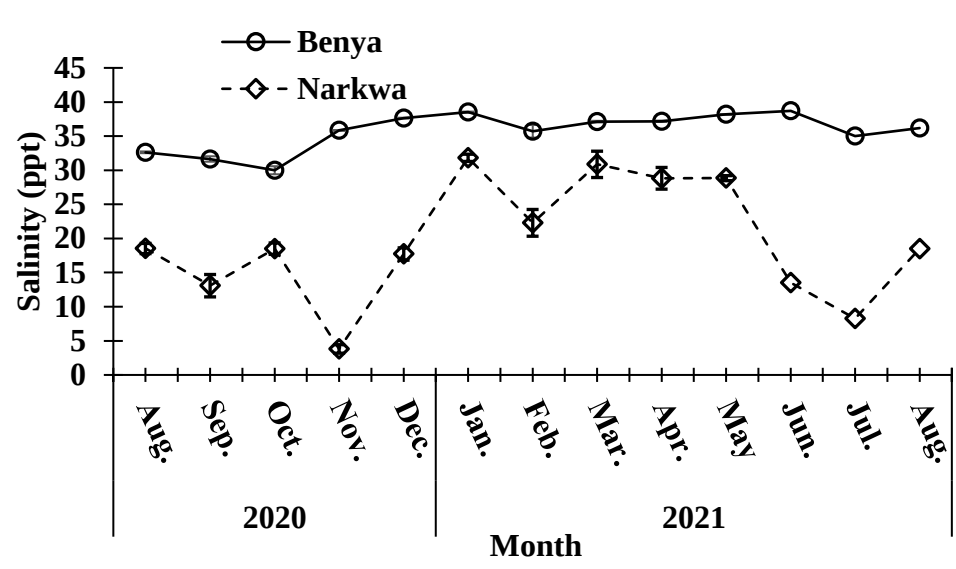

Figure 8: Temporal variations in water salinity of Benya and Narkwa lagoons from August, 2020 to August, 2021. (Error bars indicate standard errors of means).

4.1.3 Dissolved oxygen (DO)

Dissolved oxygen (DO) levels in Benya and Narkwa lagoons generally exhibited opposite pattern of variations (Figure 9). DO levels in Benya lagoon were generally lower than in Narkwa throughout the study period. It ranged from 1.3 to 3.4 mg/L, averaging at 2.5 ± 0.07 mg/L per annum. DO variability in Benya Lagoon followed a seasonal pattern, with observed episodic hypoxia (DO levels < 2.0 mg/L) in November, 2020 (wet season) and from January to March, 2021 (Dry season). Generally, the DO of the lagoon increased beginning

from April 2021, and reaching the highest level in June, 2021 (wet season). It then decreased from January to March, 2021 (Dry Season); it was lowest in November, 2020 (also in the wet season). Monthly mean DO levels in Narkwa, on the other hand, ranged from 5.02 to 8.82 mg/L, with an annual average of 6.0 ± 0.15 mg/L. Temporal variations in DO levels in Narkwa somewhat followed seasonal pattern. Increasing DO trend was observed from December, 2020 to March, 2021 with the highest DO recorded in March (dry season). Decreasing DO levels were observed in October and November, 2020, and from April to August, 2021 with the lowest DO recorded in November (wet season). Significant temporal variations in DO was observed in the two water bodies (ANOVA; F-value=3.27; df=12; p=0.000), with the mean annual DO in Benya being significantly lower than was observed in Narkwa lagoon (t=-20.77; df= 111; p=0.000).

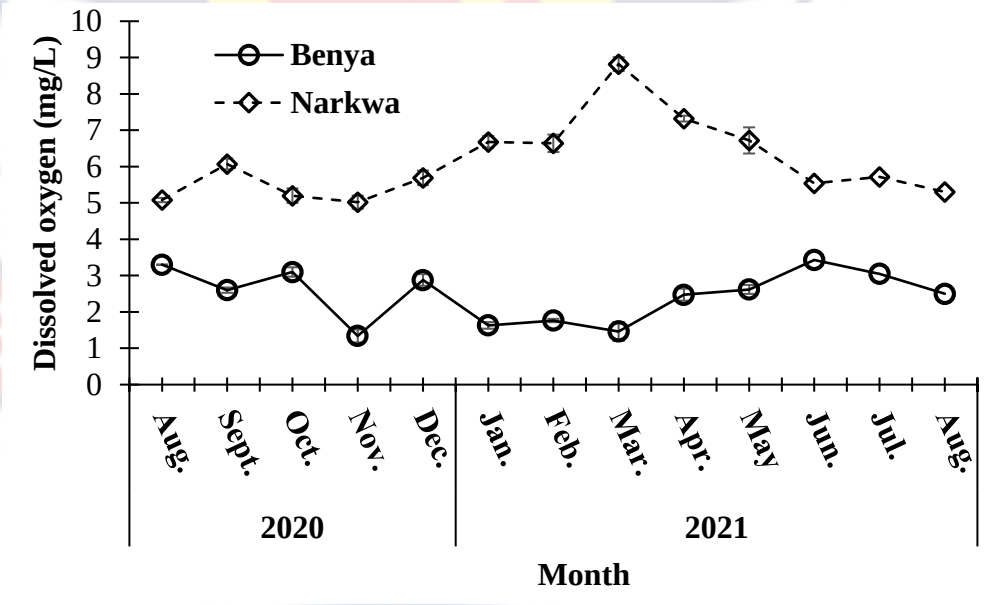

Figure 9: Temporal variations in dissolved oxygen concentrations of Benya and Narkwa lagoons from August, 2020 to August, 2021 (Error bars indicate standard errors of means).

4.1.4. pH

The acidity of Benya and Narkwa lagoons water is shown in Figure 10. The pH of Benya ranged from 6.7 (slightly acidic) to 7.9 (slightly alkaline), with an annual average of 7.2 ± 0.03. For Narkwa, the pH ranged from 6.8 (slightly acidic) to 8.09 (alkaline), with an annual average at 7.4 ± 0.05. The minimum pH levels in both water bodies were observed in October, 2020 while the maximum was observed in August, 2021. There was a significant temporal variation in pH levels in the two water bodies (ANOVA; F-value=77.76; df=12; p=0.000), with the mean annual pH level in Benya being significantly lower than was observed in Narkwa lagoon (t=-3.46; df= 111; p=0.002).

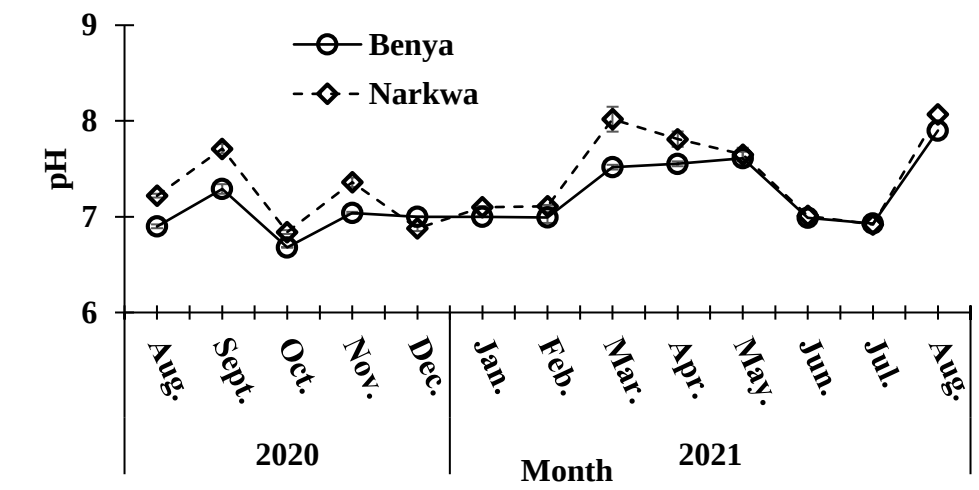

Figure 10: Temporal variations in water pH of Benya and Narkwa lagoons from August, 2020 to August, 2021 (Error bars indicate standard errors of means).

4.1.5 Turbidity

Figure 11 shows the turbidity of Benya and Narkwa lagoons during the period of this study. Temporal turbidity variabilities in both lagoons seem to have exhibited a seasonal pattern. Monthly mean turbidity in Benya ranged from 0.84 to 12.00 NTU with annual average of 6.2 ± 0.41 NTU. Increasing turbidity

levels were observed from May to August, 2021 with the highest turbidity recorded in August, 2021 (wet season). Decreasing trend was, however, observed from January to April, 2021, with the lowest turbidity recorded in February, 2021 (dry season). Monthly mean turbidity levels in Narkwa ranged from 1.9 to 44.3 NTU with annual average of 12.7 ± 1.58 NTU. Increasing trend in turbidity was observed from September to November, 2020, and from April to June, 2021, with the highest turbidity observed in June, 2021 (wet season). Lower turbidity values were observed from December, 2020 to April, 2021 with the lowest level recorded in February, 2021 (peak dry season). There was a significant temporal variation in turbidity levels in the two water bodies (ANOVA; F-value=16.44; df=12; p=0.000), and significantly higher annual mean turbidity recorded in Narkwa than in Benya (t=-4.16; df= 72; p=0.000).

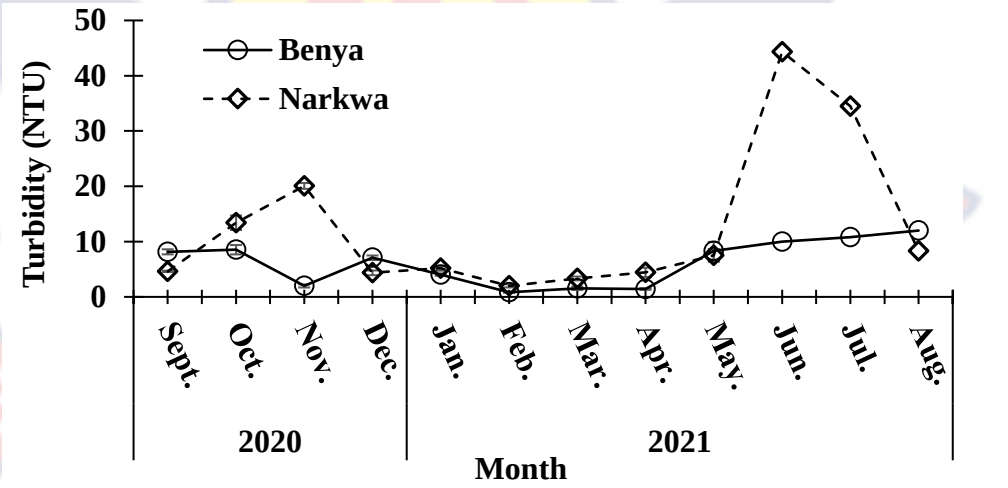

Figure 11: Temporal variations in turbidity levels of Benya and Narkwa lagoons from September, 2020 to August, 2021 (Error bars indicate standard errors of means).

4.1.6 Phosphate concentration

The monthly mean phosphate concentrations at Benya and Narkwa lagoons for the study period are presented in Figure 12. Phosphate levels in

Benya ranged from 0.28 to 0.74 mg/L, with annual average of 0.44 ± 0.02 mg/L. Monthly mean variations in Benya were characterized by a decreasing trend in the wet months from (September and October, 2020, and from April to August, 2021), with the lowest phosphate concentration recorded in October, 2020 (wet season). Increasing trend in what phosphate was observed in the dry months (November, 2020 to March, 2021), with the highest phosphate concentration observed in March, 2021 (dry season). Phosphate levels in Narkwa ranged from 0.12 to 0.58 mg/L, averaging at 0.26 ± 0.020 mg/l. Phosphate variability patterns in Narkwa on the other hand, were characterised by two peak levels in wet months (October, 2020 and May, 2021), with the minimum levels observed in dry months (January and February, 2021). There was a significant temporal variation in phosphate levels in the two water bodies (ANOVA; F-value=6.95; df=12; p=0.000), with significant higher mean annual level observed in Benya lagoon (t=6.30; df= 138; p=0.000).

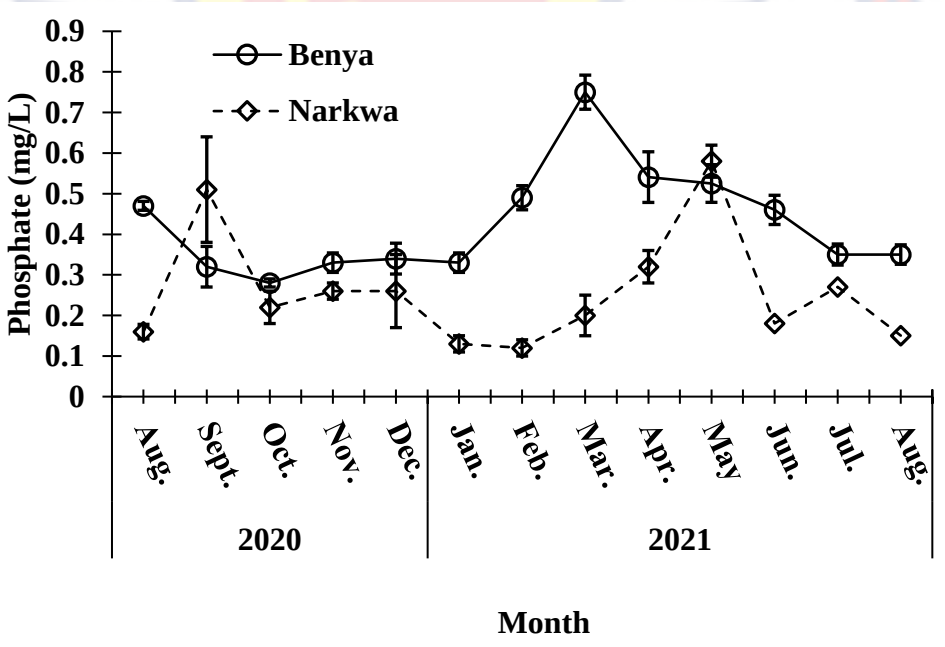

Figure 12: Temporal variations in phosphate concentrations of Benya and

Narkwa lagoons from August 2020 to August, 2021 (Error bars indicate standard errors of the mean).

4.1.7 Nitrate concentration

Figure 13 illustrates the temporal variations of mean nitrate concentrations for in Benya and Narkwa lagoons for the study period. Variations in nitrate levels in the two water bodies generally followed a similar pattern for most of the sampling months. Nitrate levels in Benya lagoon ranged from 4.4 to 13.0 mg/L, with annual mean of 8.1 ± 0.31 mg/L. Temporal variations in Benya Lagoon was characterised by general fluctuating pattern in the wet months (August to November, 2020 and from April to July, 2021), with an increasing trend observed in the dry month (January to March, 2021). Highest nitrate concentration in Benya lagoon was recorded in March, 2021 (dry season), whiles the lowest was recorded in July, 2021 (wet season). Monthly mean nitrate levels in Narkwa lagoon ranged from 0.0 to 10.2 mg/L, with annual mean of 4.5 ± 0.43. Variations in nitrate levels was depicted by decreasing trend in the wet months (September to November, 2020 and April to July, 2021), with the lowest concentrations (recorded in November, 2020 and July, 2021 (wet seasons). An increasing trend was observed in the dry months (December, 2020 to March, 2021), with the highest concentration recorded in March. Significant temporal variations in nitrate levels were observed in the two water bodies (ANOVA; F-value=19.83; df=12; p=0.000), with a significant higher mean annual level recorded in Benya (t=6.39 20.8; df= 148; p=0.000).

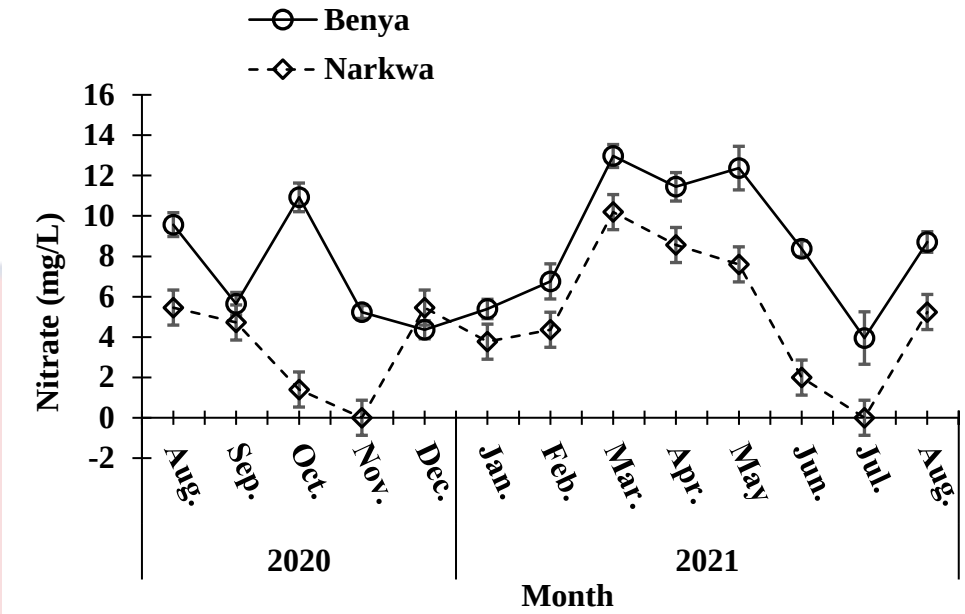

Figure 13: Temporal variations in nitrate concentration pattern of Benya and Narkwa lagoons from August 2020 to August, 2021 (Error bars indicate standard errors of the mean).

4.2.8. Chlorophyll-*a* concentration

Temporal patterns of variations in mean chlorophyll-*a* concentration in Benya and Narkwa lagoons for the study period are shown in Figure 14. Chlorophyll-*a* level in Benya lagoon ranged from 3.2 to 14.0 mg/L, with an annual mean of 6.0 ± 0.26 mg/L. Variations in monthly mean chlorophyll a concentration in Benya lagoon were characterized by general increasing trend in the wet months (April to August), and fluctuating trend in the dry month (December to March). The highest Chlorophyll-*a* concentration (14 mg/L) in Benya was recorded in February, 2021; the lowest (3.16 mg/L) was in April 2021 (wet season). Mean monthly Chlorophyll-*a* concentration in Narkwa lagoon ranged from 2.4 to 9.7 mg/L, with annual mean of 4.1 ± 0.37 mg/L. Chlorophyll-*a* pattern of variations in Narkwa Lagoon depicted general

fluctuations for most of the sampling period, except from November to January where a stable decline was observed. Highest chlorophyll-*a* concentration (9.67 mg/L) was recorded in October, 2020 (wet season) and the lowest (2.36 mg/L) in July, 2021 (wet season). Significant temporal variations in chlorophyll-*a* concentration was observed in the two water bodies (ANOVA; F-value=9.75; df=12; p=0.000), with significant higher annual mean chlorophyll-a concentration recorded in Benya lagoon (t=3.75 df= 129; p=0.000).

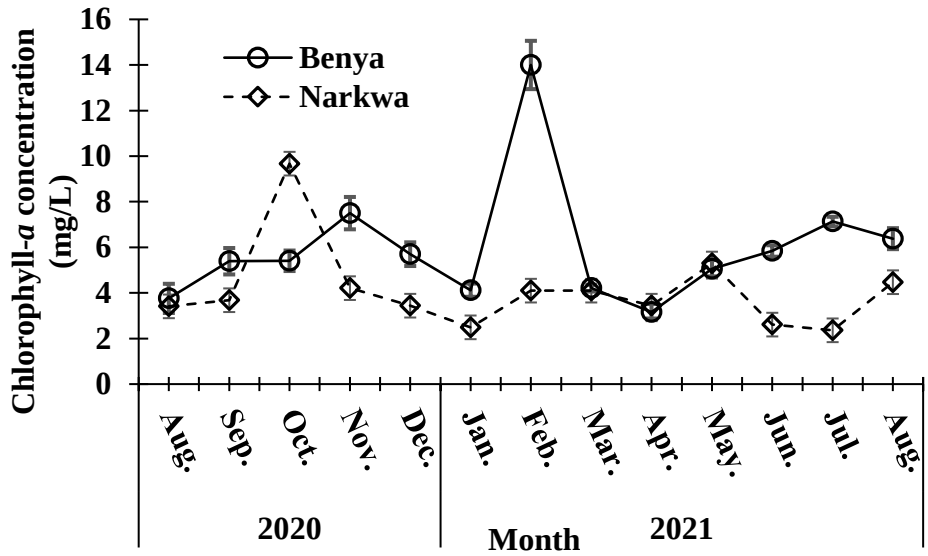

Figure 14: Temporal variations in chlorophyll-*a* concentration of Benya and Narkwa lagoons from August 2020 to August, 2021 (Error bars indicate standard errors of the mean).

4.2.9. Precipitation

Temporal pattern of variations in rainfall (precipitations) levels covering the two study areas is illustrated in Figure 15. Two peaks were observed, i.e. in October (representing the peak of the minor wet season) and in June (representing the peak of the major wet season). Low precipitations were observed in August, 2020 and February (representing the peak dry season).

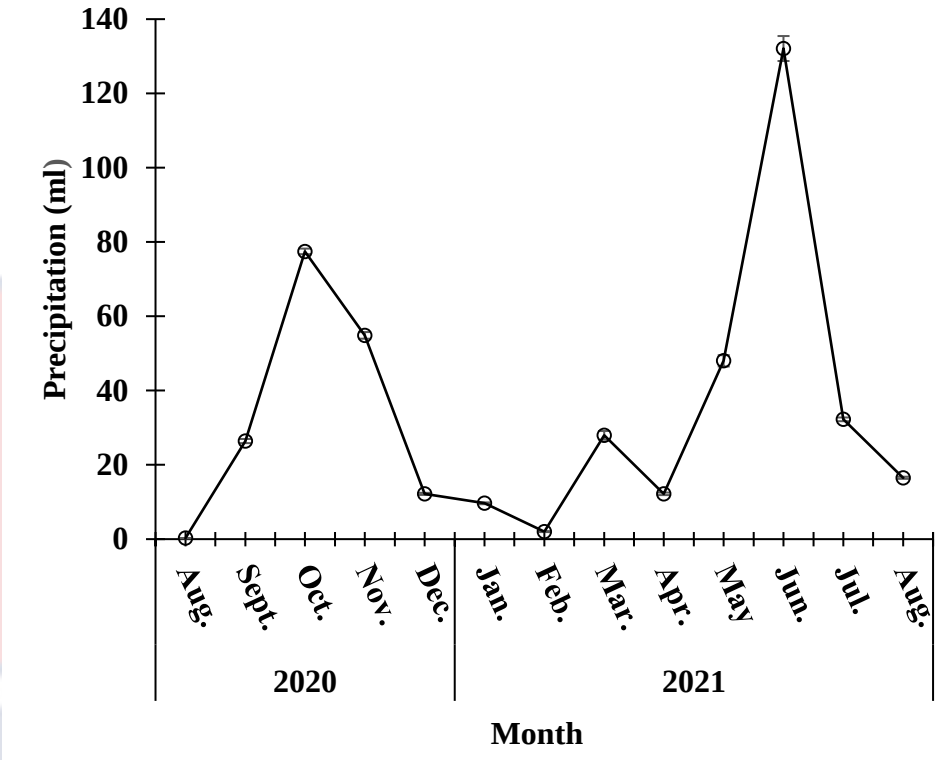

Figure 15: Temporal variations in precipitation levels at the vicinity of Benya and Narkwa Lagoons from August 2020 to August, 2021 (Error bars indicate standard errors of the mean).

4.2. Plankton Dynamics and Utilisation by *C. tulipa*

4.2.1. Plankton dynamics in Benya and Narkwa lagoons

Analysis of water samples from the Benya and the Narkwa lagoons revealed a variety in the plankton communities, which included phytoplankton, microzooplankton, mesozooplankton and fish eggs/larvae. These were grouped into ten (10) major functional groups namely: (i) Bacillariophyceae (Diatoms), (ii) Dinophyceae (Dinoflagellates), (iii) Cyanophyceae (Cyanobacteria), (iv) Chlorophyceae (Green algae), (v) Mesozooplankton (copepods/Cladocerans/Rotifers), (vi) Dictyochophyceae (Silicoflagellate), (vii) Euglenoidea (Euglenoids), (viii) Oligotrichea (Ciliates) and (ix) Spirotrichea (Ciliates) and (x) Fish eggs/larvae. These groups, excluding the

fish egg/larvae, were further classified into genus. Table 1 gives details of the breakdown of the number of genera recorded under the major plankton groups. A total of 90 genera were recorded in Benya, whiles 92 were recorded in Narkwa.

Table 1: *Number of Genera for the Plankton Functional Groups in Benya and Narkwa lagoons*

Plankton functional groups	**Number of genera recorded**	
	Benya	Narkwa
Bacillariophyceae	48	46
Dinophyceae	20	22
Cyanophyceae	9	7
Chlorophyceae	1	2
Dictyochophyceae	1	1
Euglenoidea	2	2
Oligotrichea	7	6
Spirotrichea	1	1
Mesozooplankton	1	5
Total	90	92

Figure 16 shows the temporal variations in the abundance (density) of plankton in the Benya and Narkwa lagoons. Monthly plankton densities in the Benya ranged from 7.91 $\times 10^5$ cells/ind. L^{-1} to 79.9 $\times 10^5$ cells/ind. L^{-1}, with major peak density occurring in February (dry season), and the minor peak densities in July and August (wet season). Monthly plankton densities in Narkwa Lagoon, on the other hand, ranged from 8.03 ×105 cells/ind. L^{-1} to 55.8 ×105 cells/ind. L^{-1}, the peak density recorded in April (onset of the wet season) and minor peaks observed in January and February.

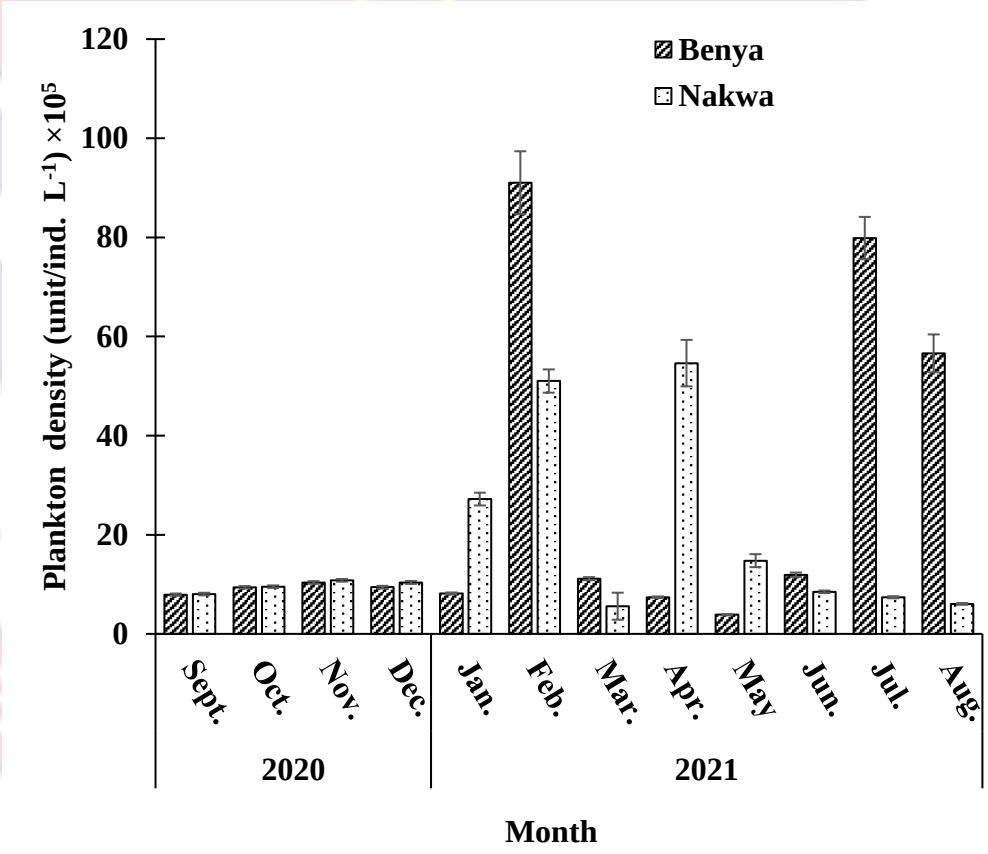

Figure 16: Temporal patterns of variations in mean plankton abundance in Benya and Narkwa lagoons from September, 2020 to August, 2021.

The overall (pooled monthly data) compositions of the plankton functional groups recorded in this present study in the Benya Lagoon is illustrated in Figure 16. The order of magnitude of their compositions is as

follows; Bacillariophyceae > Dinophyceae > Oligotrichea > Cyanophyceae > Chlorophyceae > Spirotrichea > Euglenoidea > Mesozooplankton > Dictyochophyceae > Fish egg/larvae. For brevity, only plankton functional group and taxon with a total composition ≥ 5.0 % were considered in all the compositions analyses, and those with total composition values < 5.0 % were added together and designated as "Others". Bacillariophyceae (diatoms) generally dominated the plankton community (≈61%), followed by Dinophyceae (dinoflagellates) (≈27%), and Oligotrichea (ciliates) (≈9%). All the other groups together constituted 3% of the plankton groups compositions (Figure 17a). Three taxa dominated (> 80%) the diatoms group in Benya Lagoon; *Thalassiosira* (≈ 51%), *Nitzschia* (≈ 22 %) and *Navicula* (≈10 %) (Figure 17b). The dinoflagellates were dominated (>70 %) by four harmful taxa, *Prorocentrum* (≈ 40%), *Dinophysis* (≈27%), *Protoperidinium* (≈11%) (Figure 17c). The group Oligotrichea (ciliates) were predominated by the genus *Strombilidium* (≈ 96%) (Figure 16d). Table 2 shows the frequency of occurrence of dominant taxa recorded in Benya Lagoon in this study. Six genera together accounted for more than 70 % of the plankton communities in the Benya Lagoon. However, four genera, namely *Thalassiosira*, *Nitzschia*, *Navicula* and *Prorocentrum* were present for all the sampling months, thus, recording a 100 % (year-round occurrence). The genera *Strobilidium* and *Dinophysis* had 58.33 % and 41.67 % occurrence respectively

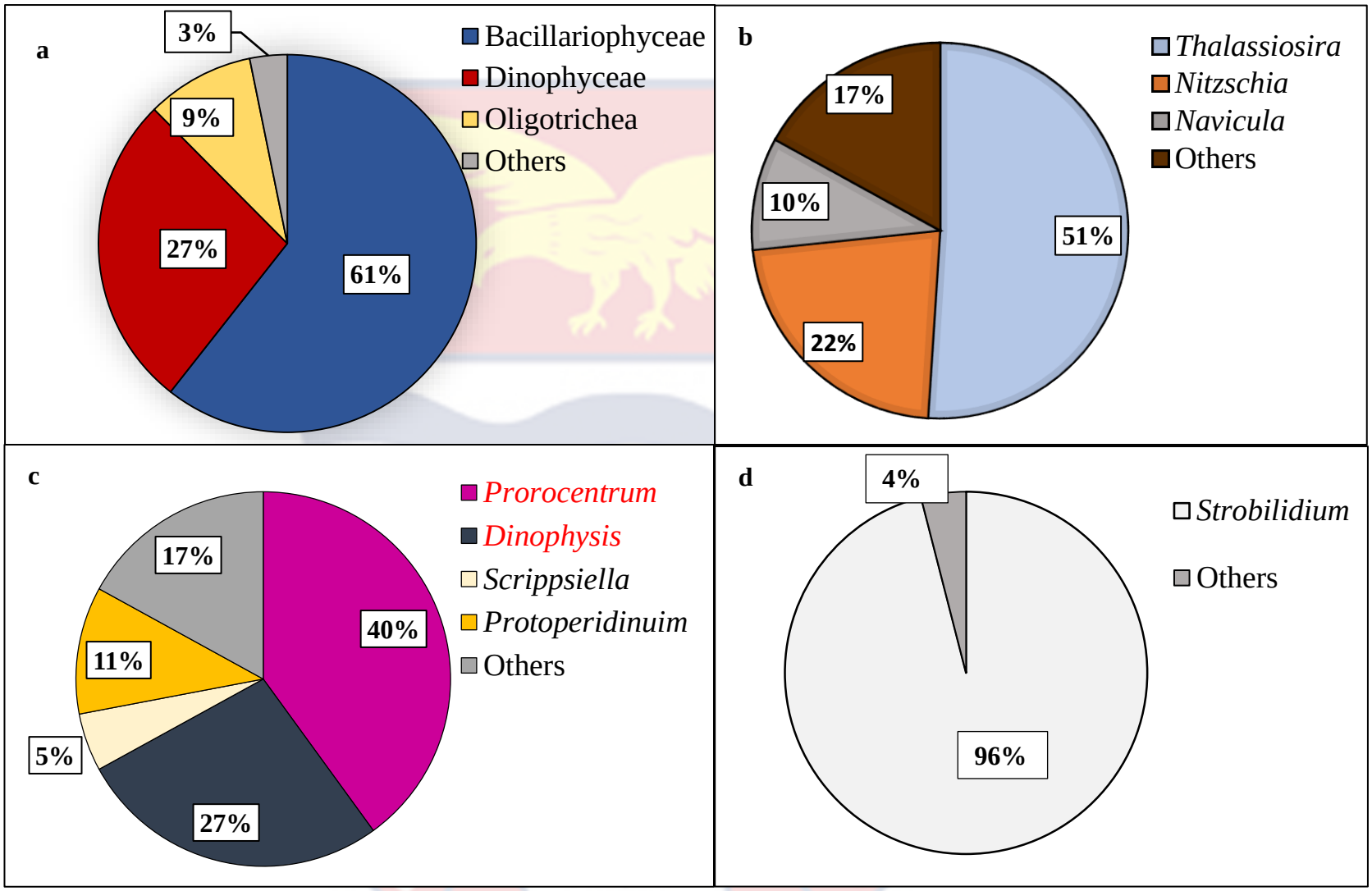

Figure 17: Overall composition of (a) plankton functional groups (b) Bacillariophyceae (c) Dinophyceae (d) Oligotrichea in the Benya lagoon.

Table 2: *Percentage Frequency of Occurrence of Dominant Plankton Genera in Benya Lagoon (B= Bacillariophyceae, D = Dinophyceae, O = Oligotrichea)*

Genera	Overall compositions (%)	Frequency of occurrence (%)
Thalassiosira (B)	30.93	100
Nitzschia (B)	13.53	100
Prorocentrum (D)	10.76	100
Navicula (B)	5.87	100
Strobilidium (O)	8.85	58.33
Dinophysis (D)	7.31	41.67

Temporal variations of plankton groups in Benya Lagoon is shown in Figure 18. Result from this study shows predominance of diatoms in almost all the months in the year, except in August where higher composition of dinoflagellates (> 80%) was observed. Peak diatoms compositions were observed in September, 2020 (wet season), December, 2020 (dry season) and February, 2021 (dry season). Higher cell densities of centric diatoms of the genus *Coscinodiscus* (1.53×10^5 cells L^{-1}) and pennate diatoms of the genus *Pleurosigma* (1.37×10^5 cells L^{-1}) appeared as to have contributed to the September peak. They were, however, succeeded by pennate diatom genera, Navicula (1.66×10^5 cells L^{-1}) and *Nitszchia* (1.32×10^5 cells L^{-1}) in the December peak, and again by the centric diatoms of the genus *Thalassiosira*

(6.08×10^6 cells L^{-1}) in the February peak. Relatively high compositions of *Oligotrichea* were recorded in May (20.16 %) and June (31.84%) due to high densities of *Strobilidium* spp. observed in May (6.90×105 ind./ L^{-1}) and July (2.52×10^6 ind./L^{-1}). Refer to Table in Appendix 1 for details on monthly plankton genera counts.

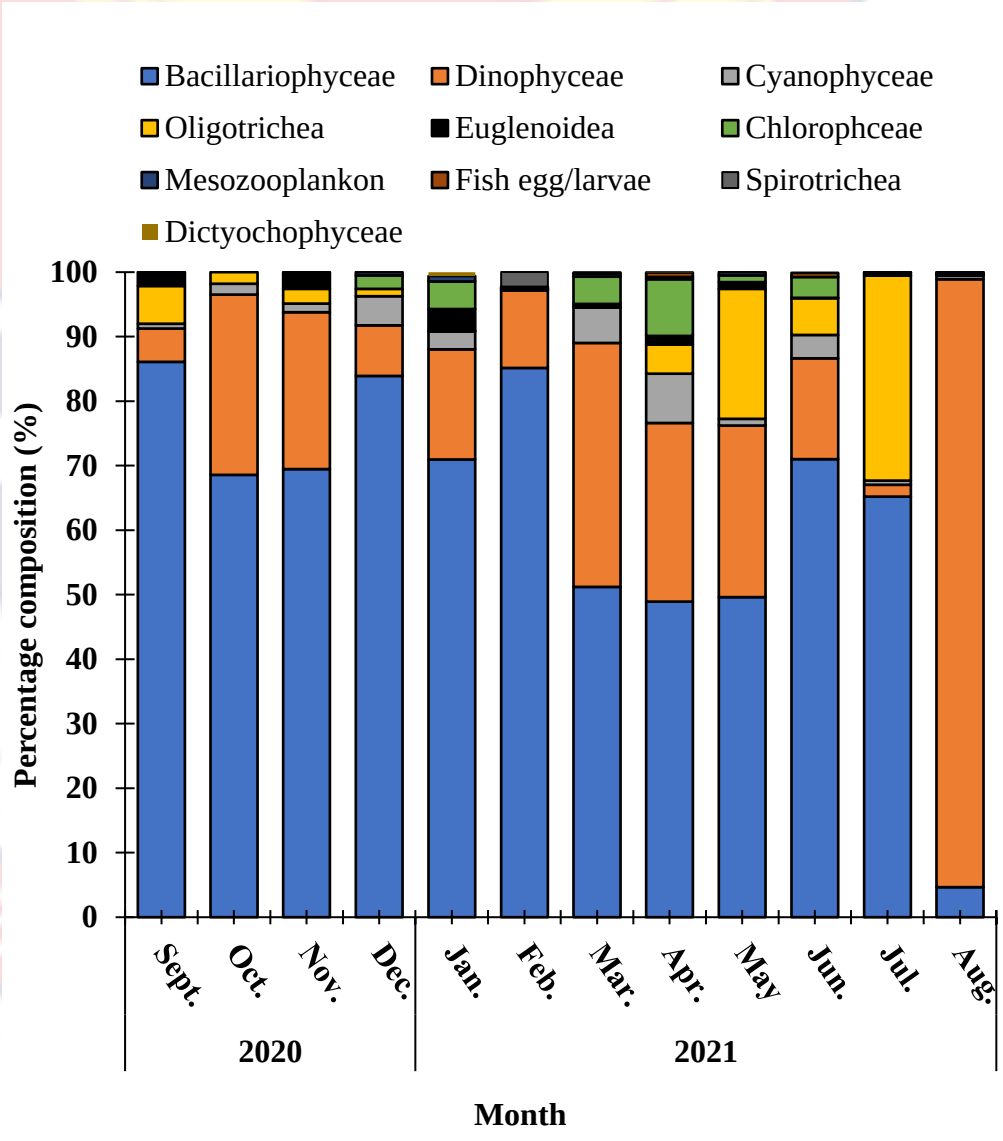

Figure 18: Temporal variations in plankton group compositions in Benya lagoon from September, 2020 to August, 2021.

Plankton groups compositions in Narkwa lagoon is illustrated in Figure 19a. The order of magnitude of compositions is as follows; Bacillariophyceae >

Dinophyceae > Chlorophyceae > Euglenoidea > Oligotrichea > Cyanophyceae Fish egg/larvae > Mesozooplankton > Dictyochophyceae >Spirotrichea. Unlike the Benya lagoon, the plankton community in Narkwa lagoon were generally dominated by the Dinophyceae (≈ 72 %), followed by Bacillariophyceae (≈ 25%), and the other groups together (≈ 3 %). Six harmful taxa together constituted ≈ 86% of the Dinophyceae group in Narkwa Lagoon *Prorocentrum* (≈25 %), *Peridinium* (≈20 %), *Gymnodinium* (≈14 %), *Heterocapsa* (≈11 %), *Karenia* (≈8%) and *Gyrodinium* (≈7%) (Figure 19b). The Bacillariophyceae group were dominated by *Nitzschia* (≈19%), *Cylindrotheca* (≈12%), Navicula (≈12%), *Chaetoceros* (≈11%), *Thalassiosira* (≈7%) and *Pleurosigma* (≈6%) (Figure 19c).

Table 3 shows the frequency of occurrence of dominant taxa recorded in Narkwa lagoon in this study. Six phytoplankton genera, *Prorocentrum, Nitzschia*, *Gymnodinium*, *Heterocapsa, Gyrodinium* and *Karenia,* accounted for more than 50 % of the plankton communities in the Narkwa lagoon. However, two genera, namely *Prorocentrum* and *Nitzschia* were present for all the sampling months, thus, recording a 100 % (year-round occurrence). *Gymnodinium, Heterocapsa, Gyrodinium* and *Karenia* had 91.67 %, 83.33 %, 75% and 33.33 occurrence, respectively.

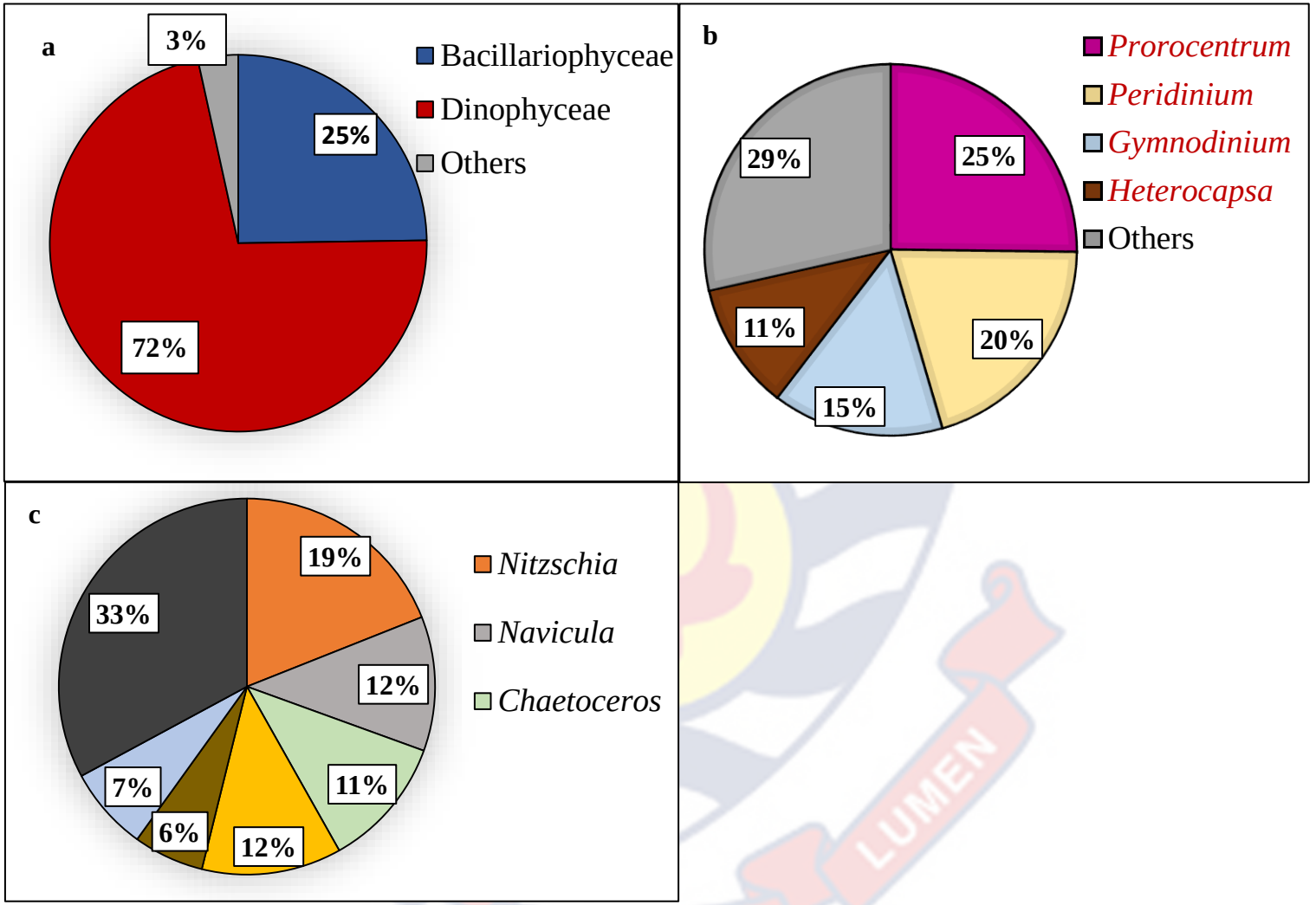

Figure 19: Overall compositions of (a) plankton functional groups: (b) Dinophyceae (c) Bacillariophyceae in the Narkwa lagoon.

Table 3*: Percentage Frequency of Occurrence of Dominant Plankton Genera in Narkwa Lagoon (B= Bacillariophyceae, D = Dinophyceae).*

Genera	**Overall compositions (%)**	**Frequency of occurrence (%)**
Prorocentrum (D)	18.08	100
Nitzschia (B)	5.00	100
Gymnodinium (D)	10.72	91.67
Heterocapsa (D)	7.95	83.33
Gyrodinium (D)	5.17	75
Karenia (D)	5.34	33.33

Temporal variations in plankton community compositions in Narkwa Lagoon (shown in Figure 20), indicated a seasonal succession between two major plankton groups. While diatoms preponderance was observed between September and November, with peak in September (wet season) (occasioned by the dominance of genus *Coscinodiscus* and *Pleurosigma*), they were succeeded by the dinoflagellates from January to May, with peak in March (dry season). The seasonal dominance of dinoflagellates observed in Narkwa lagoon, was occasioned by the seasonal proliferation of harmful taxa; namely, *Peridinium*, *Prorocentrum*, *Gymnodinium* and *Gyrodinium*.

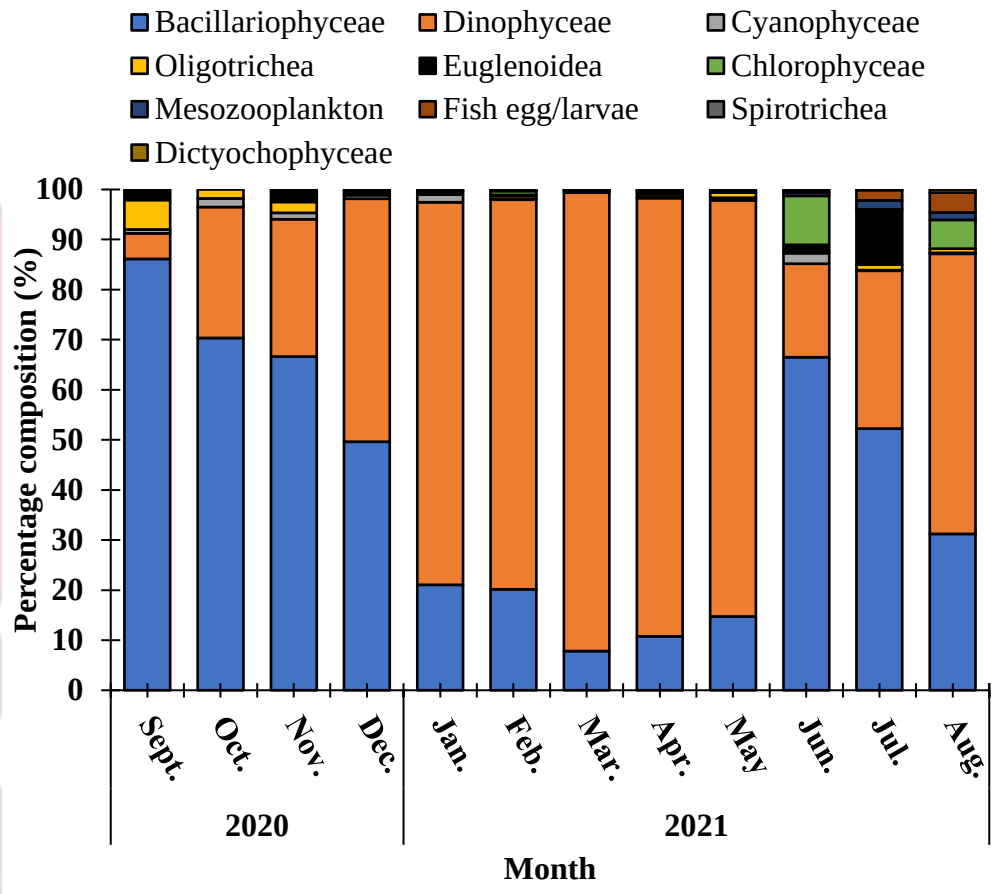

Figure 20: Temporal variations in plankton group composition in Narkwa lagoon from September, 2020 to August, 2021.

4.2.2. Relationships between measured physicochemical parameters and plankton abundance

Pearson correlation coefficients between measured physicochemical factors and the abundance (expressed as percentage compositions) of the plankton groups in the two water bodies are presented in Table 4. Temperature had a significant positive correlation with Dinophyceae composition (r=0.23; p=0.026), but negatively correlated with Oligotrichea composition (r=-0.22; p=0.032). Salinity positively correlated with Cyanophyceae composition (r=0.25; p=0.014), however, negatively with Euglenoidea composition (r=-0.31; p=0.03). Dissolved oxygen positively correlated with Dinophyceae

(r=0.41; p=0.00) and Dictyochophyceae (r=0.21; p=0.043) compositions, but negatively with Bacillariophyceae (r=-0.36; p=0.00), Cyanophyceae (r=-0.32; p=0.02) and Spirotrichea (r=-0.24; p=0.021) compositions. pH recorded a positive correlation with the Dinophyceae (r=0.40; p=0.000), Dictyochophyceae (r=0.22; p=0.031), and fish eggs/larvae (r=0.27; p=0.008) compositions, but negatively correlated with Bacillariophyceae composition (r=-0.44; p=0.00). Turbidity correlated positively with the composition of mesozooplankton (r=0.45; p=0.00) and Euglenoidea (r=0.35; p=0.001) compositions. Phosphate positively correlated with Cyanophyceae composition (r=0.32; p=0.002), but negatively correlated with Dinophyceae composition (r= -0.23; p=0.022). Nitrate recorded a positive correlation with Cyanophyceae composition (r=0.32; p=0.001), but negatively correlated with Bacillariophyceae (r=-0.22; p=0.032) and Euglenoidea (r=-0.28; p=0.006) compositions. Chlorophyll-*a* concentration correlated positively with composition of the Spirotrichea (r=0.63; p=0.000). The measured physicochemical parameters were also analysed as predictors of Bacillariophyceae (Table 5) and Dinophyceae (Table 6) (the two most dominant plankton groups) abundance in Benya and Narkwa Lagoons, using multiple linear regression. While pH and phosphate were the two significant ($p < 0.05$) predictors of Bacillariophyceae composition, pH, phosphate in addition to nitrate, were the most significant ($p<0.05$) predictors of Dinophyceae composition in the two lagoons. The measured hydrographic factors accounted for 38.40 % and 35.25 % of variation in the data for Bacillariophyceae and Dinophyceae abundance, respectively.

Table 4: *Pearson's Correlation Coefficient (r) between the Measured Physicochemical Parameters and Plankton Groups Abundance*

	Bac.	**Dino.**	**Cya.**	**Chlo.**	**Mesozoo.**	**Dict.**	**Eug.**	**Fish egg**	**Olig.**	**Spiro.**
Temperature	-0.13	0.23*	-0.14	-0.06	-0.09	0.05	-0.16	-0.08	-0.22*	0.11
Salinity	-0.13	0.07	0.25*	0.19	-0.15	-0.04	-0.31*	-0.01	0.15	0.13
DO	-0.36*	0.41*	-0.32*	-0.13	0.17	0.21*	-0.02	0.06	-0.18	-0.24*
pH	-0.44*	0.40*	-0.06	0.14	-0.09	0.22*	-0.13	0.31*	-0.08	-0.14
Turbidity	0.10	-0.14	-0.06	-0.12	0.45*	-0.01	0.35*	0.04	0.01	-0.20
Phosphate	0.19	-0.23*	0.32*	0.12	-0.20	-0.19	-0.06	-0.14	0.10	0.17
Nitrate	-0.22*	0.17	0.32*	0.07	-0.15	-0.02	-0.28*	0.01	0.03	0.01
Chl-*a*	0.18	-0.10	-0.17	-0.11	-0.15	-0.12	-0.19	-0.07	0.01	0.63*

*Significant at $p < 0.05$, Bac=Bacillariophyceae, Din=Dinophyceae, Cya=Cyanophyceae, Chlo=Chlorophyceae, Mesozoo. =Mesozooplankton, Dict=Dictyochophyceae, Eug=Euglenoidea, Fish=Fish eggs/larvae, Olig=Oligotrichea, Spiro=Spirotrichea.

Table 5: *Multiple Linear Regression Model of Bacillariophyceae (Diatoms) Composition (Factor) and the Measured Physicochemical Parameters (Predictors).*

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Bacillariophyceae (Diatoms)						
Constant	198.6	64.4	(70.7, 326.5)	3.09	0.003	
Temperature	1.10	1.77	(-2.42, 4.63)	0.62	0.536	1.70
Salinity	-0.414	0.302	(-1.014, 0.185)	-1.37	0.173	1.93
Dissolved Oxygen	-2.15	1.26	(-4.66, 0.36)	-1.70	0.092	2.34
pH	-22.06	5.80	(-33.59, -10.54)	-4.80	**0.000***	1.53
Turbidity	0.037	0.299	(-0.557, 0.632)	0.12	0.901	1.52
Phosphate	53.7	16.2	(21.4, 86.0)	3.31	**0.001***	1.77
Nitrate	-1.488	0.794	(-3.067, 0.091)	-1.87	0.064	2.09
Chl-*a*	-0.005	0.780	(-1.554, 1.545)	-0.01	0.995	1.31

*Significant at $p<0.05$, $R^2=38.40\%$, F=6.78, DF= 8

Table 6: *Multiple Linear Regression Model of Dinophyceae (Dinoflagellates) Composition (Factor) and the Measured Physicochemical Parameters (Predictors).*

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Dinophyceae						
Constant	-11.09	6.29	(-23.59, 1.40)	-1.77	0.081	
Temperature	0.133	0.173	(-0.211, 0.478)	0.77	0.443	1.70
Salinity	-0.0051	0.0294	(-0.0636, 0.0534)	-0.17	0.863	1.93
Dissolved Oxygen	0.150	0.123	(-0.095, 0.395)	1.22	0.227	2.34
pH	1.756	0.566	(0.630, 2.882)	3.10	**0.003***	1.53
Turbidity	-0.0264	0.0292	(-0.0845, 0.0317)	-0.90	0.369	1.52
Phosphate	-5.38	1.59	(-8.53, -2.22)	-3.39	**0.001***	1.77
Nitrate	0.1712	0.0776	(0.0169, 0.3254)	2.21	**0.030***	2.09
Chl-*a*	0.0318	0.0761	(-0.1195, 0.1832)	0.42	0.677	1.31

*Significant at $p<0.05$, $R^2=35.25\%$, F=5.92, DF= 8

4.2.3 Plankton ingestion and selectivity by *C. tulipa* populations

Stomach content analysis of *C. tulipa* in Benya and Narkwa Lagoons showed that the oyster populations explored all the plankton functional groups in the water columns as food resource. Table 7 gives details of the taxonomic diversity of ingested prey items belonging to plankton functional groups identified in this study. Overall (monthly pooled data) compositions of ingested plankton by oyster populations in Benya Lagoon is shown in Figure 21. The order of magnitude of the ingested plankton compositions is as follows; Bacillariophyceae > Dinoflagellates > Cyanophyceae > Mesozooplankton >Euglenoidea > Oligotrichea > Chlorophyceae > Dictyochophyceae > Spirotrichea. Just like the in the water column, Bacillariophyceae dominated (≈47%) the ingested plankton by oyster populations in Benya, followed by Dinophyceae (≈36%), Cyanophyceae (≈11%), and all other groups together (≈6%) (Figure 21a). The ingested diatoms were dominated by six taxa which together constituted about 70% of the overall ingested diatoms: *Thalassiosira* (≈20%), *Navicula* (≈18 %), *Nitzschia* (≈13 %), *Coscinodiscus* (≈7%), *Cylindrotheca* (≈7%) and *Amphora* (≈5%) (Figure 120b). Ingested dinoflagellates were dominated by three taxa which together made up over 60% of all the ingested dinoflagellates: *Prorocentrum* (≈56%), *Durinskia* (≈7%) and *Protoperidinium* (≈5%) (Figure 21b). The ingested cyanobacteria were dominated by *Chroococcus* sp (≈75%) and *Merismopedia* sp. (≈20%), which constituted 95% of all the ingested cyanobacteria (Figure 21c) by the mangrove oyster populations at Benya for the study period.

Table 7: *Number of Taxa of the Plankton Functional Groups Ingested by C. tulipa Populations in Benya and Narkwa lagoons*.

Plankton functional groups	**Number of genera recorded**	
	Benya	Narkwa
Bacillariophyceae	42	43
Dinophyceae	17	17
Cyanophyceae	6	5
Chlorophyceae	1	1
Dictyochophyceae	1	1
Euglenoidea	2	2
Oligotrichea	9	6
Spirotrichea	1	1
Mesozooplankton	1	3
Total	80	79

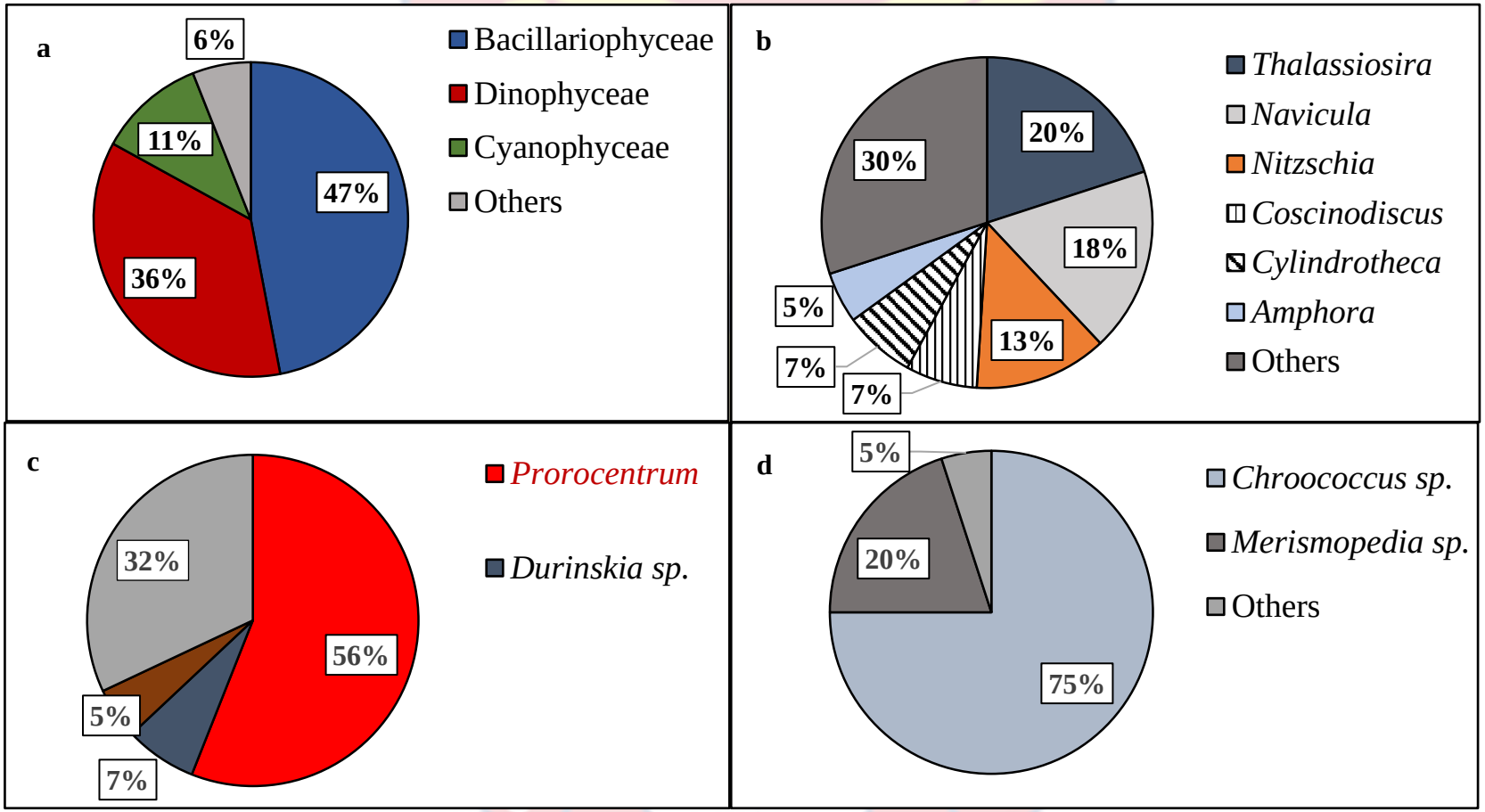

Figure 21: Overall compositions of (a) plankton groups (b) Bacillariophyceae (c) Dinophyceae (d) Oligotrichea, ingested by *C. tulipa* populations in the Benya Lagoon.

The overall compositions of ingested plankton by oyster populations in Narkwa lagoon is illustrated in Figure 22. The order of magnitude of the ingested plankton percentage compositions is as follows; Bacillariophyceae > Dinoflagellates > fish egg/larvae> Cyanophyceae > Mesozooplankton > Oligotrichea > Euglenoidea > Chlorophyceae > Dictyochophyceae > Spirotrichea. The ingested plankton groups in *C. tulipa* populations in Narkwa Lagoon were dominated by Bacillariophyceae (≈49%), followed closely by Dinophyceae (≈42%). All other plankton groups together constituted ≈ 9% of the ingested plankton (Figure 22a). The ingested diatoms were dominated by *Nitzschia* (≈43%), *Amphora* (≈20%) and *Navicula* (≈9%), with all others taxa together constituting ≈ 28% (Figure 22b). The ingested dinoflagellates, on the other hand, were dominated by *Peridinium* (≈38%), *Prorocentrum* (≈35%) and *Gymnodinium* (≈9%), with all other ingested dinoflagellates adding up to ≈ 22 % (Figure 22c). The numeric frequency of ingested plankton in oysters varied temporally in Benya and Narkwa Lagoons (Table in Appendices B1 and B2). The highest frequency of ingested plankton in Oysters in Benya Lagoon was recorded in September, 2020 influenced by proliferation of *Prorocentrum* spp., whiles the lowest numeric frequency was recorded in August, 2021. In the Narkwa Lagoon, comparatively high frequency of ingested plankton was recorded in May, influenced by high numbers of ingested *Nitzschia* and *Peridinium* spp, with the lowest frequency recorded in June.

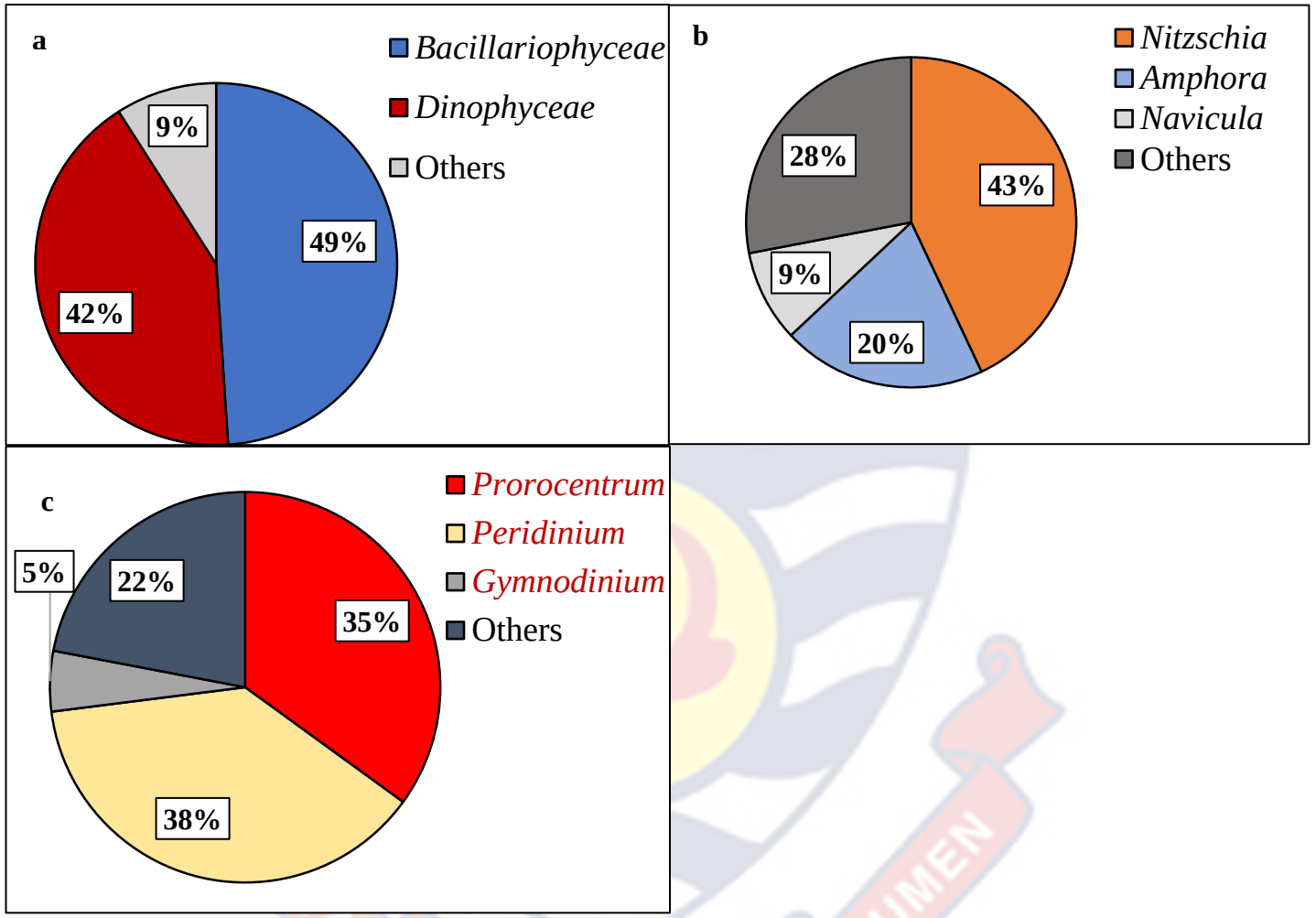

Figure 22: Overall compositions of (a) plankton groups (b) Bacillariophyceae (c) Dinophyceae (d) Oligotrichea, ingested by *C. tulipa* populations in the Narkwa Lagoon.

Pattern of temporal variations in compositions of plankton groups ingested by the *C. tulipa* population in Benya lagoon as shown in Figure 23, was characterized by seasonal peaks of diatoms dominance in February and March (dry season), May and July (representing wet season). Members of the genus *Navicula* were major component in the February peak, succeeded by genus *Thalassiosira* in March. *Nitzschia* and *Navicula* were responsible for the May peak, while *Navicula* and *Thalassiosira* featured prominently in the July peak. Ingested dinoflagellates fluctuated temporally, and the peak ingestion was observed in August and September (wet season), with genus *Prorocentrum* as the predominant taxon. Relatively higher proportions of ingested cyanobacteria were recorded in April and June (wet season), with *Chroococcus* sp. as the major ingested cyanobacteria. Unlike Bacillariophyceae, Dinophyceae and Cyanophyceae that recorded all year-round ingestion, patchy temporal ingestion was observed for other plankton groups (Oligotrichea, Euglenoidea, Mesozooplankton and Fish eggs/larvae) Appreciable proportions of Mesozooplankton were also recorded in October and March.

Selectivity analysis of ingested plankton by *C. tulipa* populations in the Benya lagoon, using the modified version of Ivlev's electivity index (Ei), is presented in Figure 24. Temporal analysis indicated monthly variations in the plankton group selectivity. Cyanobacteria (predominated by *Chrooccocus* sp.) and mesozooplankton were preferentially selected almost all year round. Plankton groups such as Oligotrichea, Euglenoidea and fish eggs/larvae recorded patchy selection even though their proportions in the water column were relatively lower. Dinoflagellates, other the hand, were selected in most months of the year, except in March and May where low rejections (<-0.5)

where observed. Strangely, diatoms, which formed the larger proportion of the ingested plankton, were observed to have been selectively rejected throughout the year.

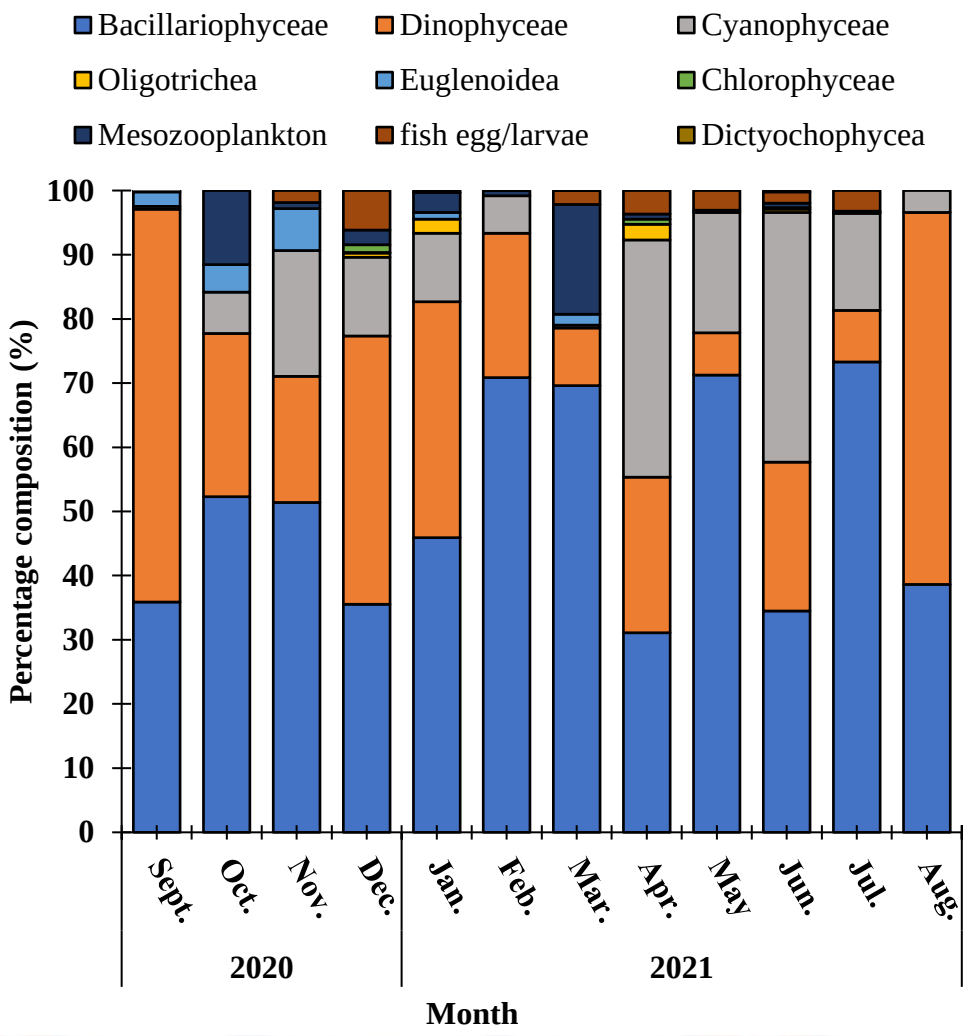

Figure 23: Temporal variations in compositions of ingested plankton groups by *C. tulipa* in Benya lagoon from September, 2020 to August, 2021.

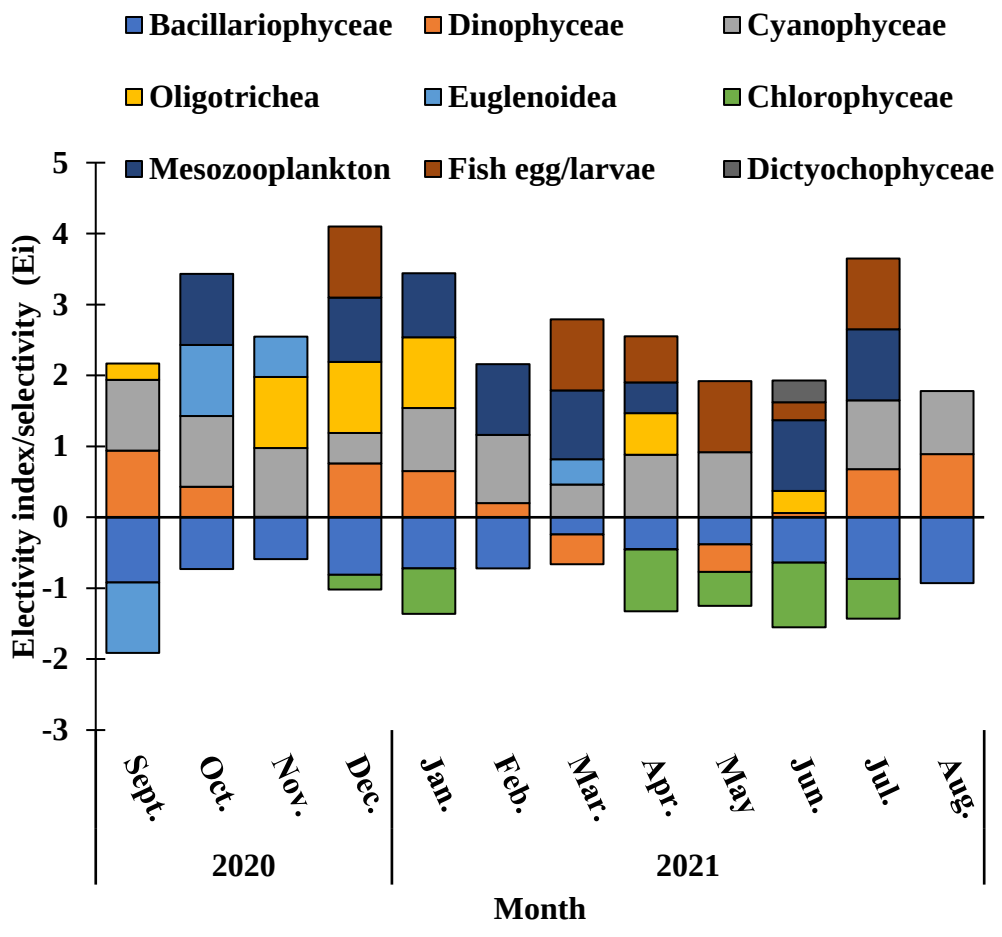

Figure 24: Temporal variations in plankton selectivity of *C. tulipa* in Benya lagoon from September, 2020 to August, 2021.

Figure 25 shows temporal variations in ingested plankton group compositions by *C. tulipa* population in Narkwa Lagoon. The pattern of variations in ingested plankton compositions in the stomach of *C. tulipa* was characterised by seasonal variabilities, with diatoms and dinoflagellates being the two dominant ingested plankton groups. Compositions of ingested diatoms declined from September to February, followed by an increase from March to June, before declining again in July and August. Peak composition of diatoms was recorded in April, influenced by high proportions of ingested *Amphora* sp.

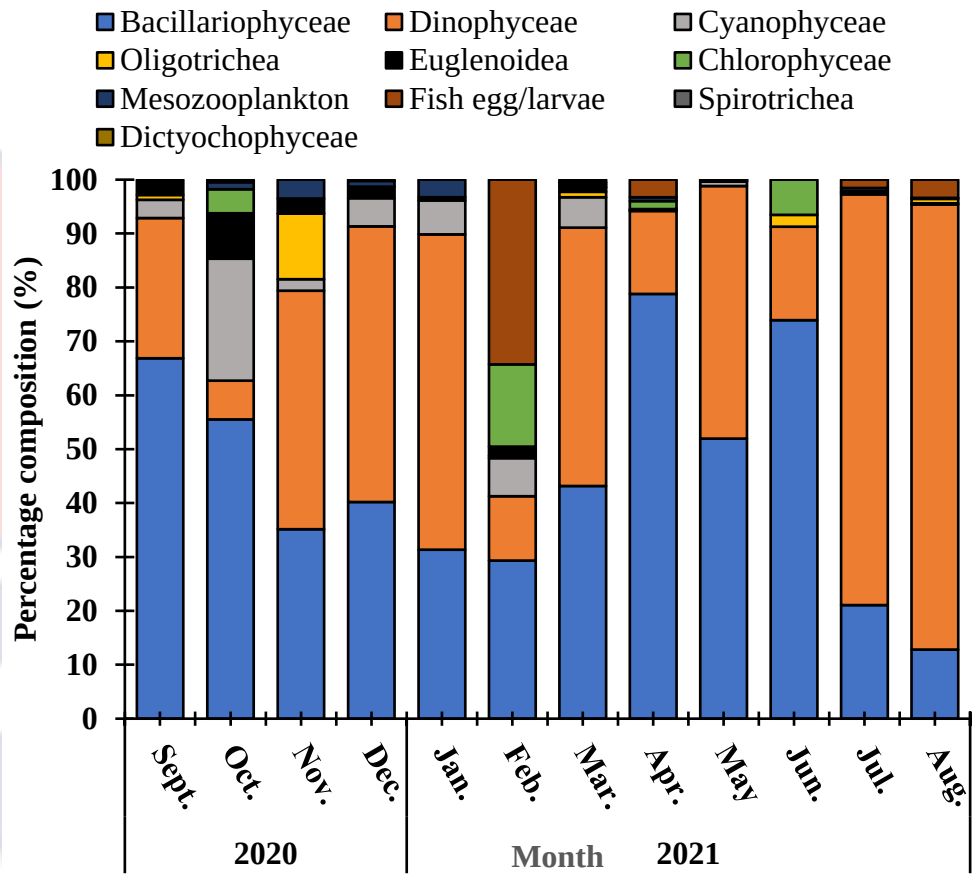

Figure 25: Pattern of variations in compositions of ingested plankton groups by *C. tulipa* in Narkwa Lagoon from September, 2020 to August, 2021.

The pattern of variations in electivity (selectivity) of ingested plankton groups compositions by *C. tulipa* populations in Narkwa Lagoon is illustrated in Figure 26. Just like the *C. tulipa* populations in Benya, similar pattern of all year-round preference for cyanobacteria and microzooplankton ciliates (Oligotrichea) was observed among *C. tulipa* populations in the Narkwa lagoon. Seasonal preference was, however, observed for diatoms, dinoflagellates, mesozooplankton, fish eggs/larvae, and Euglenoidea. Chlorophyceae (represented by *Dunaliella* sp.) were generally rejected.

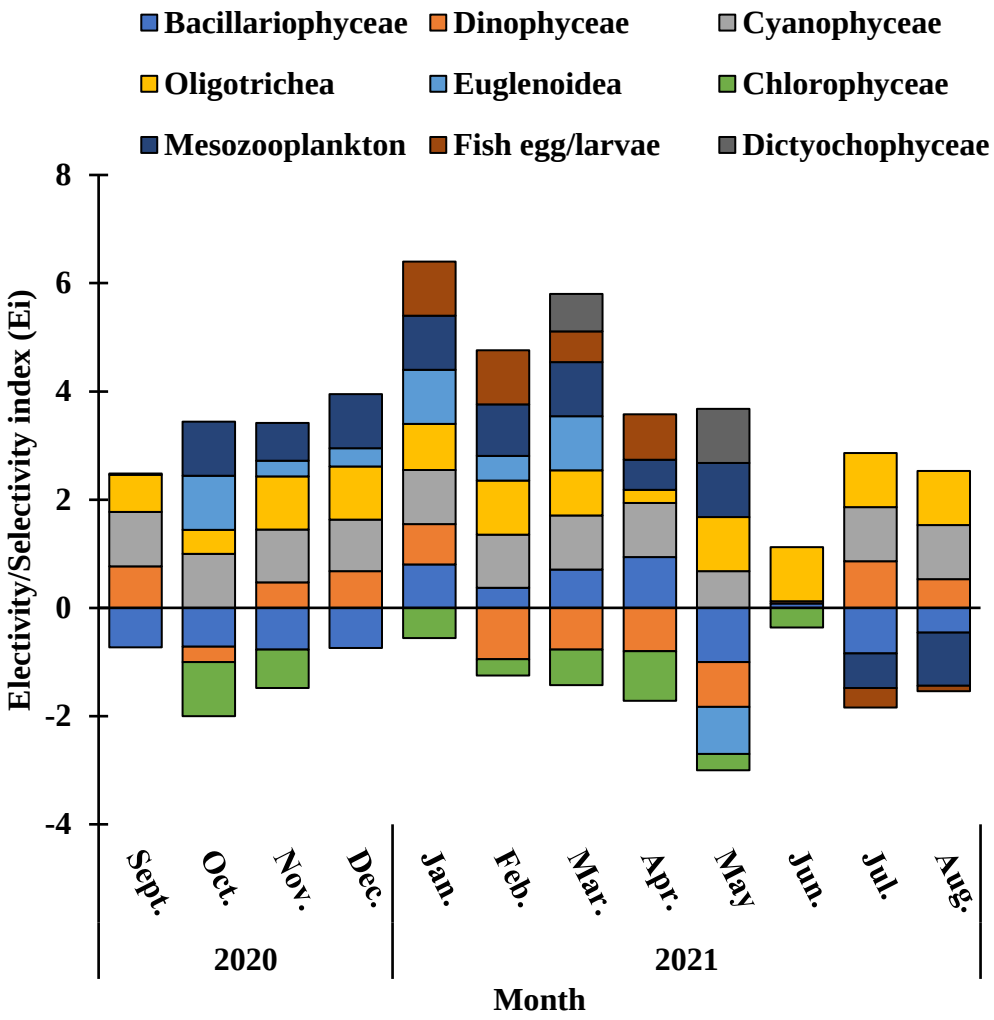

Figure 26: Temporal variations in plankton selectivity of *C. tulipa* in Narkwa Lagoon from September, 2020 to August, 2021.

4.2.4 Temporal distribution of ingested potential toxic phytoplankton by *C. tulipa* in Benya and Narkwa lagoons.

Ten (10) phytoplankton recorded genera were identified to be potentially toxic based on the United Nations Educational Science and Cultural Organization (IOC-UNESCO) Taxonomic Reference List of Toxic Microalgae directory; http/www.marinespecies.org/HABs. Seasonal oscillations and variations in taxonomic compositions of ingested harmful/toxic phytoplankton taxa were observed for the duration of the study. Pattern of temporal variations

in compositions of the ingested potentially toxic phytoplankton in Benya lagoon is shown in Figure 27. *Prorocentrum* spp. (prominently made up of *P. micans* and *P. gracile*) dominated the ingested potentially toxic phytoplankton groups in almost all the sampling months, except in February and March (peak of the dry season). Peak compositions of potentially toxic phytoplankton in the diet of oyster populations in Benya Lagoon was recorded in August, with the genera *Prorocentrum* (*P. micans* and *P. gracile*) and *Dinophysis* (*D. acuminate* and *D. caudata*) together forming ≈50 % of the total ingested plankton. Just like their occurrence in the water, *Prorocentrum* spp. were ingested throughout the period of study, suggesting all year-round ingestion.

Temporal variations in compositions of ingested potentially toxic phytoplankton taxa recorded in Narkwa lagoon is shown in Figure 28. In Narkwa lagoon three (3) major genera, *Prorocentrum*, *Heterocapsa* and *Gymnodinium*, were observed to have been the dominant potential toxic phytoplankton taxa, with the genus *Prorocentrum* occurring throughout the year. Variations in monthly compositions of potential toxic phytoplankton taxa ingested by *C. tulipa* in Narkwa lagoon also showed two seasonal peaks; one in January, with *Ostreopsis* sp. and *Gymnodinium* spp. as the major ingested phytoplankton taxa, and the second peak observed July and August, dominated solely by *Prorocentrum* spp. Figure 29 shows the photographs of some toxic phytoplankton encountered in Benya and Narkwa lagoons.

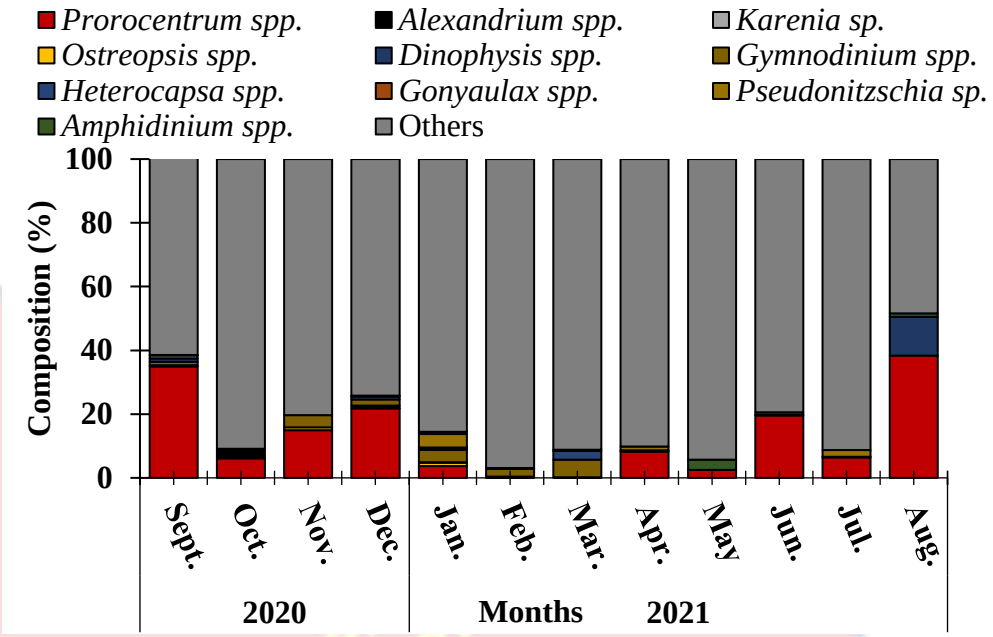

Figure 27: Temporal variations in composition of potential toxic phytoplankton genera ingested by *C. tulipa* in Benya lagoon from September, 2020 to August, 2021.

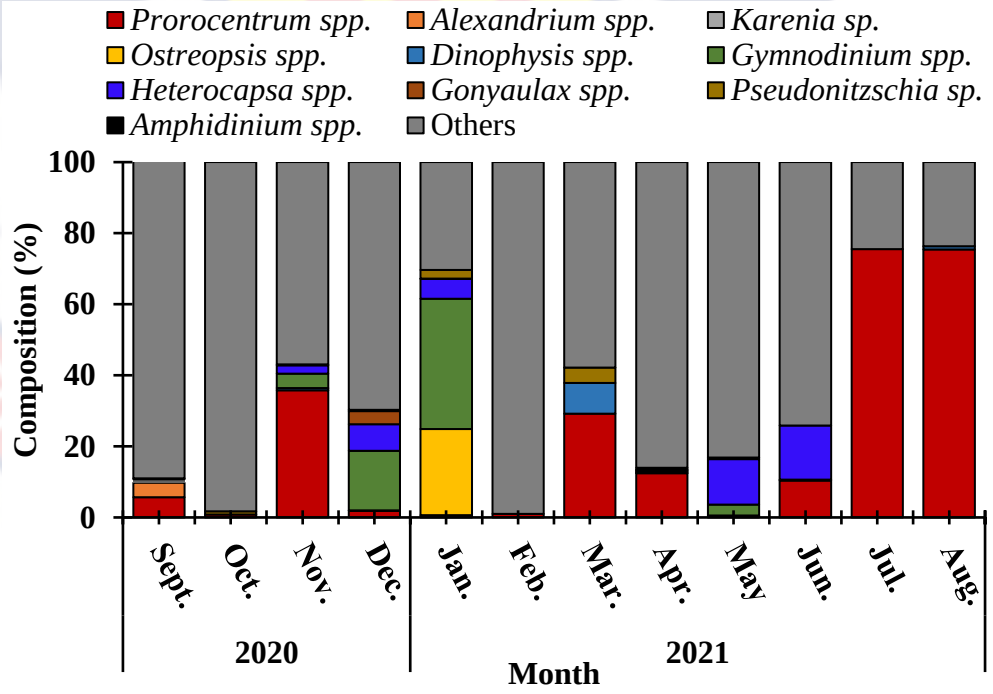

Figure 28: Temporal variations in composition of potential toxic phytoplankton genera ingested by *C. tulipa* in Narkwa lagoon from September, 2020 to August, 2021

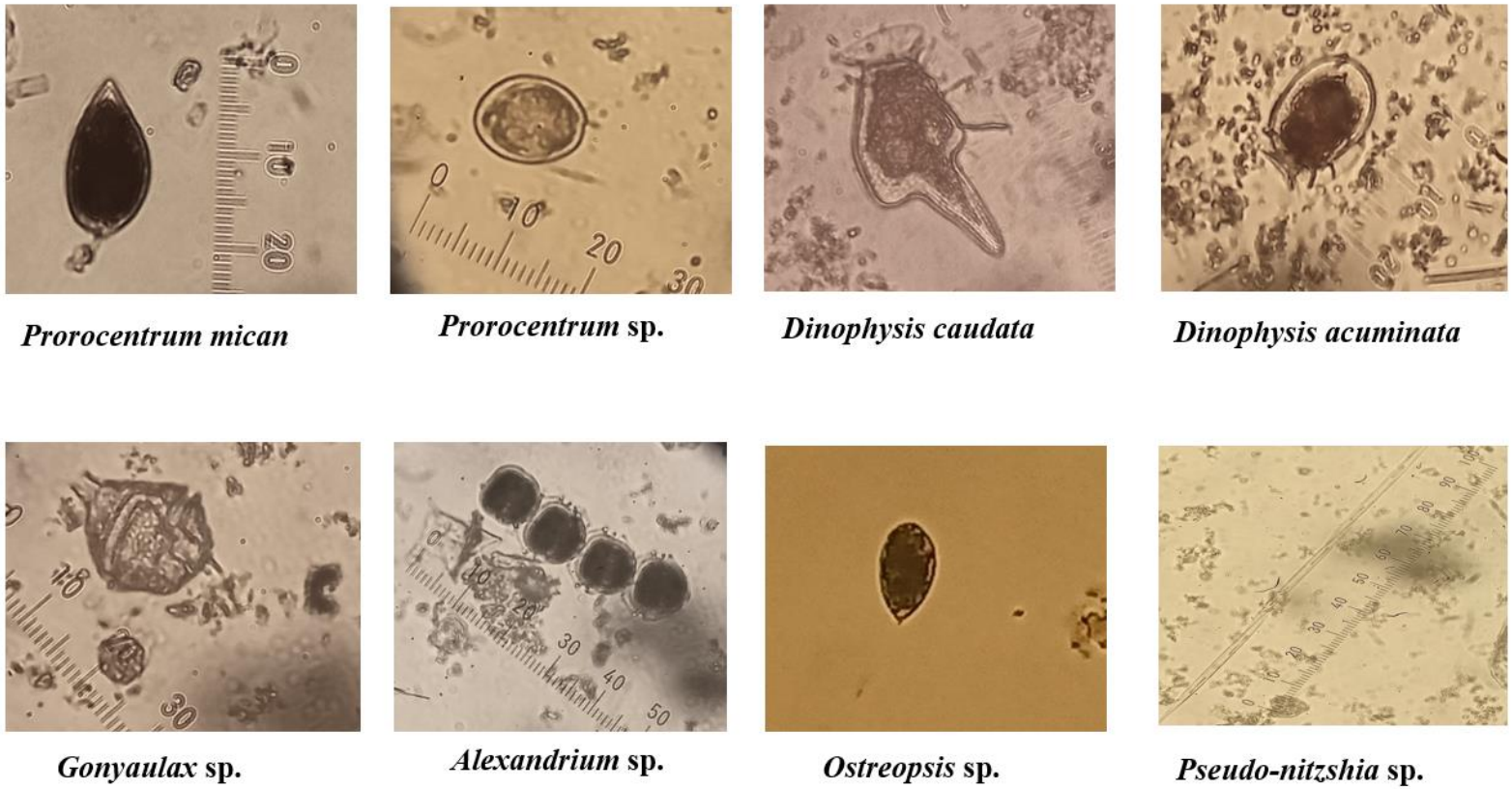

Figure 29: Photographs of some toxic phytoplankton species found in water and diet of *C. tulipa* at Benya and Narkwa lagoons

4.3. Ecophysiology of *C. tulipa*

4.3.1. Morphometry, weight, meat yield and condition index

The size-frequency distribution of *C. tulipa* sampled from Benya and Narkwa lagoons are shown in Figure 30a and 30b, respectively. A total of 548 and 515 specimens of *C. tulipa* were sampled from the Benya and Narkwa, respectively. The shell height (SH) of *C. tulipa* from Benya and Narkwa ranged from 3.0-10.10 and 3.0 -10.20 cm, respectively. The two oyster populations exhibited unimodal size distribution, with a modal shell height class of 5.0 cm - 5.9 cm. A summary comparison of the morphometry of the two *C. tulipa* populations, from two sample t-test analysis is presented in Table 8. *C. tulipa* population in Benya were observed to have a significantly higher (t=7.60; df=1051; p=0.000) shell height (SH) than their counterparts in Narkwa, even though no significant differences (t=0.00; df=1051; p=0.996) were observed for their shell lengths (SL) and shell widths (SW). Shell shape indices analysis indicated that *C. tulipa* population from Narkwa lagoon had a significantly higher (t=-9.42; df=1051; p=0.000) shell elongation and were more compact (globular) (t=-6.70; df=1051; p=0.000) than those from Benya. No significant differences (t=1.40; df=1051; p=0.16) in live weight and shell weight were observed among *C. tulipa* populations from the two lagoons. Wet meat weight was significantly higher (t=-4.61; df=1051; p=0.001) in *C. tulipa* from Narkwa than those in Benya. However, no difference (t=1.23; df=1051; p=0.220) was observed in dry meat weight in both lagoons. *C. tulipa* in Narkwa recorded significantly higher (t=-9.79; df=1051; p=0.001) meat yield and were in significantly (t=-2.77; df=1051; p=0.010) better condition than their counterparts in Benya

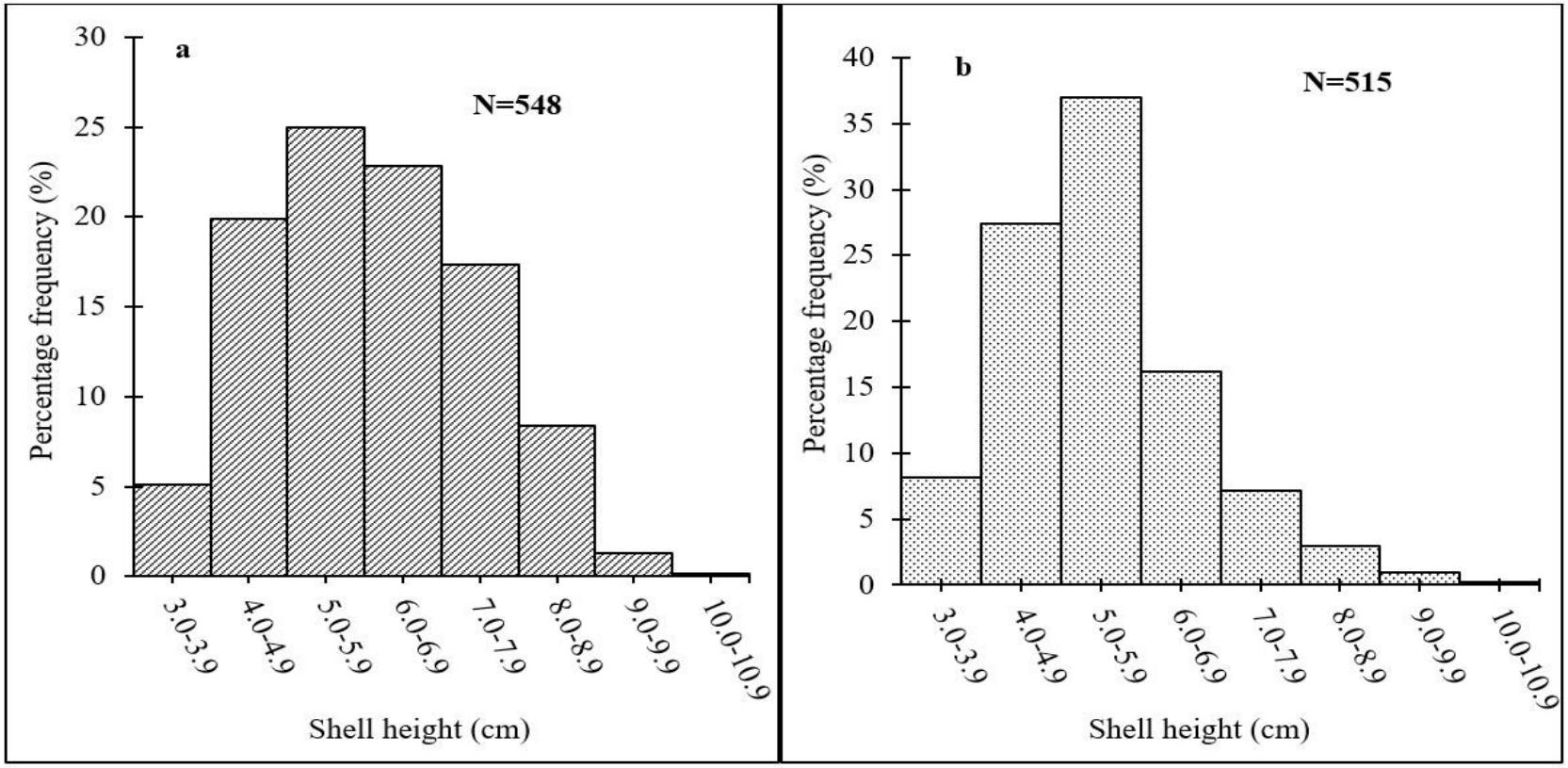

Figure 30: Size-frequency distribution of *C. tulipa* populations in (a) Benya and (b) Narkwa Lagoons.

Table 8: *Student t-test Comparison of Morphometric and Ecophysiological Parameters of C. tulipa Populations from Benya and Narkwa Lagoons*

Oyster ecophysiological parameters	**Benya Mean ± SE (Range) N=548**	**Narkwa Mean ± SE (Range) N=515**	***t-value***	***p-value***
Shell height (SH)(cm)	6.03 ± 0.06 (3.00-10.10)	5.43±0.05 (3.0 -10.20)	7.58	**0.000***
Shell length (SL) (cm)	4.07 ± 0.04 (1.70 - 6.80)	4.08 ± 0.4 (2.10 -7.20)	-0.10	0.918
Shell width (SW) (cm)	2.19 ± 0.02 (0.80 - 5.80)	2.18 ± 0.02 (1.00-7.10)	0.41	0.682
Elongation (SL/SH)	0.69 ± 0.01 (0.38-1.17)	0.76 ± 0.01 (0.40-1.69)	-9.42	**0.000***
Compactness (SW/SH)	0.37± 0.09 (0.15-0.72)	0.41± 0.01 (0.15-1.29)	-6.70	**0.000***
Convexity (SW/SL)	0.57 ± 0.01 (0.25-1.11)	0.55 ± 0.01 (0.22-1.92)	0.06	0.949
Live weight (LW)(g)	21.02 ± 0.48 (3.00 - 69.80)	20.08 ± 0.47 (4.40-71.60)	1.40	0.161
Shell weight (SW)(g)	14.83 ± 0.35 (2.20-50.57)	14.77 ± 0.35 2.40-53.40)	0.12	0.905
Wet meat weight (WMW) (g)	2.87 ± 0.06 (0.30-8.56)	3.34 ± 0.08 (0.60-11.20)	-4.61	**0.000***
Dry meat weight (DMW)(g)	0.52 ± 0.01 (0.04-20.60)	0.50 ± 0.01 (0.02-2.03)	1.23	0.218
Meat yield (MY) (%)	14.46 ± 0.17 3.61-31.25)	17.14 ± 0.21 (8.13-34.04)	-9.79	**0.000***
Condition index (CI) (%)	92.91 ± 1.51 (14.66-233.50)	99.73 ± 1.94 (20.78-611)	-2.77	**0.006***

Pattern of monthly mean condition indices of *C. tulipa* in Benya and Narkwa lagoons is shown in Figure 31. A significant temporal variation in CI of *C. tulipa* in the two lagoons was observed (ANOVA; F=18.56; df=12; p=0.000). In Benya, the seasonal pattern of variations was characterised by a steady increase from August, 2020 to the peak (130.76) in October, 2020 (wet season). This was followed by fluctuations observed between November, 2020 and May, 2021, before a gradual increase was observed from June to August, 2021. The minimum CI (55.91) was recorded in February, 2021 (dry season). Pattern of variations of CI of oyster populations in Narkwa was characterised by two seasonal peaks; the major peak (155.82) was observed in October, 2020 (wet season) and the second minor peak (102.43) in April, 2021, which were interspersed with two seasonal minimums observed in December, 2020 (Dry season) (79.38) and in July, 2021 (wet season) (68.95). Pattern of variations in Meat yield of *C. tulipa* in Benya and Narkwa lagoons is presented in Figure 32. Temporal (seasonal) variations in MY generally followed similar patterns as variations in the CI. Seasonal pattern of variations in MY of *C. tulipa* in Benya was characterised by a steady increase from August, 2020 to the peak (18.85 %) in November, 2020 (minor wet season), followed by decline from December, 2020 to the first seasonal minimum (11.43%) in February, 2021 (dry season). Meat yield was observed to have increased steadily again from March to May, 2021, followed by a decrease to the second seasonal minimum (11.04%) in June, 2021 (wet season). A sharp increase (16.21%) was then observed in July, 2021 (minor peak). Seasonal variations in MY of oysters in Narkwa were characterised by a peak (27.86%) in October, 2020 (minor wet season),

followed by constant monthly fluctuations for the rest of the year. Minimum MY (14.72%) was recorded in July, 2021.

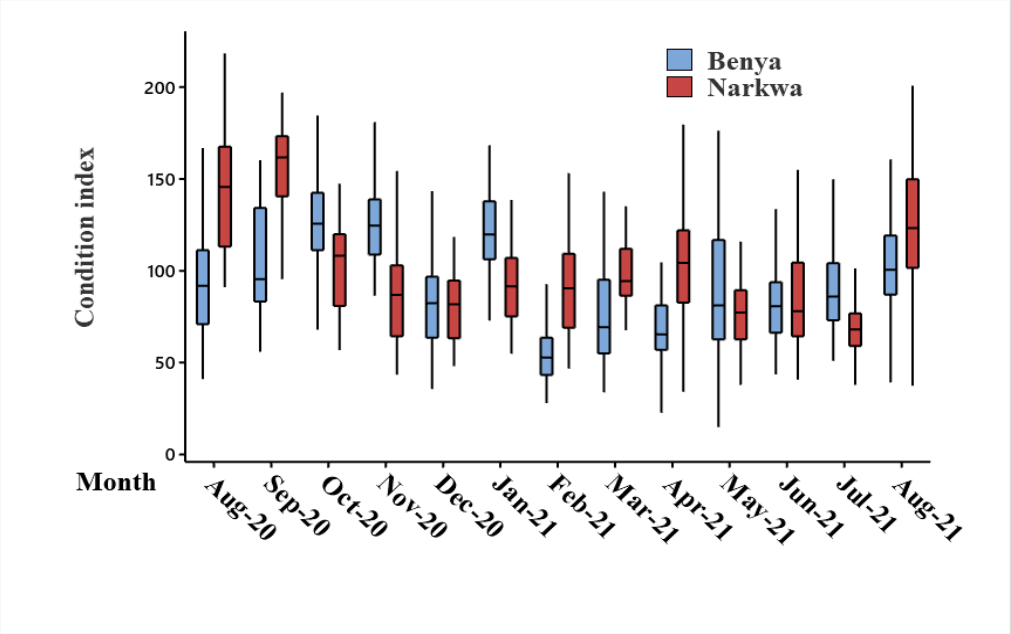

Figure 31: Temporal pattern of variations in condition index of *C. tulipa* in Benya and Narkwa lagoon.

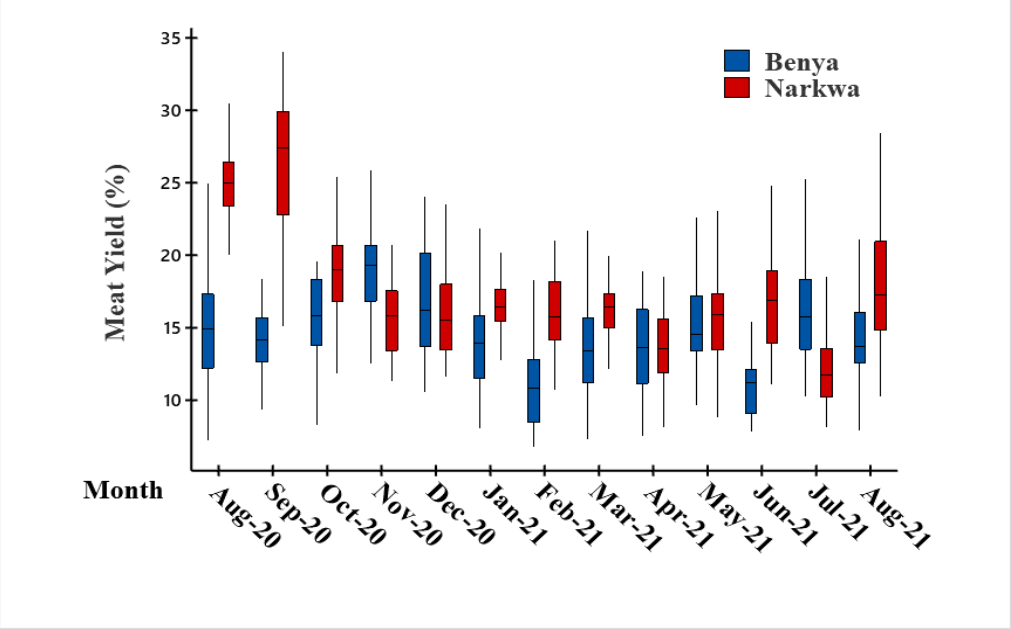

Figure 32: Pattern of variations in monthly meat yield of *C. tulipa* in Benya and Narkwa lagoon.

4.3.2. Influence of hydrographic parameters and oyster condition index and meat yield

A multiple linear regression analysis of *C. tulipa* condition index and hydrographic factors and biotic factors (plankton groups abundance) (physicochemical parameters) revealed that dinoflagellate and cyanobacteria abundance (biotic factors), in addition to pH, phosphate and chlorophyll a concentration (abiotic factors), in the water bodies are significant ($p < 0.05$) predictors of condition index of *C. tulipa* (Table 9). Also, a multiple linear regression analysis of the oyster meat yield versus hydrographic factors and biotic factors indicated that cyanobacteria abundance, pH, turbidity and phosphate were observed to be significant ($p< 0.05$) predictors of meat yield in *C. tulipa* (Table 10). The measured biotic and abiotic factors accounted for 38.33 % and 40.50 % of variation in the data for oyster condition index and meat yield, respectively.

4.4 Laboratory Rearing of *C. tulipa* Larvae on Local Microalgae Isolates

4.4.1. Growth performance characteristics of microalgae isolates

Three different species of microalgae were successfully isolated from waters sampled from the coastal waters of Ghana, 1 km off the coast of Elmina. These species were *Nannochloropsis* sp., *Pseudanabaena* sp. and *Rhodomonas* sp. (Figure 33). Their growth performances are shown in Figure 34. The growth of the microalgae generally followed a similar pattern, even though there were marked differences in the daily increase in cell densities, peak cell densities and days of peaking. Whist *Nannochloropsis* sp. peaked at 230500 cells/ml on the fifth day.

. Table 9: *Multiple Linear Regression Model of C. tulipa Condition Index (Response) and the Measured Physicochemical and Biotic Factors (Predictors).*

Term	Coef	SE Coef	95% CI	t-value	p-value	VIF
Constant	4.833	0.772	(3.298, 6.369)	6.26	0.000	
Biotic factors						
Dinophyceae	-0.00254	0.00118	(-0.00489, -0.00019)	-2.15	**0.034***	1.97
Cyanophyceae	-0.0280	0.0118	(-0.0516, -0.0045)	-2.37	**0.020***	1.51
Chlorophyceae	-0.00039	0.00909	(-0.01847, 0.01770)	-0.04	0.966	1.60
Copepoda	-0.0180	0.0123	(-0.0425, 0.0066)	-1.46	0.149	2.25
Dictyochophyceae	0.107	0.118	(-0.127, 0.342)	0.91	0.366	2.59
Euglenoidea	-0.0076	0.0101	(-0.0278, 0.0126)	-0.75	0.457	1.39
Fish egg/larvae	-0.0150	0.0280	(-0.0707, 0.0406)	-0.54	0.592	1.99
Oligotrichea	0.00047	0.00399	(-0.00747, 0.00841)	0.12	0.907	1.27

Abiotic factors						
Temperature	-0.0382	0.0201	(-0.0782, 0.0018)	-1.90	0.061	1.78
Salinity	0.00083	0.00355	(-0.00623, 0.00790)	0.23	0.815	2.19
Dissolved Oxygen	-0.0062	0.0146	(-0.0353, 0.0228)	-0.43	0.671	2.56
pH	0.1838	0.0772	(0.0301, 0.3374)	2.38	**0.020***	2.22
Turbidity	-0.00560	0.00394	(-0.01345, 0.00225)	-1.42	0.160	2.15
Phosphate	-0.673	0.208	(-1.087, -0.258)	-3.23	**0.002***	2.38
Nitrate	0.00445	0.00936	(-0.01417, 0.02308)	0.48	0.635	2.37
Chl. *a*	-0.02004	0.00926	(-0.03848, -0.00161)	-2.16	**0.033***	1.51

*Significant at ($p<0.05$), R^2=38.33%, DF=16, F=3.07, p=0.000

Table 10*: Multiple Linear Regression Model of Oyster Meat Yield (Response) and the Measured hydrographic and Biotic factors (Predictors).*

Term	Coef	SE Coef	95% CI	t-value	p-value	VIF
Constant	-0.2728	0.0750	(-0.4221, -0.1235)	-3.64	0.000	
Biotic factors						
Dinophyceae	-0.000318	0.000115	(-0.000546, -0.000090)	-2.77	**0.007***	1.97
Cyanophyceae	-0.00053	0.00115	(-0.00282, 0.00176)	-0.46	0.646	1.51
Chlorophyceae	0.001281	0.000883	(-0.000477, 0.003039)	1.45	0.151	1.60
Copepoda	0.00097	0.00120	(-0.00141, 0.00336)	0.81	0.419	2.25
Dictyochophyceae	-0.0149	0.0115	(-0.0377, 0.0080)	-1.30	0.199	2.59
Euglenoidea	-0.001347	0.000986	(-0.003309, 0.000614)	-1.37	0.175	1.39
Fish egg/larvae	-0.00478	0.00272	(-0.01019, 0.00063)	-1.76	0.083	1.99
Oligotrichea	0.000465	0.000388	(-0.000307, 0.001237)	1.20	0.234	1.27

Abiotic factors						
Temperature	-0.00292	0.00195	(-0.00681, 0.00097)	-1.50	0.139	1.78
Salinity	-0.000616	0.000345	(-0.001303, 0.000071)	-1.79	0.078	2.19
Dissolved Oxygen	0.00268	0.00142	(-0.00015, 0.00550)	1.88	0.063	2.56
pH	0.02117	0.00751	(0.00623, 0.03611)	2.82	**0.006***	2.22
Turbidity	-0.000998	0.000383	(-0.001761, -0.000235)	-2.60	**0.011***	2.15
Phosphate	-0.0605	0.0203	(-0.1008, -0.0202)	-2.99	**0.004***	2.38
Nitrate	-0.000209	0.000910	(-0.002020, 0.001602)	-0.23	0.819	2.37
Chl. *a*	-0.000372	0.000901	(-0.002164, 0.001420)	-0.41	0.681	1.51

*Significant at ($p<0.05$), R^2=40.50%, DF=16, F=3.36, p=0.000

after inoculation, *Pseudanabaena* sp and *Rhodomonas* sp. peaked at 240151.26 cells/ml and 150200.5 cells/ml, respectively on the sixth day.

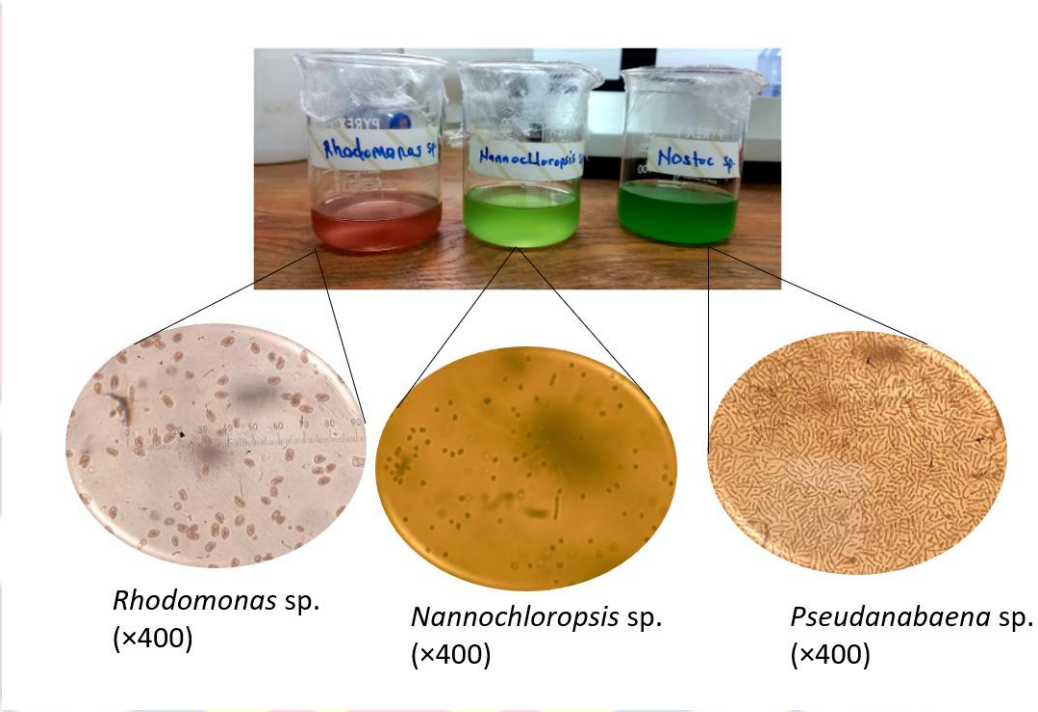

Figure 33: Photographs of the three local microalgal isolates

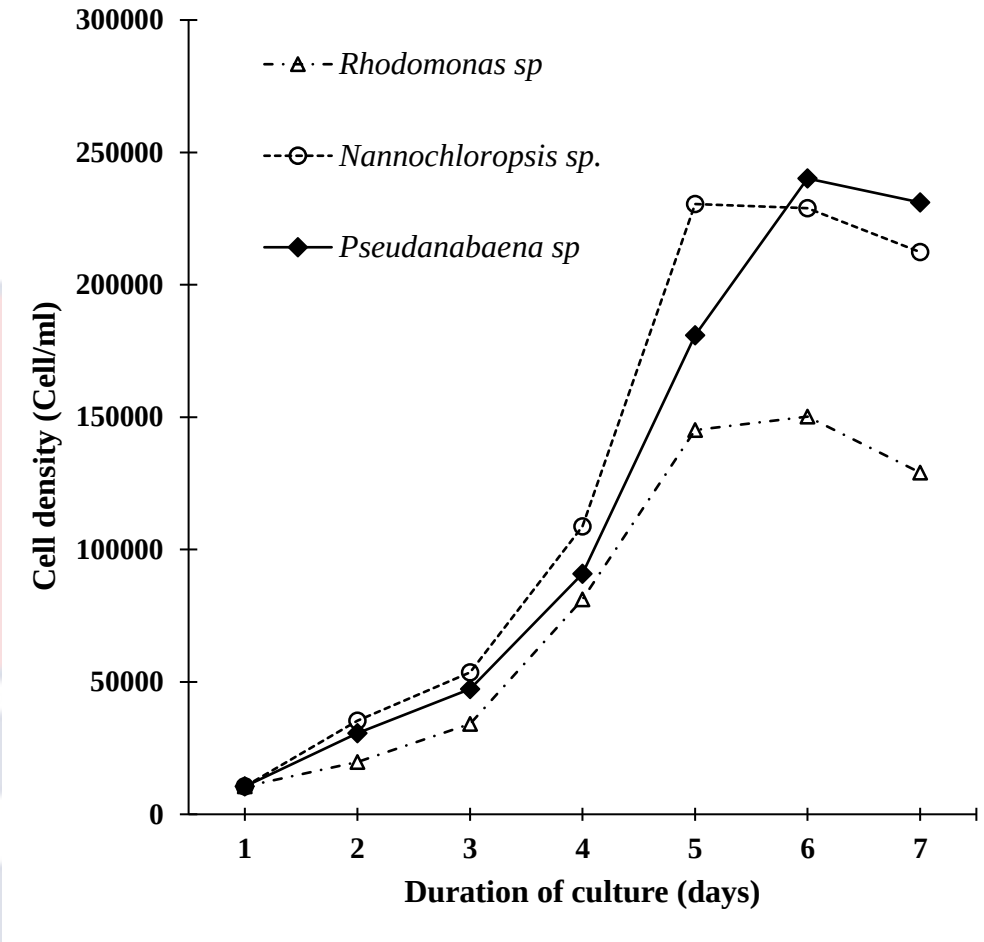

Figure 34: Growth patterns of the local microalgae isolates cultured in the laboratory.

Table 11 shows the equivalent spherical diameter (ESD), biovolume (BV), carbon weight (CW) and carbon energy content (CE) estimated for each of the algae. These estimates were recorded during the stationary phase of the growth of the microalgae. *Rhodomonas* sp. recorded the highest value of BV, CW and CE, followed by the *Pseudanabaena* sp., whilst *Nannochloropsis* sp. recorded the lowest value.

Table 11: *Biovolume, Carbon and Carbon Energy Content of the Microalgae Isolated from Water Samples off the Coast of Elmina, Ghana*

Microalgae	Equivalent Spherical Diameter (ESD) (µm)	Biovolume (µm^3)	Carbon weight (pgCCell^{-1})	Carbon energy (J)
Rhodomonas sp.	7.6 ± 0.13	238.9 ± 24.76	36.95 ± 3.60	1.7×10^{-6} J
Nannochloropsis sp.	2.5 ± 0.12	8.182 ± 0.15	1.55 ± 0.34	7.13×10^{-8} J
Pseudanabaena sp.	2.0 ± 0.34 (×3.5)	42.42 ± 1.61	7.29 ± 0.04	1.05×10^{-7}J

4.4.2 In-vitro fertilisation and growth performance of *C. tulipa* larvae

Over 6 million eggs pooled from five (5) adult female oysters (measuring 4.5 to 8.6 cm Shell height) were inseminated with sperms pooled from 5 males (measuring 3.00-5.1 cm) at the rate of 5 eggs to 1ml sperm suspension (2ml sperm in 200ml filtered seawater). An estimated 89.19 % fertilisation success was recorded. The fertilized egg (mean diameter 63.19 ± 1.83 µm, n = 45) went through various stages of development from 2-cell stage (30 minutes post-fertilisation), through to the trochophore stage before hatching into D-veligar (24 hours post-fertilisation). An estimated 90.54 % of D-larvae yield was recorded. The instantaneous growth pattern and specific growth rate (%/day) of mangrove oyster larvae (48 hours post-fertilisation, mean shell height = 65.12 ± 3.15 µm, n=59), reared under five different diet (microalgae) treatments for 21 days, are illustrated in Figures 35 and 36, respectively. Generally, the laboratory reared *C. tulipa* larvae exhibited significantly marked differences in specific growth rate (SGR) under the different diet treatments (ANOVA; F= 47.63, df=4, p = 0.000). A Tukey's post-hoc analysis of specific growth rate showed that, oyster larvae fed on mixed species of the microalgae isolates recorded a significantly better growth performance (Higher mean SGR and final mean shell height). There was, however, no significant difference in growth performance between oyster larvae that were fed on mixed diet and the natural water diet ($p > 0.05$). Oyster larvae fed on *Pseudanabaena* sp. exhibited the poorest growth performance, with an observed SGR and mean final SH of 5.61 ± 0.26 %and 199.2 ± 2.78, respectively. However, no significant difference ($p > 0.05$) in final mean SH and SGR observed in larvae that were fed on *Nannochloropsis* sp. and *Pseudanabaena* sp. The result shows that diet

treatment and the days of culture significantly accounted for the observed differences in larval growth (F=44.56; df=10; p=0.001).

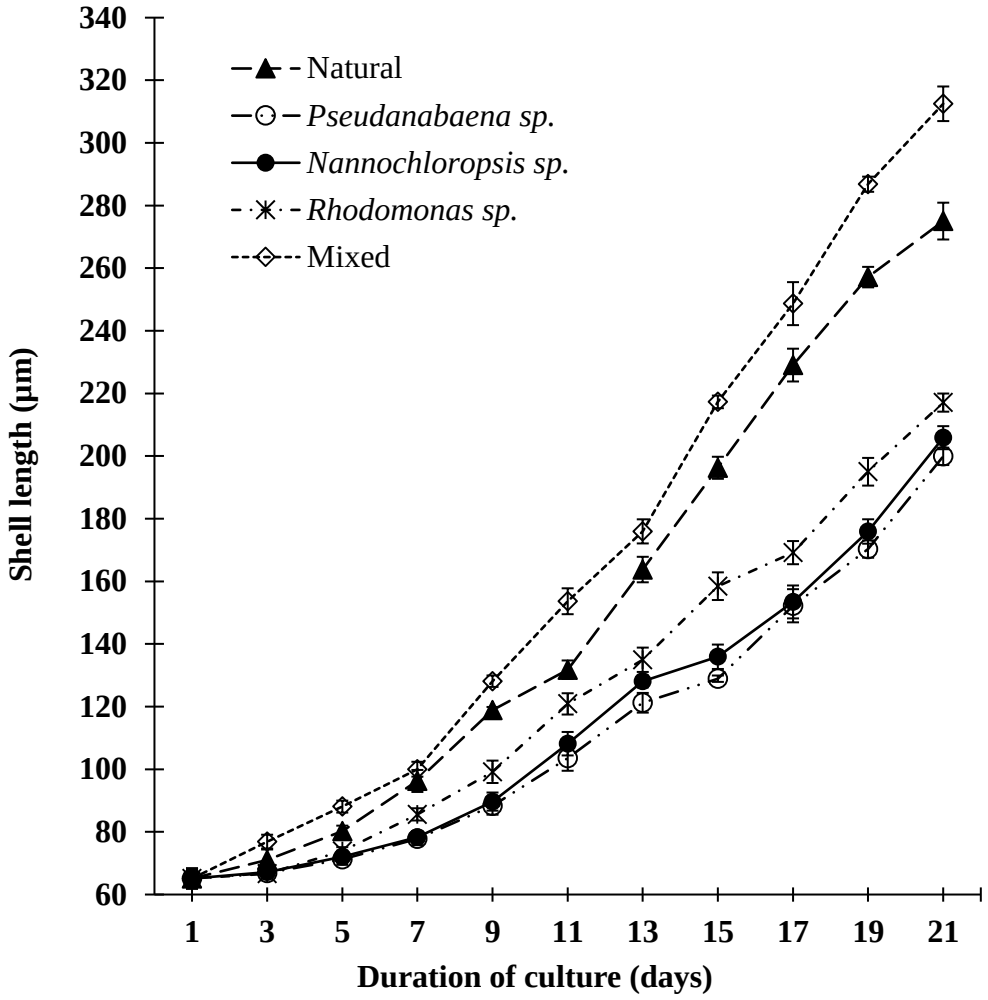

Figure 35: Growth of laboratory reared *C. tulipa* larvae fed on different diets (local microalgae isolates) over 21 days (Error bars indicate standard error of means).

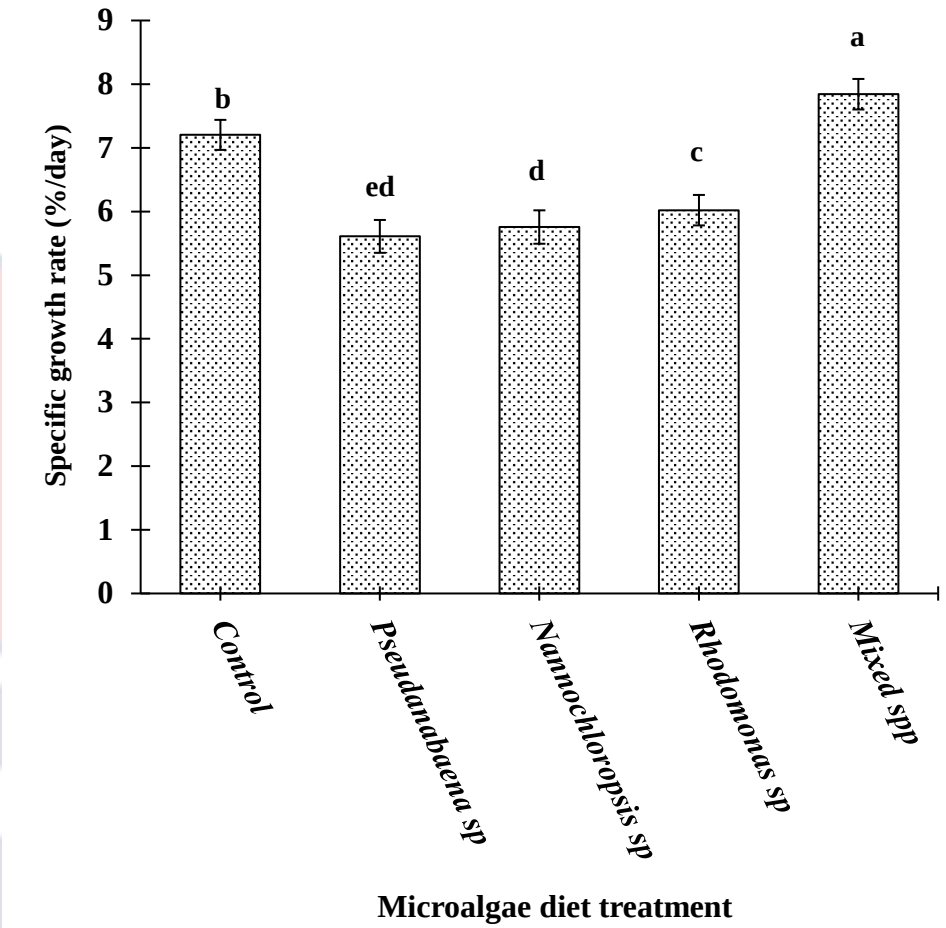

Figure 36: Mean daily growth rate of laboratory reared *C. tulipa* larvae fed on different diet (microalgae) over 21 days. Means that do not share same letter are significantly different. (Error bars indicate standard error of means)

4.3.3. Survival of laboratory reared *C. tulipa* larvae fed on different local microalgae isolates

The instantaneous survival pattern and the final mean survival rate (%) of *C. tulipa* larvae reared on different local microalgae diet treatments are illustrated in Figures 37 and 38, respectively. Generally, survival of the oyster larvae followed a similar pattern. There was a sharp decline in larval survival for the first three days of rearing in all treatments, followed by a relatively steady decline in survival till the end of the rearing period. There were

significant differences in the mean survival rate of *C. tulipa* larvae fed on different diet treatment (ANOVA; F=181.39; df=4; p=0.000). Larvae fed on mixed species of the three microalgae diet showed a significant higher final mean survival rate (48.73 ± 0.95 %) (p<0.05). This was followed by the larvae fed on *Rhodomonas* sp. (43.72 ± 1.03 %). There was no significant difference between the final mean survival rate of the larvae fed on *Pseudanabaen*a sp. and *Nannochloropsis* sp. (p>0.05). Interestingly, larvae reared under the natural diet treatment recorded significantly lowest final mean survival rate (30.95 ± 1.38 %) (F=) than the survival rate of any other treatments.

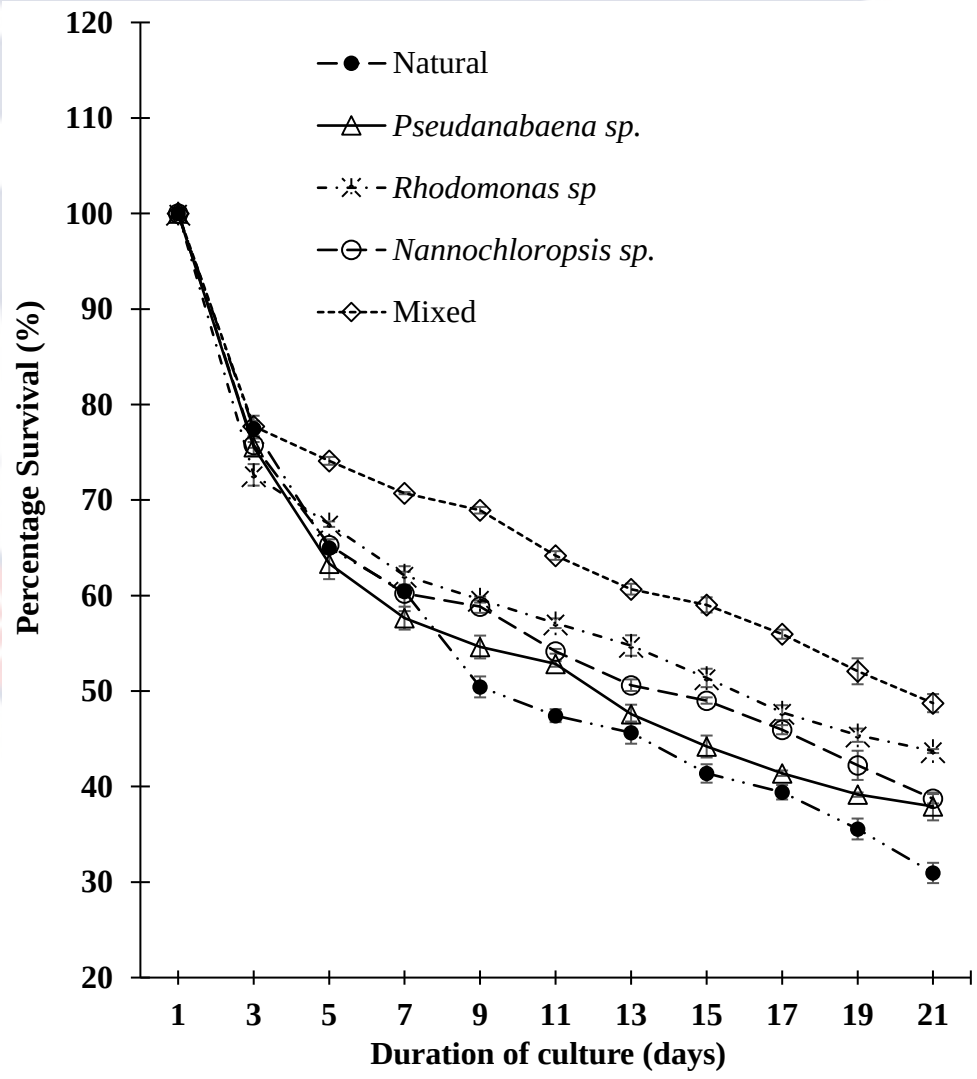

Figure 37: Instantaneous mean survival rates of laboratory reared *C. tulipa*

larvae fed on different microalgae diets over 21 days. (vertical bars indicate standard error of means).

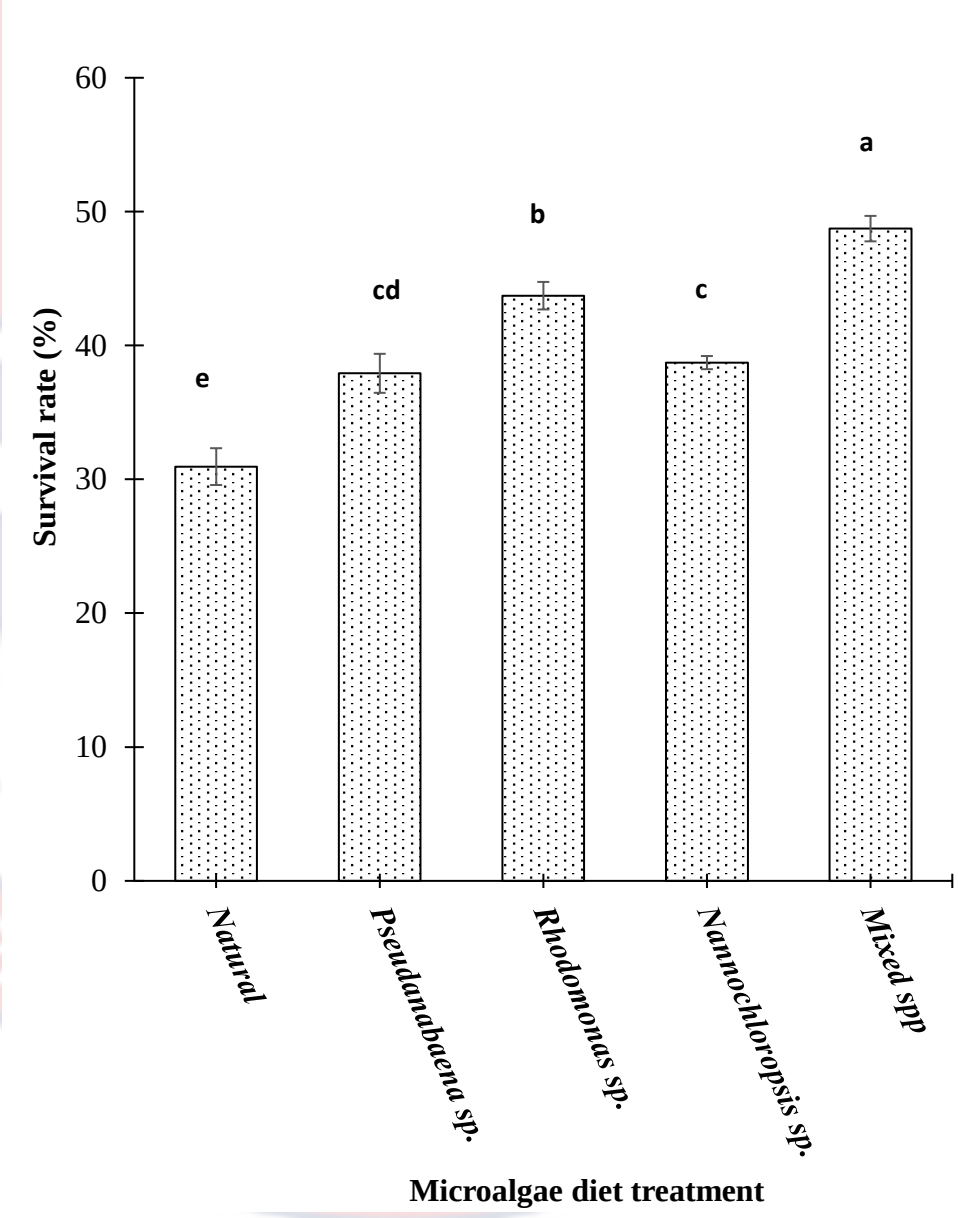

Figure 38: Final mean survival rates of *C. tulipa* larvae fed on different diets (microalgae) for 21 days. (Means that do not share a letter are significantly different $p<0.05$).

CHAPTER FIVE

DISCUSSION

The interpretation of the main findings in this study is dealt with in this chapter.

5.1. Physicochemical Parameters

The physicochemical characteristics of natural water systems, e.g. temperature, oxidation oxygen, nitrate, phosphorus and so on, give important information about water quality. The functions and biodiversity of aquatic organisms can be affected by variations in these water quality parameters (Dzakpasu, 2019; Mustapha, 2008). They are therefore able to be used as an environmental indicator on the health of aquatic ecosystems (Okyere, 2015). The hydrographic parameters measured in Benya and Narkwa lagoons, to some extent, give indication of the ecological health of these water bodies. Like other coastal ecosystems, the measured physicochemical parameters in Benya and Narkwa lagoons in this study, exhibited marked spatio-temporal variations. The variability in the physicochemical parameters of coastal lagoons is said to result from interactions between several biotic and abiotic factors, which have important consequences for understanding lagoon responses to increased anthropogenic pressures and changing climate (Srichandan et al., 2015). In coastal aquatic ecosystems, salinity and temperature variations are often assumed to be the main factors that influence spatial and temporal patterns of biological communities (Herrera-Silveira, 1996), and the interactions between these water properties are said to explain, generally, most of the seasonal changes in one lagoon and differences between lagoons (Comin, Menendez & Fores, 1987; Herrera-Silveira, 1996; Nixon, 1982). Other environmental

variables, such as, oxygen, pH, or turbidity also play critical role in the functioning of coastal lagoons (Pérez-Ruzafa et al., 2019). Spatial differences and seasonal variations were observed within key parameters considered in this study, namely temperature, salinity, DO, PH, turbidity, nitrates, phosphates, chlorophyll a and precipitation.

Water surface temperature generally controls the living organisms and their activities, influence many of the chemical responses that occur in natural water systems, and significantly affects the solubility of gasses in water (Bozorg-Haddad et al., 2021). Temperature values between 26.5 ℃ and 31.2 ℃ recorded in this study (Figure 7) fall within the temperature range that has been reported to characteristise shallow tropical coastal water bodies (Abowei, 2010; Chagas & Suzuki, 2005; Davies, Ugwumba, Abolude, 2008; Herrera-Silveira, 1996; Nayak & Behera, 2004; Dzakpasu & Yankson, 2015; Dzakpasu, 2019; Okyere, 2015; Tay, Asmah & Biney, 2009; Yap, Rahim Ismail, Azrina, Ismail & Tan, 2006). The temperature of natural water systems is said to be influenced by many factors including ambient temperature (Bozorg-Haddad, Delpasanda & Loáiciga, 2021). High temperatures observed in the dry month and low temperatures in the wet month in the two lagoons studied are, thus, a reflection of the climate conditions in southern Ghana (Dzakpasu, 2019). Seasonal variabilities in water temperature have also been reported for other tropical lagoons elsewhere; Açu Lagoon in Brazil (Chagas & Suzuki, 2005), the Chilika Lagoon in India (Nayak & Behera, 2004), and the Lagos Lagoon in Nigeria (Akhiromen et al., 2022).

This study found no significant differences in the mean annual temperatures in the two study water bodies. This could be attributed to

proximity within the same climatic zone. Temperature ranges observed in the present study closely compare with that recorded in earlier studies in the same water bodies (Asare et al., 2019; Chuku 2019; Obodai, 1997; Sowah, 2019), and other estuarine water bodies in Ghana (Dzakpasu et al., 2012; Dzakpasu, 2019; Osei, 2019).

Salinity is reported to be the main factor influencing the distribution of organisms in tropical coastal lagoons. (Barletta et al., 2005), and it is said to be closely linked to climatic conditions (Dzakpasu, 2019; Waller, Burchett & Dando, 1996). The seasonal variations in salinity in both lagoons could be linked to seasonal variations in local climatic conditions; decreasing salinity through dilution from influx of freshwater from river discharge and run-offs in the wet season, and increasing salinity as a result of evaporation and tidal incursions in the dry season. The relatively narrow salinity range (typical of marine ecosystems) that characterised the Benya lagoon, could be attributed to the constant connection with the ocean due to the artificial modification of the opening of the lagoon, and the apparent absence of visible freshwater connection. Discharge of hypersaline water from a number of saltpans located along the banks of the Benya lagoon could be contributing to its high salinity, even in the peak of wet (rainy) season. For the Narkwa lagoon, even though the connection to the ocean remained opened throughout the study period, copious of amount freshwater dilution from the Okye River during the wet season, and tidal incursion in the dry months on the other hand could have accounted for drastic seasonal fluctuations and wide salinity range. Seasonal variabilities in salinity in other tropical lagoons and estuarine environments elsewhere, linked with seasonal freshwater influx from river discharges and run-offs, have been

reported for Chilika lagoon in India (Srichandan et al., 2014), and the Bach Dang estuary (Vietnam) (Rochelle-Newall et al. 2011). Salinity ranges recorded in the present study in the Benya and the Narkwa lagoons were closely similar to those recorded in earlier studies for the same systems (Asare et al., 2019; Chuku 2019; Obodai, 1997; Sowah, 2019), other coastal water bodies in Ghana (Dzakpasu, 2012; Osei, 2019), and other tropical lagoons elsewhere (Chagas & Suzuki, 2005; Nayak & Behera, 2004).

Dissolved oxygen concentration is among the key chemical components that support the growth and survival of living aquatic organisms (Osei, 2019). DO is a significant factor affecting the aquatic system trophodynamics and water quality. It is removed or mixed into water by different physical, chemical or biological reactions (Prasad, Srinivasu, Varma, Raman & Ray, 2014). A low dissolved oxygen content in the water is an indicator of possible contamination and plays a major role in determining water quality, pollution control as well as treatment processes (Bozorg-Haddad et al., 2021). Mean monthly DO levels recorded in Narkwa were significantly higher than that recorded in Benya throughout the study period (Figure 9). The differences in DO levels in the two lagoons could be due to differences in the extent of physical (mixing) and biological processes (respiration or photosynthesis) in the lagoons. Occurrence of lower dissolved oxygen (hypoxic) conditions in coastal habitats have been directly linked to increase in population and industrial activities which generates industrial and municipal wastes, leading to increased terrestrial fluxes of nutrients into these ecosystems (Diaz & Rosenberg, 1995; Steckbauer et al., 2011 as cited in Hsieh et al., 2021). The low DO levels (with intermittent hypoxic conditions) recorded in Benya lagoon could therefore be due to

microbial decomposition of loads of organic waste emanating from pig-sties, refuse dumps, human effluents, fish processing activities that are more prevalent at the banks of the lagoon. Microbial respiration and decomposition of organic biomass have been reported to lead to increasing rates of oxygen consumption in aquatic ecosystems (Hsieh, 2021; Kemp et al., 2005). This assertion collaborates that made by Adesalu and Kunrunmi (2012) in the Tomaro and Ajegunle creeks and Akhiromen et al., (2022) in the Lagos lagoon, who suggested that the low concentration of oxygen found at these study sites can be attributed to the exposure of the water bodies to wastewater containing oxygen demanding substances and household sewage. The shady nature (brought about by the thick mangrove cover at the banks of the lagoon) and the comparatively small surface area ($0.013km^2$) of Benya possibly limit air-water interaction, which could possibly further contribute to low dissolved oxygen in the system. Increasing DO levels in Benya were observed to have increased with precipitations, indicating that rainfall- induced mixing could have accounted for this increasing observation. However, contrasting scenario was made in the Narkwa, where increasing DO levels were observed in the dry months, and low turbidity and high irradiance during the dry months may have enhanced primary production which could have accounted for the increasing DO levels in the dry months. The DO ranges recorded in Benya and Narkwa, respectively, in the present study were similar to that recorded in earlier studies (Asare et al., 2019; Chuku 2019; Obodai, 1997; Sowah, 2019) for the same water bodies. Barring the intermittent hypoxia situations observed in Benya lagoon, the annual mean DO in Benya and Narkwa lagoons falls within DO levels suitable for the survival of aquatic organisms (Behar, 1997; Dzakpasu, 2019).

The variabilities in pH values recorded in Benya and Narkwa lagoons reflected little or no influence of season, except the relatively stable (low variations) pH regime observed from December to February (dry season) (Figure 10). According to Srichandan et al., (2015), processes such as carbon dioxide absorption by phytoplankton and macrophytes, tidal mixing and net dilution of seawater by freshwater, low primary productivity, and decomposition of organic matter could be associated with seasonal variations in pH values in aquatic ecosystem. The pH range recorded in the current study for Benya and Narkwa varied from slightly acidic to slightly alkaline, and falls within the accepted range for estuarine and other aquatic ecosystems. For example, Wood (1967) indicated a pH range for estuarine systems. Nayak and Behera (2004) observed a pH range in a tropical lagoon in India. Clearly, the pH values recorded in Benya and Narkwa were within the required range for coastal ecosystem processes and for sustenance of aquatic life. The pH values were also similar to that reported by earlier studies in the same water bodies (Chuku, 2019; Sowah, 2019), other estuarine systems in Ghana (Okyere, 2015; Dzakpasu, 2019), and tropical coastal lagoons elsewhere (Nayak & Behera, 2004; Tarafdar et al., 2021).

Phytoplankton and algae growth, resuspension of sediment from the bottom and city waste water are all factors that have an impact the turbidity of aquatic systems (American Public Health Association, 1999; Dzakpasu, 2019). High turbidity is said to impede light penetration into water systems, which in turn reduces primary production. Turbidity pattern observed in the two water bodies in the present study (Figure 11) somewhat reflected the climatic condition (particularly the rainfall pattern) for the study vicinity, suggesting that

colloidal materials from freshwater influx and runoffs from rains played a role in the turbidity of the two water bodies. The high and wider fluctuations in turbidity values recorded in the Narkwa lagoon may be as a result of loads of silt and suspended particles transported by the influx of freshwater from the Okye River. Unlike the Benya lagoon that has mangrove vegetation cover along its banks (expect the areas with saltpans), the exposed banks of the Narkwa are prone to erosion caused by run-offs during rains. This could also increase the suspended particle loads in the lagoon, thus, contributing to high turbidity. The turbidity ranges observed in Benya and Narkwa lagoons in this present study were relatively lower than what have been reported earlier for the same systems (Chuku, 2019; Sowah, 2019) and for other coastal water bodies (Dzakpasu, 2012; Okyere, 2015; Osei, 2019). The differences in turbidity levels could be attributed to volume of freshwater influx and sediment transport (influenced by anthropogenic activities upstream).

The balance of nitrogen and phosphorus is generally influence the growth of phytoplankton or primary productive biomass (Redfield, 1934 as cited in Asiedu, 2020). This is largely due to the fact that nitrogen is an important component of amino acids and chlorophyll which is essential for plant photosynthesis. Phosphorus, on the other hand, is critical in converting other nutrients into usable building blocks for phytoplankton growth (Asiedu, 2020). Nitrogen and phosphorus in aquatic systems take their sources from organic (animal/human) waste, chemical fertilizers, and industrial sources, and unlike phosphorus, nitrogen can also be added to water via atmospheric deposition and biological nitrogen fixation (Galloway et al., 2003; Whitall, Mason & Pait, 2012). Nitrates and phosphates (which are the useful forms of nitrogen and

phosphorus readily available for plant and phytoplankton use) occur naturally in low concentrations in aquatic ecosystems (Nartey, Edor, Doamekpor & Bobobee, 2012). Excess of these nutrients can result in explosive growth of phytoplankton (algal blooms), changes in phytoplankton community composition and oxygen deficiency (hypoxia/anoxia) in coastal water bodies (Whitall et al., 2012). In the current study, concentrations of phosphate were generally lower than nitrate. This is not surprising because phosphorus is said to be bonded in rocks and sediments, making them unavailable and generally limiting in aquatic ecosystems (Smil, 2000). Seasonal variabilities in nitrate and phosphate concentrations were observed in both lagoons. Generally, increasing levels of nitrates were observed in the dry season, whiles decreasing levels were observed in the wet season. Similar seasonal trend in nitrate concentration has been reported for other estuarine systems in Ghana (Okyere, 2015; Osei, 2019), and denitrification has been ascribed to be responsible for the decreasing levels in the wet seasons in the case of the Densu estuary (Osei, 2019). Also, similar trend in seasonal variability in nitrate concentrations has been reported in the Chilika lagoon in India (Tarafdar et al., 2021). On the other hand, increasing phosphate levels in Narkwa Lagoon in the wet season could as a result of runoffs carrying fertilizers and excreta from the catchment area as ascribed by Dzakpasu (2019). The significantly high concentrations of nitrate and phosphate in the Benya (Figures 11 and 12) could be coming from direct dumping of human effluents and other organic wastes into the lagoon. It is also worth noting that, both lagoons had high levels of nitrate and phosphate values recorded in this current study; and these were above the recommended levels of 1.0 mg/l and 0.1 mg/l, respectively in estuarine and coastal waters for avoidance of algal

blooms (Behar, 1997; Dzakpasu, 2019). The two lagoons are located close to human settlements and therefore prone to receiving excess nutrients from sources linked to anthropogenic activities. This result corroborates findings from other studies that have also reported high (well above the permissible) nitrate and phosphate levels in other estuarine systems in recent times in Ghana (Dzakpasu, 2019; Okyere, 2015; Osei, 2019). This nutrient enrichment, if not checked, could lead to significant economic and social costs.

In aquatic ecosystems, chlorophyll-*a* concentrations are indicators of the abundance of phytoplanktons, biomass and primary production (Jiménez-Quiroza, Martell-Duboisb, Cervantes-Duartec & Cerdeira-Estrada, 2021; Shepard et al., 2012), and thus can be an effective measure of trophic status (Lee, 1999). Nonetheless, extreme levels of chlorophyll a can be indicative of poor water quality (Ogamba, Chindah, Ekweozor & Onwuteaka, 2004; Onuoha & Vyverman, 2010). Chlorophyll a concentration has been used as a proxy for food availability for bivalve growth and carrying capacity modelling (Shepard et al., 2012). Temporal variabilities in chlorophyll a concentration was observed in Benya and Narkwa lagoons in this current study, which did not seem to follow any discernable seasonal pattern, except that, the peak chlorophyll a concentration in Benya was recorded in February (dry season), and the lowest in April (onset of the wet season) (Figure 14). Higher chlorophyll-*a* in dry season could be linked to high solar irradiance and improved water clarity (reduced turbidity) during this period which probably enhances phytoplankton growth. Conversely, no plausible reason could be ascribed to the observation made in the Narkwa lagoon, where the highest and lowest chlorophyll-*a* concentrations were recorded in October and July (both in wet season),

respectively. According to Pennock (1985), chlorophyll-*a* concentration in estuarine and coastal water systems are said to be subjected to spatial and temporal variabilities, due to a diverse set of physical, chemical and biological factors that characterise these aquatic systems. The significantly higher annual mean chlorophyll a concentration recorded in Benya lagoon could be due to over enrichment of the lagoon largely linked to anthropogenic sources, resulting in significantly higher nutrient levels recorded in that system.

5.2. Plankton Dynamics in Benya and Narkwa lagoons

Plankton generally play crucial ecological role in the energy transfer within numerous food webs in aquatic ecosystems, and are also useful indicators of water quality (Akhiromen et al., 2022). Plankton, particularly phytoplankton, are generally considered important food resources to bivalve shellfish including oysters. Whiles many of these phytoplankton species ingested by filter feeding bivalves are harmless, a few species are said to produce biotoxins, which accumulate within flesh of the bivalves, and can be transported to higher trophic levels within the food web, posing health threat to human consumers (Hinder et al., 2011; Silva et al., 2016; Swan & Davidson, 2011). Plankton dynamics and community structure were found to be different across the two coastal lagoons in this study. This is evidenced by the differences in the number of taxa recorded under the plankton functional groups, and the overall number of taxa in each of the two lagoons. The plankton communities in the two coastal lagoons was, however, found to be highly diverse, with a total number of genera for Benya and Narkwa lagoons. The number of plankton genera recorded for each of the two lagoons in this study were higher than what was reported from a much larger Lagos Lagoon (Nkwoji et al., 2010). This could be attributed to

differences in prevailing hydrographic factors at play in these ecosystems. This observation is consistent with Dalu et al. (2014), who observed differences in phytoplankton community structure within different coastal estuarine environments, and expressed how this could be potentially utilised to identify the differences between estuarine ecosystems.

Plankton abundance also exhibited seasonal variabilities across the two lagoons (Figure 16). The highest mean plankton density in Benya lagoon recorded in the dry season (February), whiles that of Narkwa was observed at the onset of the wet season (April). Seasonal variabilities in phytoplankton abundance have been reported for other tropical lagoons (Akhiromen et al., 2022; Srichandan et al., 2015). The plankton communities encountered in this study in the two lagoons were made up of phytoplankton, microzooplankton, mesozooplankton and fish eggs/larvae. The phytoplankton, which were the dominant and the most diverse community were composed of six groups, namely, Bacillariophyceae, Dinophyceae, Cyanophyceae, Euglenophyceae, Chlorophyceae and Dictyochophyceae, which were closely similar to the phytoplankton groups reported in Lagos lagoon (Akhiromen et al., 2022) and for another tropical lagoon (Chilika lagoon) in India (Srichandan et al., 2015). Phytoplankton are generally known to be a predominant component in the plankton community (Kamiyama, 2011). The predominance of diatoms in Benya lagoon in the current study is consistent with reports made along the coastal waters of Ghana (Denutsui, 2019) and from many other tropical estuarine environments such as the Lagos lagoon in Nigeria (Nwankwo, 1996; Nkwoji, 2010), the Chilika Lagoon in India (Tarafdar et al., 2021), a mangrove estuary in Philippine (Canini, Metillo & Azanza, 2013), and the Na Thap

Estuary of Thailand (Lueangthuwapranit, Sampantarak & Wongsai, 2011). However, according to Akhiromen et al. (2021), high occurrence of pollution indicator species such as *Nitzschia* and *Navicula* can indicate the extent of anthropogenic influence on aquatic systems, including indiscriminate dumping of municipal waste, untreated sewage and industrial effluents into the aquatic system. Diatom community structure is said to have a bearing on water quality (Morin, Gómez, Tornés, Licursi & Rosebery, 2016). The relatively high proportions of *Nitzschia* and *Navicula* recorded in the two lagoons could further give some level of credence to the anthropogenic-induced pollution occurring in these lagoons.

The plankton community compositions were also observed to have exhibited temporal oscillations and seasonal turnover or successions from one plankton community type to another. Diatoms were most abundant (>50%) throughout the study period in the Benya lagoon, except during the onset of the wet season (April and May) where their compositions were below 50%, and also in August where their dominance was completely taken over by two harmful dinoflagellate genera; *Dinophysis* and *Prorocentrum*. Similar findings of low diatom abundance during wet (monsoon) season has been reported in a tropical lagoon in India (Tarafdar et al., 2021). Contrastingly, dinoflagellates dominated the plankton group compositions in Narkwa, and the pattern of variabilities in the temporal compositions of plankton groups revealed a classic case of seasonal turnover or succession between diatoms and dinoflagellates. Whiles diatoms abundance was higher in the wet season (from September to November and June and July), A shift in dinoflagellates dominance was observed from the dry season to the onset of the wet season (December to May),

occasioned by the proliferation of harmful genera namely; *Peridinium Prorocentrum*, *Gymnodinium* and *Gyrodinium*. This observation in Narkwa lagoon corroborates observations from studies elsewhere where dinoflagellates abundance was observed in warmer seasons (Esqueda-Lara, Hernández-Becerril & Robles-Jarero, 2005). According to Schrader (1981), seasonal turnover or succession in plankton community compositions are well known phenomena in aquatic systems which undergo distinct hydrographic changes. Also, a number of studies have emphasized the seasonal oscillations in phytoplankton assemblages which is attributable to seasonal variations in physical mixing dynamics, temperature and light regime (Diehl, Berger, Ptacnik, & Wild, 2002; Leterme, Jendyk, Amanda, Ellis, Brown, Kildeaet, 2014; Vajravelu Martin, Ayyappan & Mayakrishnan, 2018). This further gives some credence to the assertion of the possible influence of seasonality in the spatio-temporal variabilities in plankton community compositions within estuarine ecosystems (Dalu, Froneman & Richoux, 2014). Plankton distributions have been reported to also have strong correlation with various physical, chemical, biological, and hydrological factors as well as their interactions (Paerl, Rossignol & Hall, 2010; Srichandan et al., 2015). According to Salleh and Ruslan (2010), phytoplankton distribution is said to be closely linked with their physiological requirements such as dissolved oxygen, pH and nutrients which can impact on the overall health of an aquatic ecosystem. From the Pearson's correlation analysis (Table 2), a negative relationship was observed between dissolved oxygen, pH, nitrate and diatoms abundance. This could possibly explain the dominance of diatoms in the significantly lower oxygen and pH conditions that prevailed in the Benya lagoon. On the other

hand, a positive relationship was observed between temperature, dissolved oxygen, pH and dinoflagellate abundance, indicating that relatively higher temperature, dissolved oxygen and pH in Narkwa lagoon could have contributed the higher abundance of dinoflagellates in that lagoon. pH and nutrients (nitrate and phosphate) were generally observed to be the most significant predictors of diatoms and dinoflagellates abundance in the two lagoons.

5.3. Plankton Ingestion and Selectivity by *C. tulipa* Populations in Benya and Narkwa lagoons

Water samples analyses indicated that the mangrove oyster *Crassostrea tulipa* population in Benya and Narkwa lagoons were exposed to diverse plankton functional groups, and the results from the oyster stomach contents analyses revealed that the oyster populations in Benya and the Narkwa explored all the plankton functional groups in the water columns as food resource. However, marked differences were observed between the number of plankton taxa recorded in the water column and that ingested by the oyster populations in both lagoons, with relatively lower number of ingested plankton taxa (Table 1). This could be due to selective ingestion of some plankton taxa by the oyster populations. The ingested plankton communities by *C. tulipa* population in Benya and Narkwa lagoons were composed of Phytoplankton (diatoms, dinoflagellates, cyanobacteria, euglenoids, chlorophytes and silicoflagellates), Microzooplankton (ciliates), Mesozooplankton (copepods, rotifers and cladocerans) and fish eggs/larvae. This observation is consistent with the assertions that oysters in addition to microalgae, ingest other non-algae cells including zooplankton (copepods and rotifers), eggs and larvae of various

species, as well as detrital matter and fragments of seagrasses (Bayne, 2017; Haines & Montague, 1979; Newell, 1988; Weissberger & Glibert, 2021). That notwithstanding, phytoplankton generally formed the bulk constituent (>90%) of the food items in *C. tulipa* in this study. Phytoplankton-dominated foraging behaviour have also been reported for the Pacific oyster *C. gigas* (Kasim & Mukai, 2009; Miossec, Le Deuff, & Goulletquer, 2009), Eastern oyster, *C. virginica* (Abgrall, Miron & Ouellette, 2010) and clam (*Ruditapes philippinarum*) (Kasim & Mukai, 2009). All these observations further corroborate the general assertion that phytoplankton are the main food source of filter-feeding bivalves. Diatoms dominated the plankton groups ingested *by C. tulipa* populations in the two lagoons. This observation is also consistent with the findings of Adite et al. (2013b) who also found diatoms as the main diet of *C. gasar* (=*tulipa*) cultured in a coastal lagoon in Benin. Importance of diatoms in the diet of oysters and other bivalves have been emphasized by other authors (Kasim & Mukai, 2009; Rouillon et al., 2005; Villalejo-Fuerte et al., 2009).

Temporal analyses of ingested plankton communities in oyster stomach content also revealed marked differences between the relative compositions of the plankton groups in the water column and that found in the oyster stomach. For instance, the dominance of diatoms and dinoflagellates in Benya and Narkwa lagoons, respectively, were not similarly reflected by their compositions in the oyster stomach in most of the sampling months. This suggested selective feeding in *C. tulipa*. Temporal variabilities in ingested plankton group compositions were characterised by seasonal oscillations and turnovers in ingested plankton group compositions among the *C. tulipa* populations in Benya and Narkwa lagoons (Figure 23 and 24). For example,

whiles members of the genus *Navicula* was the most ingested diatoms by *C. tulipa* populations in Benya lagoon in the February (dry season) peak, there was a shift to the genus *Thalassiosira* in March. *Nitzschia* and *Navicula* were the most ingested responsible for the May (wet season) peak, while *Navicula* and *Thalassiosira* were prominently featured in the oyster diet in July (wet season) peak. Dinoflagellate ingestion peaks were recorded in August and September (minor wet season), with genus *Prorocentrum* as the main taxon responsible for the peaks. Higher compositions of cyanobacteria (due to increased number of *Chroococcus* sp.) among the ingested plankton groups were observed in April and June (wet season) even though their proportions within the water columns were relatively lower throughout the sampling period. Appreciable proportions of ingested mesozooplankton (copepods) were also recorded in October and March. Peak composition of diatoms among ingested plankton by oysters in Narkwa lagoon was recorded in April, influenced by high numbers of *Amphora* sp. Peak dinoflagellates composition was observed in August occasioned by high numbers of ingested *Prorocentrum* and *Dinophysis* spp. Temporal variabilities in ingested phytoplankton compositions have also been reported in *C. virginica* by Weissberger and Glibert (2021).

Temporal analyses of plankton electivity (selectivity) (*Ei*) were carried out with the aim to unravel preferential selection of plankton functional groups by *C. tulipa* populations in Benya and Narkwa. Estimated values (which ranged from +1 to – 1) related the plankton group proportions in the stomach to that in the water column, where positive value indicated ‘preferred’, negative value indicated ‘not preferred’, and zero (0) indicated consumption proportional to availability (Weissberger & Glibert, 2021). Generally, cyanobacteria

(represented by *Chrooccocus* sp.), mesozooplankton (copepods/rotifers/cladocerans), ciliates (Oligotrichea) were recorded as the preferred prey item for the *C. tulipa* populations in Benya and Narkwa lagoons for most of the sampling months. Euglenoids and fish eggs/larvae recorded seasonal (patchy) selection even though their proportions in the water column were relatively lower. Although diatoms and dinoflagellates formed the cored components of ingested plankton in *C. tulipa* in this study, they were generally observed to have been the 'not preferred' food in the period when they recorded highest abundance in the water column. This observed selectivity of less abundant plankton groups over highly abundant groups by *C. tulipa* could be necessitated by the need to maximize energy and nutrient uptake (Rosa et al., 2013). All plankton species are said to be unequal in terms of nutritional quality for bivalves, and oysters and other bivalves are reported to exhibit preferential utilisation of plankton species on the bases of their food value, particle size, food concentration and other intrinsic qualities of the food particle (Cucci et al., 1985; Kiørboe & Mohlenberg, 1981; Rouillon & Navarro, 2003; Weissberger & Glibert, 2021). Nonetheless, Rouillon et al. (2005), opined that it is more useful to look at the preferential usage of plankton by bivalves as a mechanism for maximum use of food resources under highly variable conditions in marine coastal ecosystems.

5.4. Temporal Compositions of Ingested Potential Toxic Phytoplankton by *C. tulipa* in Benya and Narkwa lagoons

Stomach content (diet) analysis showed that phytoplankton are an important food source for *C. tulipa* populations in Benya and Narkwa lagoons, similar to many other bivalves. However, according to Swan and Davidson

(2011), whilst a great number of phytoplankton explored as food by bivalve filter-feeders are benign, a few species produce toxins which can accumulate in the tissue and organs of these bivalves and cause health complications to their human consumers. For sustainable exploitation of bivalve molluscs and development of aquaculture, it is essential to monitor and predict the occurrence of harmful toxic phytoplankton. (Silva et al., 2016; Swan & Davidson, 2011). Analyses of the stomach contents of *C. tulipa* populations in Benya and Narkwa lagoons identified significant compositions of ingested potential toxic phytoplankton. Nine (9) genera of dinoflagellates (*Prorocentrum*, *Dinophysis*, *Alexandrium*, *Gymnodinium*, *Karenia*, *Gonyaulax*, *Ostreopsis*, *Heterocapsa*, *Amphidinium*) and One (1) genus of diatom (*Pseudo-nitzschia*) were identified to be potentially toxic (potentially causatives of diarrheic, paralytic shellfish, Azaspiracid and neurotoxic shellfish poisoning). This result is consistent with earlier by Denutsui (2019). This study reported on occurrence of toxic dinoflagellates. The study further reported on positive test of bloody cockles (*Anadara* sp.) to lipophilic toxins, Okadaic acids (OA) and Dinophysistoxins (DTX2) (toxins responsible for shellfish poisoning) along the coast of Ghana. Denutsui (2019), however, did not encounter *Pseudo-nitzschia* spp. which are also notorious for shellfish poisoning and fish kills (Swan & Davidson, 2011). Ingestion of toxic phytoplankton by *C. tulipa* in Benya and Narkwa lagoon could imply high risk of shellfish poisoning among oyster consumers in these coastal communities.

Temporal pattern of distribution revealed seasonal variabilities in the compositions of ingested potential toxic phytoplankton *C. tulipa* populations in Benya and Narkwa lagoons. *Prorocentrum* spp. formed major component of the

ingested potential toxic phytoplankton throughout the year in both lagoons. One seasonal peak ingestion was recorded in Benya in August, and the lowest ingestion recorded in February. Two seasonal peak ingestions were, however, recorded in Narkwa. The January (dry season) peak ingestion composed mainly of two genera (*Gymnodinium* and *Ostreopsis*), whiles the August (wet season) peak was dominated by *Prorocentrum* spp. The quantity of toxic phytoplankton ingested by filter feeding shellfish depends on the availability of other harmless phytoplankton species, but an increase in feeding rates of shellfish in the presence of a mixed phytoplankton assemblage of toxic species may increase the toxicity burden more than feeding on a monospecific diet. (Bricelj & Shumway, 1998; Swan & Davidson, 2011). Periodic analysis of oyster stomach content in addition to water sample assessment, as done in this current study, can therefore help in predicting patterns of toxic burdens on oyster populations to guide their exploitation.

5.5. Ecophysiology of *Crassostrea tulipa*

5.5.1. Oyster morphometry, weight, meat yield and condition index

Changes in weight is said to be the most appropriate variable for estimating growth in bivalve, but increase in the longest shell dimension (i.e. increase in length and height) is usually chosen as an indicator of size because it is more easily measured (Gosling, 2003; Vakily, 1989). In this study, shell dimensions (shell height, shell length and shell width), oyster weights (whole live weight, meat weight, dry meat weight and shell weight), in addition to shell shape/form (elongation, compactness and convexity) were used as the growth performance indicators of oysters. The shell height of *C. tulipa* populations in Benya and Narkwa lagoons as recorded in this current study is summarized by

the shell height-frequency distribution, which may reflect the size structure of the oyster populations (Figure 28a and b). The shell size ranges (3.0-10.20 cm and 3.0-10.10 cm for Benya and Narkwa, respectively) of the two studied populations were comparatively similar, with a unimodal shell height (5.0 to 5.9 cm). This result corroborates that which was reported in earlier studies by Asare et al. (2019) in Narkwa. Osei et al. (2021), however, recorded a relatively wider size range (2.0 to 14.6 cm), with a unimodal shell height (4.0-4.9 cm) for the mangrove oyster population at Densu estuary. *C. tulipa* population in Benya recorded a significantly higher mean shell height than their counterparts in Narkwa, even though no significant differences were found between their shell length and shell width. The differences in mean shell height of the two populations could be attributed to growth overfishing occurring in Narkwa where bigger-sized oysters are targeted for exploitation leading to lower proportion of bigger-sized oysters in the population. Narkwa lagoon is one of the hotspots for oyster exploitation along the coast in Ghana (Asare et al., 2019). In terms of weight, no significant difference was observed for the live weight, shell weight and the dry meat weight of the two populations. However, a significantly higher meat weight was estimated for the Narkwa population than their relatives in Benya lagoon. Mean shell height, shell length, shell width and live weights of the two oyster populations as recorded in this study were similar to what was reported earlier for the oyster population in Narkwa (Asare et al., 2019). Ansa and Bashir (2007), however, recorded comparatively longer mean shell heights (6.26 cm and 6.02 cm), heavier mean live weight (24.79g and 22.68g), with relatively lighter meat weight (1.87g and 1.76g) for mangrove oyster population in Port Harcourt, Nigeria. With respect to shell morphology

(shape) indices, oysters in Narkwa lagoon had significantly more elongated and compact (globular) shells than their relatives in the Benya lagoon, even though no significant difference was observed for shell convexity among the two oyster populations. Osei (2020), observed no differences in shell morphology of the oyster population in the Densu estuary. The differences in shell morphology of the Benya and Narkwa populations could be ascribed to differences in the type of substrate of attachment for the two oyster populations or the nature of spatial constraints as they grow in clusters (Quayle 1988; National Research Council, 2004). *C. tulipa* population in Benya lagoon are attached to the roots of the red mangrove (*Rhizophora* sp.) fringing the lagoon, whilst those in Narkwa attached to the sandy-mud substratum of the lagoon.

Condition index and meat yield are usually used as commercial quality indicators for bivalve exploitation (Crosby & Gale, 1990; Davenport & Chen, 1987; Yankson, 2004; Yildiz et al., 2011). According to Crosby and Gale (1990), condition index is widely regarded as a useful estimate of the nutritive status of bivalves, and can be used as an indicator of environmental stress (pollutant, diseases, etc). Oysters in Narkwa recorded significantly higher mean condition index and meat yield than those in Benya lagoon. Temperature, salinity, pH, DO, food and other factors have been linked to variations in bivalve condition index and meat yield (Gosling, 2003; Hemachandra & Thippeswamy, 2008; Sreedevi et al., 2014). In this study, chlorophyll a concentration (proxy for food quantity), dinoflagellates and cyanobacteria compositions in water in addition to pH, phosphate were observed to be the significant predictors of condition index of *C. tulipa* in the two lagoons (Table 9). Cyanobacteria composition in addition to pH, turbidity and phosphate levels in water were

observed to be significant predictors of meat yield in *C. tulipa* in Benya and Narkwa lagoons. Temporal assessment of condition index and meat yield of oysters could give indication of the period in the year when they are in best condition for exploitation to ensure maximum production (Yankson, 2004). *C. tulipa* populations in Benya and Narkwa exhibited temporal oscillations and seasonal variabilities in condition index and meat yield, with the two parameters generally following similar pattern of temporal variations in both Lagoons. This indicates a strong link between the two parameters. Peak condition index and meat yield in oysters at Benya were recorded in October. For Narkwa, peak oyster condition index and meat yield were observed in September. Gametogenesis and spawning activities dictated by hydrographic factors, are said to be main drivers of temporal oscillations in bivalve condition index and meat yield (Yildiz et al., 2011). Period of gametogenic activity is reflected by increase in condition index, but spawning activity significantly reduces flesh/meat weight and ultimately a reduction in condition index in bivalves (Gosling, 2003; Krampah, Yankson & Blay, 2016; Quayle & Newkirk, 1989). A comparison of the overall means of the growth parameters, condition index and meat yield which served as the indicators of the ecophysiological state of *C. tulipa* populations in Benya and Narkwa lagoons are shown in Table 8. Generally, mangrove oyster population in Narkwa exhibited better ecophysiological state, in terms better shell morphological indices, condition index and meat yield, than their relatives in Benya. A number of factors have been asserted to affect the growth and physiology of oysters, with hydrographic factors (food, tidal exposure, temperature, salinity, DO, pollutant, pest and diseases) reported to interactively play a significant role in these two aspects

(Asare et al., 2019; Angell, 1986; Gosling, 2003; Quayle & Newkirk, 1989; Yankson, 2004;). The underpinning assumption, according to Asare et al. (2019), is that growth and physiological condition of oysters are enhanced when these factors are within their tolerance range or animals have not been exposed to extreme changes in the environmental conditions for a long time. All the hydrographic parameters measured in Benya and Narkwa in this study were found to be within the tolerance range of *C. tulipa* (Obodai, 1997; Mahu et al., 2022; Osei, 2019;). Nonetheless, the differences observed in growth and ecophysiological parameters (condition index and meat yield) of the two populations could therefore be attributed to disparities in environmental conditions that characterised the two lagoons. Oysters in Narkwa lagoon were observed to have recorded better growth and ecophysiological conditions probably due to relatively favourable environmental conditions that prevailed in the Narkwa. *C. tulipa* is said to thrive well in estuarine (brackish) environments, with growth rates varying depending on local conditions (Mahu et al., 2022).

5.6. Laboratory Rearing of *C. tulipa* Larvae on Local Microalgae Isolates

5.6.1. Growth performance characteristics of microalgae isolates

Microalgae used in aquaculture must meet various criteria, and one important criterion is the ease of culture (Brown Mular, Miller, Trenerry & Farmer, 1999; Renaud Thinh, Lambrinidis & Parry, 2002; Spolaore, Joannis-Cassan, Duran, & Isambert, 2006). In this study, the growth performance characteristics of three local microalgae isolates (*Rhodomonas* sp., *Nannochloropsis* sp. and *Pseudanabaena* sp.) were therefore assessed prior to them being used as feed for the rearing of *C. tulipa* larvae in the laboratory. The

growth of the three microalgae isolates generally followed similar pattern, albeit marked differences in the daily increase in cell densities. This could be due to the unequal rate of the cell division (Moazami et al., 2012). Growth of microalgae, according to Moazami et al. (2012), can be classified into four phases; lag phase, log phase/exponential, stationary phase and finally death phase. The lag phase is the early stage of culture where the cell division is relatively slow when the cells are acclimatizing to the culture surrounding. The microalgae begin to undergo active cell division subsequently and the cell density increases exponentially. The stationary phase is the growth stage where cell division ceases and cell density stabilises. Death phase is observed when cell density decline is observed. In this study, the three microalgal isolates generally exhibited lag phase from the first to the third day of culture. Exponential growth was observed from the forth and firth day of culture for the Rhodomonas sp. and *Nannochloropsis* sp., whiles that of the *Pseudanabaena* sp. was observed from the fourth to the sixth day. Lafarga-De la Cruz et al. (2006), recorded optimal growing condition up to the fourth day for *Rhodomonas* sp. under different culture conditions. Microalgae have a wide variety of shape and size ranging from submicron species like picoplanktonic prochlorphytes to diatoms with greater than 1 mm in diameter (Hillebrand et al., 1999; Reynolds 1984;). However, according to Brown (2002), microalgae used as feed in the hatchery must be of appropriate sizes for ingestion, e.g. from 1 to 15 µm for filter feeders. The size of the three microalgae isolates as represented by the Equivalent Spherical Diameters (ESD) in this study were within the appropriate size range as asserted for filter feeders by Brown. The (ESD) recorded for the *Rhodomonas* sp. in this study was relatively bigger than that

estimated in literature (Acheampong, 2011; Broglio et al. 2003). On the other hand, ESD estimated for *Nannochloropsis* sp. in this study was comparatively smaller than that reported by Wang Yin (2013). Khan et al., (2017) reported that different strains of *Pseudanabaena* cultured under different illumination and temperature conditions exhibited varied ESD. By virtue of the ESD of the microalgae cells, *Rhodomonas* sp. recorded the highest estimated biovolume, carbon weight and carbon energy, whiles *Nannochloropsis* sp. recorded the lowest estimated biovolume, carbon weight and carbon energy.

5.6.2. Rearing performance of *C. tulipa* larvae fed with microalgae isolates

Microalgae is expected to continue to be the most suitable feed the larval rearing of bivalves in the hatchery despite recent advances in the development of artificial feed (Cheng et al., 2020; Yarnold, Karan & Hankamer, 2019; Yin, Wang, Yang & Xie, 2019). With the increasing demand for seafood globally, microalgae will therefore continue to play a crucial role in the rapid expansion of bivalve shellfish production sector to meet the growing demand. This means that research into isolation and culture of new strains or species of microalgae to support shellfish bivalve aquaculture development particularly in sub-Saharan Africa is essential.

The high level of fertilisation rate observed in this study is comparable to those reported by Yankson (1990) and Obodai (1997) for the same species. The embryonic development of *C. tulipa* as observed in this study were also similar to that described in earlier studies (Yankson, 1990, Obodai, 1997). The three local microalgae diets significantly supported growth and survival of oyster larvae at different scales, with the larvae fed on mixed diet recording the overall best performance with respect to growth and survival. Oyster larvae

reared on the natural-sourced diet exhibited better growth, but poor survival than any of the individual microalgae diets. The poor survival of oyster larvae under natural diet treatment could largely be attributed to infections resulting from proliferation fungal-like growths in the untreated water. With regard to the individual scale, oyster larvae fed on *Rhodomonas* sp. recorded a superior performance in terms of growth and survival than the *Nannochloropsis* sp. and *Pseudanabaena* sp. Observed differences in growth and survival of oyster larvae fed on different diet treatment, may be due to differences in their biochemical compositions, particularly lipid content and fatty acid composition (Cheng et al., 2020; Sigrún, 2019). The result from this study support the assertions that different microalgae species can significantly affect oyster larval growth and survival rates, with some species showing better performance than others (Ye et al., 2022). However, mixed cultures of microalgae, in most cases, provide better nutrition and growth for oyster larvae than single species cultures (Araya, Mingant, Petton, & Robert, 2012; Cheng et al., 2020; Helm, 2004). The nutritional composition of microalgae greatly affects the growth of oyster larvae, emphasizing the importance of choosing appropriate microalgae species for optimal oyster larval rearing through continuous research (Cheng et al, 2020; Ponis et al., 2006).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary

Key findings of this study are summarised as follows:

Significant temporal variations were generally observed among all the measured hydrographic parameters in the two water bodies. However, annual mean salinity, dissolved oxygen and turbidity were significantly higher in Narkwa lagoon, whiles phosphate, nitrate and chlorophyll a (phytoplankton biomass) were significantly higher in Benya. Nitrates and phosphates were above the recommended limits in the two water bodies

Higher number of plankton functional groups were recorded in Narkwa than in Benya, with diatoms and dinoflagellates generally dominating the plankton functional groups compositions in Benya and Narkwa, respectively. The diatoms in Benya were dominated by genera: *Thalassiosira*, *Nitzschia* and *Navicula*, with all-year-round occurrence. The dinoflagellates, on the other hand, were dominated by two harmful/toxic genera; *Prorocentrum* (year-round occurrence) and *Dinophysis* (seasonal occurrence). The dinoflagellates in Narkwa lagoon were dominated by five harmful/toxic genera: *Prorocentrum* (all-year-occurrence), *Gymnodinium*, *Heterocapsa*, *Gyrodinium* and *Karenia*. The diatoms in Narkwa were dominated by *Nitzschia* (with all-year-round occurrence). Temporal variations in plankton functional group compositions were generally observed in the two water bodies, with a clear case of seasonal succession between diatoms and dinoflagellates observed in Narkwa Lagoon.

There were various degrees of correlation between physico-chemical parameters and plankton functional group compositions. Phosphate and pH were the most significant predictors of diatoms composition (abundance), whiles phosphate, nitrate, in addition to pH were the most outstanding predictors of dinoflagellates abundance in the two water bodies.

Diatoms and dinoflagellates co-dominated the diet compositions of oyster populations in the two water bodies possibly due to their predominance in the water column. However, a diet electivity analysis indicated a preferential selection for less abundant groups such as cyanobacteria, mesozooplankton and microzooplankton. There were temporal variations in ingested potential toxic phytoplankton by oyster population in each of the two water bodies, with *Prorocentrum* spp dominating and occurring year-round.

In terms of shell morphometry, oysters in Benya recorded significant higher shell height than those in Narkwa. Conversely, Narkwa oysters were more elongated, globular and recorded higher wet meat weight, meat yield and condition index, compared to the Benya oysters. Dinoflagellates and cyanobacteria abundance in water, in addition to pH and phosphate levels were observed to be the most significant predictors of condition index of *C. tulipa* in the two lagoons. On the other hand, Cyanobacteria abundance in addition to pH, turbidity and phosphate levels in water were observed to be most notable predictors of meat yield in *C. tulipa* in Benya and Narkwa Lagoons.

The three local microalgal isolates promoted growth and survival of oyster larvae on different scales, and the observed differences in growth and survival of oyster larvae could be due to differences nutritional compositions of

the different isolates. However, a combination of the three microalgae diets provided complementary nutrients for best growth and survival of *C. tulipa* larvae. Natural occurring microalgae in untreated rearing water could sustain *C. tulipa* larval growth, but microbial infections could lead to poor or lower survival rates. This observation indicated that local microalgae isolates have the potential to support hatchery-rearing of *C. tulipa* and possibly other bivalves of economic importance. This critical for the optimisation which essential for diversification of oyster aquaculture in Ghana and the rest of the sub-region.

6.2. Conclusion

Whereas previous non-seasonal data on diet compositions of *C. tulipa* were available from a study in Benin, the current study has provided empirical data on the oyster's trophic behaviour by considering the natural cycle of plankton dynamics, temporal variations in diet compositions, diet selectivity/preference and temporal variations in compositions of potential toxic phytoplankton in the diet of *C. tulipa* population in two differing coastal lagoons in Ghana. The study also reported the ecophysiology of *C. tulipa* populations in two coastal lagoons in Ghana. This scientific information will be essential in the exploitation management, conservation and aquaculture planning of the species in the two water bodies, most especially Narkwa lagoon which a hotspot for oyster exploitation in Ghana. The study further provided data for the first time on the potential of three local microalgae isolates as food resource for larval rearing of *C. tulipa* in the laboratory.

6.3. Recommendations

For sustainable oyster exploitations and aquaculture development in Ghana. It is recommended that;

1. Sanitary and nutrient control measures be put in place by the Water Resources Commission and the District Assemblies to control the possible eutrophication of coastal water bodies in view of the high levels of nutrients could be a precursor for harmful algal blooms in these ecosystems. This will help control the proliferation of HABs in these water bodies.
2. In view of the potential of local microalgae strains to sustain the oyster seed rearing in the hatchery, Fisheries Commission, NGOs and private individuals with the requisite capacity are encouraged to invest in the hatchery production of oyster seeds to promote commercial farming of oysters along the coast of Ghana.

Based on the results of this study, further studies are recommended to be undertaken to;

1. Further studies be carried out to understand the dynamics of HABs occurrence along the coast of Ghana, and monitoring and prediction mechanisms be put in place by the appropriate quarters to forestall any negative consequences associated with HABs and to guarantee the safety of bivalve shellfish to human consumers.
2. Investigate the mechanisms of food preference in *C. tulipa* under controlled conditions in the laboratory.
3. Investigate biotoxin profiles and levels in oysters and other bivalves harvested along the coast of Ghana.

4. Assess biochemical compositions of the three local microalgae isolates under various culture conditions to optimise their large-scale production in the laboratory.
5. Further studies be carried out on *C. tulipa* larval rearing until larval settlement and beyond.
6. Assess the suitability of local microalgal isolates as feed resource for the seed production of other bivalves of commercial important.

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APPENDICES

Appendix A: *Summary of Student t-test comparison of mean annual hydrographic parameters in Benya and Narkwa Lagoons*.

Hydrographic parameters	Benya	Narkwa	*t-value*	*p-value*
Temperature (°C)	28.6 ± 0.10 (26.5-29.9)	28.7 ± 0.16 (26.8-31.2)	-0.51	0.610
Salinity (ppt)	**35.7 ± 0.23** (30.0-38.5)	19.58 ± 1.00 (3.81-31.82)	15.76	0.000*
Dissolved Oxygen (mg/L)	2.5 ± 0.07 (1.3-3.4)	**6.0 ± 0.15** (5.02-8.82)	-20.8	0.000*
pH	7.2 ± 0.03 (6.7-7.9)	**7.4 ± 0.05** (6.8-8.09)	-3.22	0.002*
Turbidity (NTU)	6.2 ± 0.41 (0.84-12.0)	**12.7 ± 1.58** (1.9-44.3)	- 3.93	0.000*
Phosphate (PO_3) (mg/l)	**0.43 ± 0.02** (0.28-0.74)	0.26 ± 0.020 (0.12-0.58)	6.83	0.000*
Nitrate (NO_3-N) (mg/L)	**8.1± 0.31** (4.4-13.0)	4.5±0.43 (BDL-10.2)	6.86	0.000*
Chlorophyll a (mg/L)	**6.0 ± 0.26** (3.2-14.0)	4.1 ± 0.37 (2.4-9.7)	4.09	0.000*

***s**ignificant at $p < 0.05$

Appendix B1**:** *Monthly abundance (density) of plankton (cells//ind. ml^{-1}) in Benya Lagoon*

	2020				2021								
Taxon	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Overall total
Chlorophyceae													
Dunaliella spp.	0	0	0	20	35	26	47	**65**	4	38	30	1	266
Cyanophyceae													
Chroococcus sp.	0	0	2	11	5	6	7	**24**	2	10	2	11	80
Epigloesphaera sp.	0	0	0	0	0	0	**29**	10	2	6	0	0	47
Johannesbaptista sp.	0	0	0	2	0	0	0	1	0	0	0	0	3
Leptolyngbya sp.	0	0	0	1	10	0	0	0	0	0	0	0	11
Merismopedia spp.	0	3	2	4	2	7	1	0	0	0	2	3	24
Microcoleus sp.	0	0	0	8	0	0	0	0	0	19	0	0	27

Oscillatoria sp.	1	0	0	0	3	1	0	4	0	6	9	15	39
Trichodesmium sp.	0	12	0	0	0	0	0	0	0	0	**37**	2	51
Unidentified cyanobacteria spp.	5	0	10	14	0	0	**25**	18	0	2	0	0	74
Bacillariophyceae													
Achanthes spp.	0	0	0	3	1	2	0	4	0	1	0	1	12
Amphipleura sp.	0	0	0	0	0	0	0	0	0	0	7	0	7
Amphora spp.	4	31	16	**49**	31	11	18	9	0	5	3	1	178
Asterionellopsis sp.	2	0	18	4	19	4	0	0	1	1	0	0	49
Bacillaria sp.	0	3	3	22	0	1	2	2	0	1	2	2	38
Bacteriastrum sp.	0	0	2	1	0	0	0	0	0	0	0	0	3
*Biddulpha s*p.	0	0	0	2	0	0	0	0	0	0	0	0	2
Chaetoceros spp.	**43**	9	32	6	17	18	1	0	0	0	0	0	126

Cocconeis spp.	0	0	1	2	2	5	0	6	0	4	3	11	34
Coscinodiscus spp.	**112**	10	48	13	1	0	5	12	1	1	3	9	215
Cylindrotheca spp.	53	115	**139**	132	97	109	56	52	4	29	86	1	873
Cymatopleaura sp.	0	7	0	0	0	**26**	0	0	0	0	4	0	37
Cymbella spp.	0	3	0	**23**	6	2	0	1	3	10	2	9	59
Dactyliosolen sp.	0	0	0	**2**	0	0	0	0	0	0	0	0	2
Denticula sp.	0	0	0	0	0	0	0	**3**	0	0	1	0	4
Detonula sp.	0	0	0	1	0	0	0	0	0	1	0	0	2
Diatoma sp.	0	0	0	0	0	**10**	0	7	3	0	0	0	20
Diploneis spp.	0	0	0	2	9	2	5	6	0	3	3	2	32
Ditylum sp.	0	0	0	1	0	0	0	0	0	0	0	2	3
Entomoneis spp.	19	14	29	16	20	42	41	2	5	23	**46**	15	272
Grammatophora sp.	0	0	1	0	0	0	0	3	0	1	0	0	5

Guinardia spp.	0	7	5	**19**	**19**	0	3	2	0	0	1	0	56
Gyrosigma spp.	6	17	2	14	15	1	0	5	6	4	**20**	10	100
Haslea sp.	0	0	0	0	**3**	0	0	0	3	0	3	1	10
Hemiaulus sp.	0	0	0	0	2	0	0	5	0	0	1	1	9
Isthmia sp.	0	0	0	0	0	0	0	0	4	2	0	0	6
Lauderia sp.	0	0	0	0	2	0	0	2	0	0	0	0	4
Leptocylindricus spp.	**29**	15	7	6	4	0	0	2	3	0	9	1	76
Licmophora sp.	0	1	0	0	0	4	0	1	0	0	0	0	6
Mastogloia spp.	0	0	0	10	8	1	2	7	2	1	**15**	6	52
Melosira spp.	0	1	0	0	0	0	0	0	0	0	0	1	2
Navicula spp.	73	133	70	166	75	**710**	89	32	22	194	166	74	**1804**
Nitzschia spp.	73	14	174	130	104	**702**	189	81	69	266	**2322**	30	**4154**
Odontella spp.	6	4	3	0	6	0	1	0	0	**18**	0	0	38

Palaria sp.	0	0	0	0	0	0	1	0	0	0	1	4	6
Pinnularia spp.	0	0	5	13	3	3	1	5	2	4	8	3	47
Pleurosigma spp.	**137**	29	41	11	14	5	1	14	5	15	28	31	331
Probosca spp.	0	0	0	0	0	0	0	0	0	0	1	1	2
Pseudonitzschia spp.	0	14	11	2	**35**	6	0	0	0	0	3	1	72
Rhizosolenia spp.	1	**47**	0	4	6	0	0	1	0	0	7	2	68
Skeletonema sp.	0	8	2	0	0	0	0	0	0	0	0	0	10
Stauroneis sp.	0	0	0	0	0	0	0	7	3	0	0	0	10
Surirella spp.	0	1	0	3	0	0	0	1	0	1	1	0	7
Synedra spp.	0	**17**	13	0	13	0	0	0	0	0	0	0	43
Thalassionema spp.	25	3	2	1	10	0	0	0	0	9	1	10	61
Thalassiosira spp.	55	133	80	107	45	**6083**	149	55	53	250	**2460**	27	**9497**
Thalassiothrix spp.	42	0	0	0	0	0	0	0	0	0	0	0	42

Trachyneis spp.	0	0	0	0	14	2	7	0	0	0	0	0	23
Trigonium sp.	0	0	2	0	0	0	0	0	0	0	0	0	2
Ulnaria spp.	0	8	20	28	0	1	2	**36**	3	3	0	0	101
Dictyochophyceae													
Dictyocha sp.	0	0	0	0	5	0	0	0	0	1	0	0	6
Dinophyceae													
Alexandrium spp.	0	0	1	8	0	17	24	3	5	11	11	1	81
Amphidinium spp.	0	83	7	0	6	24	8	2	7	3	8	0	148
Ceratium spp.	0	0	0	2	0	0	1	1	0	1	0	6	11
Dinophysis spp.	2	0	4	0	1	0	0	0	0	0	1	**2238**	2246
Ebria sp.	0	25	0	1	0	0	0	0	0	0	0	0	26
Gambierdiscus sp.	0	3	2	0	0	0	0	0	0	0	0	0	5
Gonyaulax spp.	0	0	39	8	5	0	20	2	0	0	0	24	98

Gymnodinium spp.	0	55	4	13	32	88	21	10	9	3	6	1	242
Gyrodinium spp.	0	17	4	1	6	14	1	5	26	3	7	0	84
Heterocapsa spp.	0	0	9	5	1	23	29	4	20	135	28	13	267
Karenia sp.	0	0	0	0	0	18	35	13	1	0	0	0	67
Lingulodinium spp.	0	0	0	0	0	0	5	0	0	0	0	10	15
Nocticula sp.	3	2	0	3	0	0	0	0	0	0	0	0	8
Ostreopsis sp.	0	0	3	0	9	3	0	2	0	0	0	0	17
Oxyrrhis spp.	0	0	0	4	11	47	0	3	4	3	0	0	72
Pentapharsodinium sp.	0	0	11	0	1	0	0	0	0	0	1	0	13
Peridinium spp.	0	0	22	16	15	43	130	43	8	4	5	11	297
Prorocentrum spp.	24	8	84	7	31	13	61	30	10	17	17	**3003**	**3305**
Protoperidinium spp.	0	10	1	0	5	**761**	46	30	0	3	19	15	890
Scrippsiella trichoidea	12	61	63	8	17	45	43	56	12	4	44	19	384

Euglenoidea													
Euglena spp.	17	0	5	0	19	1	0	10	0	0	0	4	56
Eutreptiella spp.	0	0	10	0	9	4	5	0	4	1	5	0	38
Oligotrichea													
Amphorellopsis sp.	0	0	0	0	0	0	0	0	0	0	0	5	5
Codonellopsis sp.	0	0	0	0	0	0	0	29	0	0	1	1	31
Favella sp.	1	1	0	0	1	0	0	1	0	0	0	1	5
Helicostomella sp.	0	0	0	0	0	0	0	0	0	0	0	1	1
Leegaardiella sp.	0	0	0	0	0	0	0	0	8	23	25	0	56
Leprotintinnus spp.	0	0	0	3	0	0	0	0	0	0	0	0	3
Stenosomella sp.	0	0	0	0	0	0	0	0	0	2	0	0	2
Strombilidium spp.	46	16	23	0	0	4	0	0	69	42	**2517**	0	2717
Tintinnopsis sp.	0	0	1	5	0	0	1	3	1	1	0	4	16

Spirotrichea													
Stylonychia sp.	0	0	0	0	18	**148**	42	0	0	0	0	0	208
Mesozooplankton	0	0	4	5	7	0	1	2	2	0	4	9	34
Fish egg	0	0	0	0	0	0	1	5	0	8	0	6	20
Total (cells//ind. ml^{-1})	**791**	**940**	**1037**	**942**	**817**	**9103**	**1114**	**739**	**386**	**1193**	**7986**	**5660**	**30708**

Appendix B2: *Monthly compositions of plankton functional groups in Benya Lagoon from August, 2020 to September, 2021*

	2020			2021							
Plankton functional groups	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Bacillariophyceae	86.12	68.57	69.47	83.91	70.94	85.14	51.2	48.92	49.61	70.99	65.2	4.66
Dinophyceae	5.13	27.94	24.31	7.84	17.07	12.04	37.82	27.69	26.62	15.67	1.84	94.23
Cyanophyceae	0.75	1.69	1.34	4.54	2.78	0.15	5.53	7.66	1.03	3.6	0.63	0.55
Oligotrichea	5.87	1.8	2.3	1.13	0.12	0.04	0.09	4.53	20.16	5.7	31.84	0.21
Euglenoidea	2.13	0	2.2	0	3.39	0.09	0.45	1.34	1.03	0.08	0.06	0.07
Chlorophyceae	0	0	0	2.06	4.24	0.28	4.19	8.74	1.03	3.19	0.38	0.02
Mesozooplankton	0	0	0.38	0.52	0.85	0	0.45	0.37	0.52	0	0.05	0.16
Fish egg/larvae	0	0	0	0	0	0	0.09	0.67	0	0.67	0	0.11
Spirotrichea	0	0	0	0	0	2.28	0.18	0	0	0	0	0

Dictyochophyceae	0	0	0	0	0.61	0	0	0.08	0	0	0	0
Total compositions	100	100	100	100	100	100.02	100	100	100	99.9	100	100.01

Appendix B3: *Monthly abundance (density) plankton (cells//ind. ml-1) in Narkwa lagoon*

	2020				2021								
Taxon	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Overall Total
Chlorophyceae													
Dunaliella spp.	0	0	0	0	0	37	27	53	0	83	10	35	245
Ulothrix sp.	0	0	0	2	3	5	2	0	0	0	0	0	12
Sub-total													
Cyanophyceae													
Chroococcus sp.	0	0	2	0	1	0	0	0	0	0	0	0	3
Epigloesphaera sp.	0	0	0	0	30	0	14	6	2	15	0	0	67
Merismopedia spp.	0	3	2	0	3	1	0	1	0	0	0	0	10
Oscillatoria sp.	1	0	0	0	3	0	0	1	0	0	1	1	7

Trichodesmium sp.	0	12	0	0	5	0	0	1	2	0	0	0	20
Unidentified cyanobacteria spp.	0	0	10	4	19	1	8	0	0	0	0	0	42
Bacillariophyceae													
Achanthes spp.	0	0	0	0	3	2	0	13	1	8	2	3	32
Amphiprora spp.	1	0	0	0	0	5	0	0	0	0	0	0	6
Amphora spp.	4	31	16	34	45	27	31	27	12	29	11	18	285
*Asterionellopsis s*p.	2	0	18	0	19	3	15	0	0	9	0	0	66
Bacillaria sp.	0	3	3	2	4	3	30	2	2	10	0	11	70
Bacteriastrum sp.	0	0	2	0	1	0	0	0	0	0	0	0	3
Cerataulina sp.	0	9	0	1	1	2	0	0	0	0	1	0	14
Chaetoceros spp.	43	9	32	0	24	**624**	4	2	0	1	0	0	739
Cocconeis spp.	0	0	1	8	6	3	0	9	1	14	4	6	52

Coscinodiscus spp.	120	10	48	36	0	2	5	38	1	0	1	7	268
Cylindrotheca spp.	53	115	139	62	67	47	40	86	37	108	24	8	786
Cymatopleaura sp.	0	7	0	0	0	2	23	5	0	0	8	5	50
Cymbella spp.	0	3	0	4	13	1	12	23	0	21	10	7	94
Detonula sp.	0	0	0	2	0	0	1	0	0	0	1	1	5
Diatoma sp.	0	0	0	0	15	1	0	3	0	2	0	0	21
Diploneis spp.	0	0	0	0	4	3	1	11	1	4	2	4	30
Entomoneis spp.	19	14	29	16	6	3	9	4	1	4	17	9	131
Grammatophora sp.	0	0	1	0	6	5	0	3	0	7	0	0	22
Guinardia spp.	0	7	5	60	33	3	0	13	0	0	0	0	121
Gyrosigma spp.	6	17	2	8	12	5	5	11	1	2	40	2	111
Haslea sp.	0	0	0	0	5	2	0	0	0	6	0	0	13
Hemiaulus sp.	0	0	0	0	2	0	0	0	0	0	0	1	3

Isthmia sp.	0	0	0	1	0	0	0	0	2	0	0	0	3
Leptocylindricus spp.	29	15	7	17	5	0	0	1	4	0	0	2	80
Licmophora sp.	0	1	0	0	0	0	0	1	0	0	0	0	2
Lithodesmium sp.	0	0	0	0	0	0	0	7	0	0	0	1	8
Mastogloia spp.	0	0	0	2	1	3	3	2	0	0	0	0	11
Melosira spp.	0	1	0	1	0	0	5	0	1	0	0	0	8
Navicula spp.	73	133	70	67	79	31	57	55	7	86	73	25	**756**
Nitzschia spp.	73	14	174	145	107	120	112	188	27	176	76	29	**1241**
Odontella spp.	6	4	3	1	0	0	0	1	0	0	0	3	18
Palaria sp.	0	0	0	0	0	0	0	2	5	3	4	4	18
Pinnularia spp.	0	0	5	0	5	4	4	23	3	20	7	9	80
Pleurosigma spp.	137	29	41	3	12	12	14	26	3	12	99	8	396
Probosca spp.	0	0	0	3	1	0	0	0	0	0	0	0	4

Pseudonitzschia spp.	0	14	11	3	66	8	30	14	0	9	1	0	156
Rhizosolenia spp.	1	47	0	5	1	0	0	6	0	0	1	5	66
Skeletonema sp.	0	8	2	0	0	0	0	0	0	0	0	0	10
Stauroneis sp.	0	0	0	0	0	0	0	1	1	5	0	0	7
Surirella spp.	0	1	0	0	1	0	8	31	0	11	3	11	66
Synedra spp.	0	17	13	0	0	13	12	3	0	0	0	0	58
Thalassionema spp.	25	6	2	2	7	1	4	11	0	0	1	4	63
Thalassiosira spp.	105	133	80	0	26	102	10	9	0	3	0	3	471
Trachyneis spp.	0	0	0	0	0	2	0	6	0	11	1	0	20
Ulnaria spp.	0	8	20	31	0	0	3	1	5	5	0	0	73
Dictyochophyceae													
Dictyocha sp.	0	0	0	0	1	0	3	6	0	4	1	4	19
Dinophyceae													

Alexandrium spp.	0	0	1	23	0	3	341	26	0	17	0	0	411
Amphidinium spp.	0	83	7	0	0	2	15	0	1	0	2	0	110
Ceratium spp.	0	0	0	1	1	4	13	3	1	0	1	0	24
Dinophysis spp.	2	0	4	1	0	0	4	1	0	0	1	3	16
Ebria sp.	0	25	0	0	0	0	0	5	0	0	0	0	30
Gambierdiscus sp.	0	3	2	0	0	0	0	0	0	0	0	0	5
Gonyaulax spp.	0	0	31	39	3	3	2	5	2	0	2	4	91
Gymnodinium spp.	0	55	4	176	**1001**	**626**	**878**	26	31	10	12	11	**2830**
Gyrodinium spp.	0	17	4	18	15	3	**1232**	14	61	0	0	1	**1365**
Heterocapsa spp.	0	0	9	78	152	188	265	36	**1219**	50	79	22	**2098**
Karenia sp.	0	0	0	0	0	**1390**	2	9	0	10	0	0	**1411**
Lingulodinium spp.	0	0	8	1	0	1	0	0	0	0	0	9	19
Nocticula sp.	3	2	0	19	1	1	0	10	0	0	0	0	36

Ostreopsis sp.	0	0	3	0	**660**	16	4	0	0	0	0	0	**683**
Oxyrrhis spp.	0	0	0	0	1	3	1	6	0	0	0	0	11
Pentapharsodinium sp.	0	0	11	0	8	21	0	0	0	0	6	0	46
Peridinium spp.	0	0	22	7	116	**1480**	**2062**	93	15	0	7	45	**3847**
Prorocentrum spp.	24	8	84	20	18	21	61	**4387**	2	7	16	126	**4774**
Protoperidinium spp.	0	10	1	75	41	92	188	25	10	55	63	85	**645**
Scrippsiella trichoidea	12	61	63	44	41	118	10	24	3	10	33	34	453
Unidentified	0	0	45	5	0	0	0	0	0	0	0	0	50
Dinoflagellate													
Euglena spp.	17	0	5	0	19	11	0	0	0	0	0	0	52
Eutreptiella spp.	0	0	10	7	0	4	0	23	5	11	82	0	142
Oligotrichea													
Amphorellopsis sp.	0	0	0	0	0	0	2	1	0	0	0	1	4

Codonellopsis sp.	0	0	0	0	0	5	5	5	0	2	1	0	18
Eutintinnus sp.	0	0	0	2	0	0	1	0	0	0	0	0	3
Favella sp.	1	1	0	0	0	0	0	3	0	1	0	0	6
Leegaardiella sp.	0	0	0	0	0	0	0	0	1	0	1	0	2
Leprotintinnus spp.	0	0	0	0	0	0	0	22	0	0	0	0	22
Steenstrupiella sp.	0	0	0	0	0	3	0	0	0	0	0	0	3
Strobilidium spp.	46	16	23	0	0	0	0	0	3	0	5	0	93
Tintinnopsis sp.	0	0	1	0	0	20	1	5	4	0	1	4	36
Spirotrichea													
Stylonychia sp.	0	0	0	0	0	3	2	0	0	0	0	0	5
Mesozooplankton	0	0	4	3	4	8	0	11	0	6	13	9	58
Fish egg/larvae	0	0	0	0	0	0	3	16	0	1	15	24	59
Total (Cell/unit ml^{-1})	**803**	**952**	**1080**	**1037**	**2720**	**5101**	**5580**	**5462**	**1477**	**848**	**739**	**604**	**26403**

Appendix B4: Monthly compositions (%) of plankton functional groups in Narkwa Lagoon from September, 2020 to August, 2021

	2020				2021							
Plankton functional groups	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Bacillariophyceae	86.13	70.37	66.61	49.66	21.05	20.18	7.85	10.77	14.74	66.51	52.23	31.25
Dinophyceae	5.12	26.14	27.43	48.52	76.42	77.78	91.49	87.52	83.08	18.68	31.53	55.92
Cyanophyceae	0.75	1.69	1.28	0.67	1.54	0.039	0.39	0.17	0.51	2.12	0.14	0.16
Oligotrichea	5.88	1.8	2.2	0.19	0	0.65	0.16	0.6	1.03	0.35	1.08	0.82
Euglenoidea	2.13	0	2.11	0.67	0.7	0.29	0	0.39	0.64	1.29	11.09	0
Chlorophyceae	0	0	0	0	0.11	0.72	0	0	0	9.75	0	5.76
Mesozooplankton	0	0	0.37	0.29	0.18	0.16	0	0.18	0	0.71	1.76	1.48
Fish egg/larvae	0	0	0	0	0	0	0.054	0.27	0	0.12	2.03	3.95

Spirotrichea	0	0	0	0	0	0.18	0	0	0	0	0	0
Dictyochophyceae	0	0	0	0	0	0	0.054	0.1	0	0.47	0.14	0.66
Total compositions	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Appendix C1: *Monthly compositions (%) of ingested plankton functional groups by C. tulipa population in Benya Lagoon*

	2020			2021							
Plankton functional groups	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Bacillariophyceae	86.12	68.57	69.47	83.91	70.94	85.14	51.2	48.92	49.61	70.99	65.2	4.66
Dinophyceae	5.13	27.94	24.31	7.84	17.07	12.04	37.82	27.69	26.62	15.67	1.84	94.23
Cyanophyceae	0.75	1.69	1.34	4.54	2.78	0.15	5.53	7.66	1.03	3.6	0.63	0.55
Oligotrichea	5.87	1.8	2.3	1.13	0.12	0.04	0.09	4.53	20.16	5.7	31.84	0.21
Euglenoidea	2.13	0	2.2	0	3.39	0.09	0.45	1.34	1.03	0.08	0.06	0.07
Chlorophyceae	0	0	0	2.06	4.24	0.28	4.19	8.74	1.03	3.19	0.38	0.02
Mesozooplankton	0	0	0.38	0.52	0.85	0	0.45	0.37	0.52	0	0.05	0.16
Fish egg/larvae	0	0	0	0	0	0	0.09	0.67	0	0.67	0	0.11
Spirotrichea	0	0	0	0	0	2.28	0.18	0	0	0	0	0
Dictyochophyceae	0	0	0	0	0.61	0	0	0.08	0	0	0	0
Total compositions	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Appendix C2: *Monthly compositions (%) of ingested plankton functional groups by C. tulipa population in Narkwa Lagoon*

	2020				2021							
Plankton functional groups	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Bacillariophyceae	66.86	55.55	35.14	40.21	31.33	29.35	43.18	78.79	51.96	73.91	21.08	12.79
Dinophyceae	26	7.11	44.25	51.13	58.53	11.94	47.89	15.38	46.84	17.39	76.2	82.6
Cyanophyceae	3.43	22.67	2.17	5.21	6.31	7.05	5.68	0.12	0.85	0	0.3	0.21
Oligotrichea	0.86	0.44	12.15	0	0	0.39	0.97	0.23	0.03	2.17	0.3	0.84
Euglenoidea	2.86	8	2.82	2.11	0.58	1.76	0.81	0	0.03	0	0	0
Chlorophyceae	0	4.44	0	0	0	15.26	0.16	1.51	0.22	6.52	0	0
Mesozooplankton	0	1.33	3.47	1.10	3.24	0	0.48	0.7	0.05	0	0.6	0.21
Fish egg/larvae	0	0	0	0.22	0	34.25	0.32	3.26	0	0	1.51	3.35
Spirotrichea	0	0.44	0	0	0	0	0	0	0	0	0	0

Dictyochophyceae	0	0	0	0	0	0	0.49	0	0.03	0	0	0
Total compositions	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Appendix C3: *Distribution of monthly plankton selectivity of C. tulipa population in Benya Lagoon from September, 2020 to August, 2021*

	2020				2021							
Plankton functional group	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Bacillariophyceae	-0.92	-0.73	-0.59	-0.81	-0.72	-0.72	-0.24	-0.45	-0.38	-0.64	-0.87	-0.93
Dinophyceae	0.94	0.43	0.008	0.76	0.65	0.2	-0.42	-0.005	-0.39	0.06	0.68	0.89
Cyanophyceae	1	1	0.97	0.43	0.89	0.96	0.46	0.88	0.92	0	0.97	0.89
Oligotrichea	0.23		1	1	1			0.59		0.31		
Euglenoidea	-0.99	1	0.57				0.36					
Chlorophyceae				-0.21	-0.64			-0.87	-0.48	-0.91	-0.56	
Mesozooplankton		1		0.91	0.9	1	0.97	0.43		1	1	
Fish egg/larvae				1			1	0.65	1	0.25	1	
Dictyochophyceae										0.31		

Appendix C4: *Distribution of monthly plankton selectivity of C. tulipa population in Narkwa Lagoon from September, 2020 to August, 2021.*

	2020				2021							
Plankton groups	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Bacillariophyceae	-0.73	-0.72	-0.77	-0.74	0.8	0.37	0.71	0.94	-1	0.08	-0.84	-0.46
Dinophyceae	0.77	-0.28	0.47	0.68	0.75	-0.95	-0.77	-0.8	-0.83	0.04	0.86	0.53
Cyanophyceae	1	1	0.98	0.95	1	0.98	1	1	0.68		1	1
Oligotrichea	0.69	0.44	0.98	0.98	0.85	1	0.83	0.24	1	1	1	1
Euglenoidea	0.021	1	0.29	0.34	1	0.46	1		-0.87			
Chlorophyceae		-1	-0.71		-0.56	-0.3	-0.66	-0.92	-0.3	-0.36		
Mesozooplankton		1	0.7	1	1	0.95	1	0.56	1		-0.64	-0.98
Fish egg/larvae					1	1	0.57	0.84			-0.36	-0.1
Dictyochophyceae							0.69		1			

Appendix C5: *Distribution of monthly compositions of ingested toxic phytoplankton taxa in Benya Lagoon from September, 2020 to August, 2021.*

	2020				2021							
Phytoplankton taxon	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Prorocentrum spp.	34.89	6.17	14.95	21.78	3.73	0.14	0.21	8.23	2.54	19.56	6.43	38.40
Alexandrium spp.	0.46	2.95	0.00	0.87	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00
Karenia sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ostreopsis spp.	0.00	0.00	0.93	0.00	1.08	0.03	0.00	0.40	0.00	0.00	0.00	0.00
Dinophysis spp.	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.16	12.09
Gymnodinium spp.	1.07	0.00	3.74	1.95	3.86	2.48	5.50	0.00	0.00	0.00	0.00	0.00

Heterocapsa spp.	1.07	0.00	0.00	0.87	0.12	0.26	2.75	0.00	0.00	0.24	0.00	0.00
Gonyaulax spp.	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudonitzschia sp.	0.04	0.00	0.00	0.00	4.22	0.00	0.00	1.20	0.00	0.00	2.09	0.00
Amphidinium spp.	0.00	0.00	0.00	0.33	0.72	0.00	0.42	0.00	3.21	0.73	0.00	1.10
Others	62.47	90.88	80.37	74.21	85.54	96.90	91.12	90.16	94.25	79.47	91.32	48.41
Total compositions	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Appendix C6: *Distribution of monthly compositions of ingested toxic phytoplankton taxa in Narkwa Lagoon from September, 2020 to August, 2021.*

	2020				2021							
Phytoplankton taxon	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
Prorocentrum spp.	5.71	0.87	35.76	1.91	0.66	0.98	29.22	12.44	0.55	10.35	75.53	75.47
Alexandrium spp.	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Karenia sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00
Ostreopsis spp.	0.00	0.00	0.00	0.00	24.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dinophysis spp.	0.00	0.00	0.64	0.10	0.00	0.00	8.60	0.35	0.00	0.00	0.00	0.84
Gymnodinium spp.	0.00	0.00	4.07	16.79	36.71	0.00	0.00	0.35	3.07	0.30	0.00	0.00

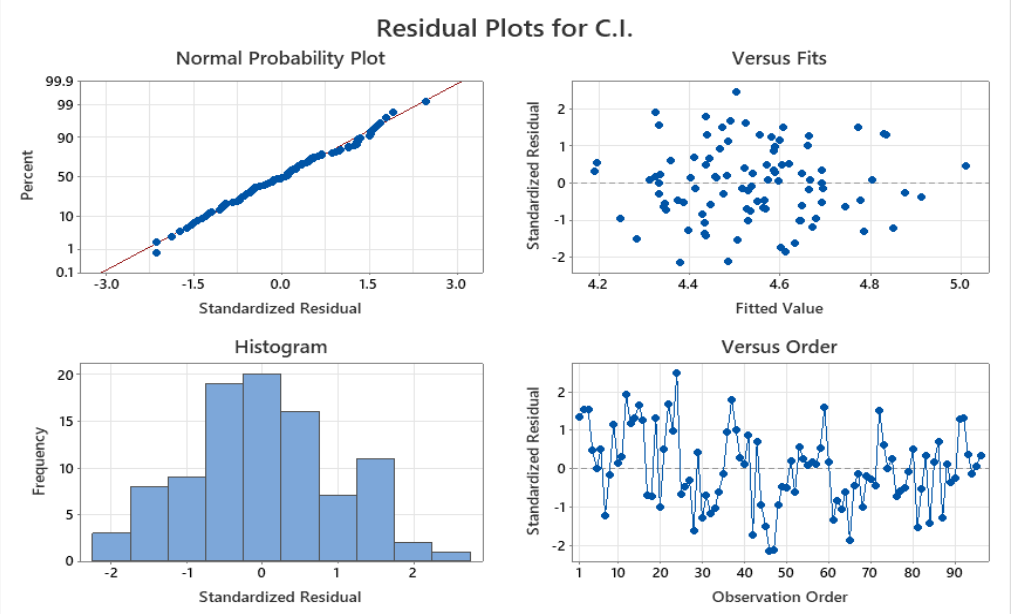
Heterocapsa spp.	0.00	0.00	2.36	7.44	5.57	0.00	0.00	0.00	12.81	15.22	0.00	0.00
Gonyaulax spp.	0.00	0.00	0.21	3.72	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudonitzschia sp.	0.29	0.87	0.00	0.29	2.42	0.00	4.38	0.23	0.33	0.00	0.00	0.00
Amphidinium spp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.11	0.00	0.00	0.00
Others	90.00	98.25	56.97	69.75	30.33	99.03	57.80	86.03	83.13	74.13	24.47	23.69
Total compositions	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Appendix D: *Size-frequency distribution of C. tulipa populations in Benya and Narkwa Lagoons*

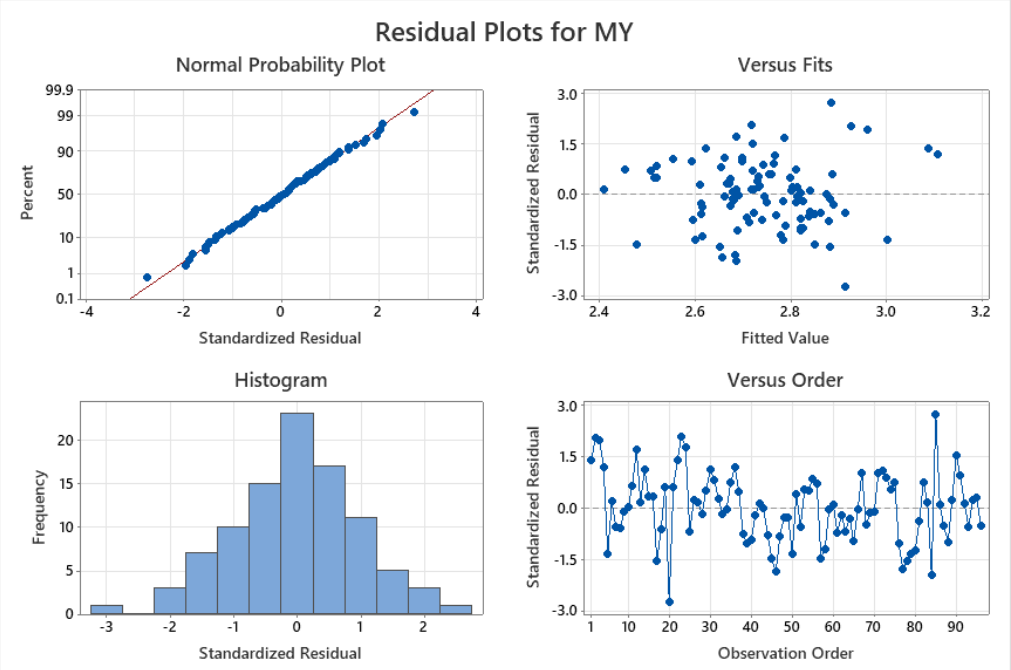
	Benya		Narkwa	
Class size	Frequency	% Frequency	Frequency	% Frequency
3.0-3.9	28	5.11	42	8.17
4.0-4.9	109	19.89	141	27.43
5.0-5.9	137	25.00	190	36.96
6.0-6.9	125	22.81	83	16.15
7.0-7.9	95	17.34	37	7.20
8.0-8.9	46	8.39	15	2.92
9.0-9.9	7	1.28	5	0.97
10.0-10.9	1	0.18	1	0.19
Total	548	100.00	514	100.00

Appendix E: Durbin-Watson statistic for Transformed responses used for multiple linear regression analysis

Durbin-Watson statistic = 1.24303



Durbin-Watson statistic = 1.30392



Appendix F1: *Instantaneous growth rate of C. tulipa larvae fed on five different microalgae fed treatment*

Day	Natural	*Pseudanabaena* sp.	*Nannochloropsis* sp.	*Rhodomonas* sp.	Mixed
1	65.12 ± 3.15	65.12 ± 3.15	65.12 ± 3.15	65.12 ± 3.15	65.12 ± 3.15
3	71.09 ± 3.29	66.83 ± 1.67	67.14 ± 1.66	66.67 ± 1. 21	76.9 ± 2.20
5	80.21 ± 1.78	71.25 ± 1.77	72.08 ± 1.90	73.85 ± 1.27	88.08 ± 1.91
7	96.27 ± 3.52	77.79 ± 2.04	78.27 ± 1.97	85.60 ± 1.95	100.02 ± 2.37
9	118.87 ± 1.02	88.33 ± 2.84	89.68 ± 2.89	99.19 ± 3.57	128.12 ± 1.83
11	131.79 ± 2.95	103.55 ± 4.03	108.167 ± 3.75	120.88 ± 3.40	153.65 ± 4.14
13	163.77 ± 4.07	121.23 ± 3.14	128.08 ± 1.94	134.96 ± 3.88	175.96 ± 3.85
15	196.27 ± 3.52	128.92 ± 1.02	135.92 ± 3.90	158.46 ± 4.42	217.31 ± 2.02
17	229.06 ± 5.23	152.21 ± 5.30	153.43 ± 5.26	169.17 ± 3.70	248.68 ± 6.86
19	257.15 ± 3.27	170.31 ± 2.77	175.92 ± 3.90	195 ± 4.42	286.81 ± 2.42
21	275.04 ± 5.88	199.92 ± 2.78	205.88 ± 3.68	217.08 ± 2.90	312.5 ± 5.52

Source of variation	DF	Adj SS	Adj MS	*F*-Value	*P*-Value	
Days	10	5367092	536709	44.56	0.000	*
Diet	4	752517	188129	15.93	0.001	*
Days×Diet	40	481795	12045	1063.58	0.000	*
Error	1535	17384	11			
Total	1589	6571415				

Appendix E2: *Instantaneous survival rate of C. tulipa larvae fed on five different microalgae fed treatment*

Day	Natural	*Pseudanabaena* sp.	*Nannochloropsis* sp.	*Rhodomonas* sp.	Mixed
1	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3	77.48 ± 0.80	75.53 ± 0.92	75.77 ± 0.30	72.63 ± 1. 13	77.72 ± 1.10
5	64.99 ± 0.22	63.35 ± 1.64	65.29 ± 0.60	67.47 ± 0.29	74.10 ± 0.41
7	60.43± 2.06	57.63 ± 1.20	60.22 ± 0.36	62.10 ± 0.96	70.72 ± 0.11
9	50.43 ± 1.10	54.61 ± 1.20	58.84 ± 0.65	59.57 ± 0.31	68.93 ± 0.35
11	47.42 ± 0.68	52.84 ± 0.30	54.16 ± 0.25	57.10 ± 0.51	64.19 ± 0.45
13	45.65 ± 1.16	47.59 ± 0.98	50.61 ± 0.61	54.77 ± 1.07	60.67 ± 0.57
15	41.37 ± 0.96	44.19 ± 1.15	49.01 ± 0.33	51.37 ± 0.96	59.02 ± 0.80
17	39.40 ± 0.76	41.40 ± 0.28	45.95 ± 0.48	47.74 ± 0.78	55.95 ± 0.48
19	35.56 ± 1.09	39.16 ± 0.23	42.22 ± 1.53	45.38 ± 0.70	52.07 ± 1.36
21	30.95 ± 1.06	37.92 ± 1.46	38.72 ± 0.49	43.72 ± 0.21	48.73 ± 0.95