UNIVERSITY OF CAPE COAST

MESOZOOPLANKTON ABUNDANCE, COMPOSITION AND RESPONSE TO GLOBAL CLIMATE CHANGE IN THE VOLTA ESTUARINE SYSTEM

BY

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DECLARATION

Candidate's Declaration

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

Candidate's Signature ..... Date .....…

Name .....

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 .....

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

Name .....……..

ABSTRACT

Projections suggest that global climate change is warming and changing the salinity of brackish waters (e.g., lagoons, estuaries), with impacts on nursery and feeding habitats for fish and other organisms. This study evaluated the response of mesozooplankton - responsible for energy transfer to higher trophic levels - to the combination of these climate change factors. It was conducted using samples from the Volta estuary. A field study on transects indicated that, in terms of species composition, mesozooplankton of the area were dominated by copepods (68%) followed by decapods (25%), cladocerans (4.5%) and rotifers (0.5%); these were related to chlorophyll-a concentration, temperature and dissolved oxygen of the estuary. Combined impacts of warming and changes in salinity were assessed on two cosmopolitan mesozooplankton - *Temora Stylifera and Paracalanus parvus* - using microcosm experiments involving different levels of salinity (22, 21, 25, 29 and 30 ppt) and warming (+0, +2 and +4 °C). Combination of these factors could explain ≈74% of the variations in egg production rate (EPR) by *Temora*; EPR of the copepod decreased (70%) with each degree of warming. In contrast, only feacal pellet production (FPP) by *Paracalanus* could be related to the combination of the two climate change factors; FPP decreased by ≈56% when the copepod was exposed to increasing temperature and salinity. It can therefore be said that different species of estuarine mesozooplankton respond differently to the combined impacts of surface warming and salinity changes expected under global climate change.

KEY WORDS

Mesozooplankton

Multiple stressors

Volta estuary

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DEDICATION

To Kwabena Owusu Amoako of Blessed Memory

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LIST OF ACRONYMS

IPCC	Intergovernmental panel on climate change
GCLME	Guinea Current Large Marine Ecosystem
GMA	Ghana Meteorological Association
UCC	University of Cape Coast
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated biphenyls
DDT	Dichlorodiphenyltrichloroethane
EPR	Egg production rate
FPR	Faecal pellet production rate
MR	Mortality rate
NM	Nautical mile

CHAPTER ONE

INTRODUCTION

Background of the Study

Estuaries provide significant ecological and economic importance. Many of these ecosystems are rich in biodiversity; and they provide a variety of habitats that serve as major nursery sites for commercial fishing species (Beck *et al*., 2001). Additionally, they serve as feeding and breeding spaces for populations of terrestrial animals, such as birds (Xia *et al*., 2020). Estuaries are noted for capturing, filtering, and recycling nutrients, pathogenic viruses, and suspended particulate matter that may be harmful to human health (Davies *et al*., 2016).

While an estuary's salinity is typically lower than that of natural sea water and varies over time and along its length, it can become hypersaline in areas where evaporative water loss is high and freshwater and tidal inputs are insufficient, according to Potter, Chuwen, Hoeksema, and Elliott (2010). These systems are highly dynamic as they provide a region with a complex interaction of physical, geological and chemical as well as different biological processes, all of which are influenced by the marine environment and the surrounding catchment (Aubry & Elliott, 2006; McLusky & Elliott, 2004).

Challenges Facing Coastal Estuaries

Despite the significant role estuarine ecosystems play, recent studies indicate that estuaries are being degraded at an alarming rate worldwide (Paerl, Peierls & Rossignol, 2014; Whitfield, 2021;). This has been attributed to the accelerating rate of urbanization and industrialization which comes along with the

constant increase in population growth (Kennish, 2002). Estuarine ecosystems have become receptacles for domestic and industrial effluents which release harmful heavy metals, polycyclic aromatic hydrocarbons and excessive levels of organic and inorganic nutrients that contribute to pollution in estuaries (Asare- Donkor *et al*., 2015; Boateng *et al*., 2020; Nyarko *et al*., 2010; Okyere, 2018; Olympio & Amos-Abanyie, 2013).

Furthermore, challenges related to global climate change, such as increasing temperatures, precipitation and rising sea levels, result in substantial impacts on estuaries world wide (Edgar *et al*., 2000) (Figure 1).

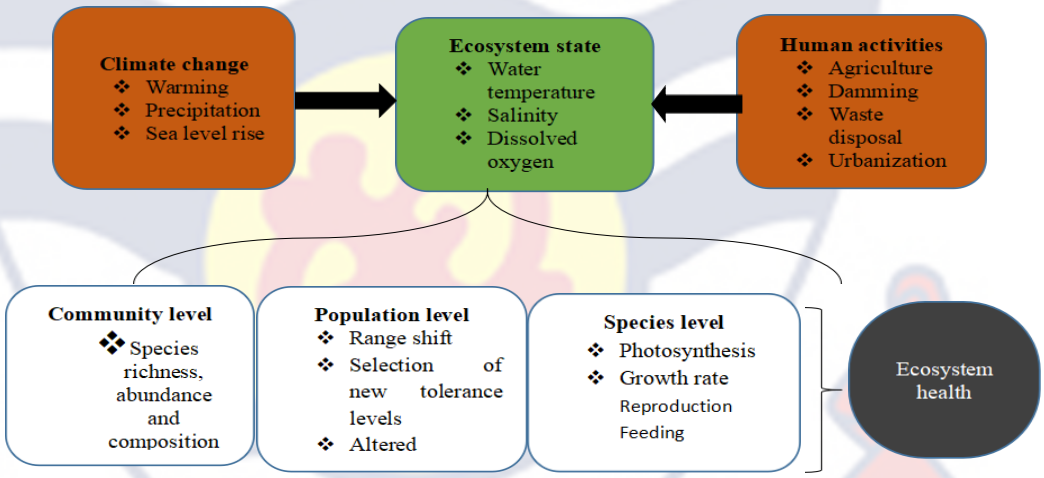

Figure 1*:* Illustration of Global change impact on coastal ecosystems. Adapted from Kunzmann et al. (2018)

Global climate change has been labelled as a key factor that is causing rapid changes in various ecosystems around the world. It may be defined as a change in the time variation of weather conditions over a period in the average climatic conditions over a long period (IPCC, 2007). These changes have been reflected in the increase in the average temperatures, melting of the polar ice, changes in the frequency and intensity of precipitation etc. Human activities including the burning

of fossil fuel and cement manufacture have emitted nearly 2000 gigatons of carbon dioxide (CO_2) into the atmosphere from the beginning of the industrialization period according to IPCC (2014) report. In just 200 years, these human activities have caused atmospheric CO_2 concentrations to rise rapidly from 280 parts per million before industrialisation to nearly 400 parts per million (IPCC, 2014; Siegenthaler *et al*., 2005)

According to the IPCC (2007 and 2013) reports, global temperatures are expected to rise by 1–5°C in the twenty-first century (Figure 2), with anthropogenic activities identified as its primary driver as reported by the National Research Council (2012); National Academy of Sciences (2014) and the Australian Academy of Sciences (2015).

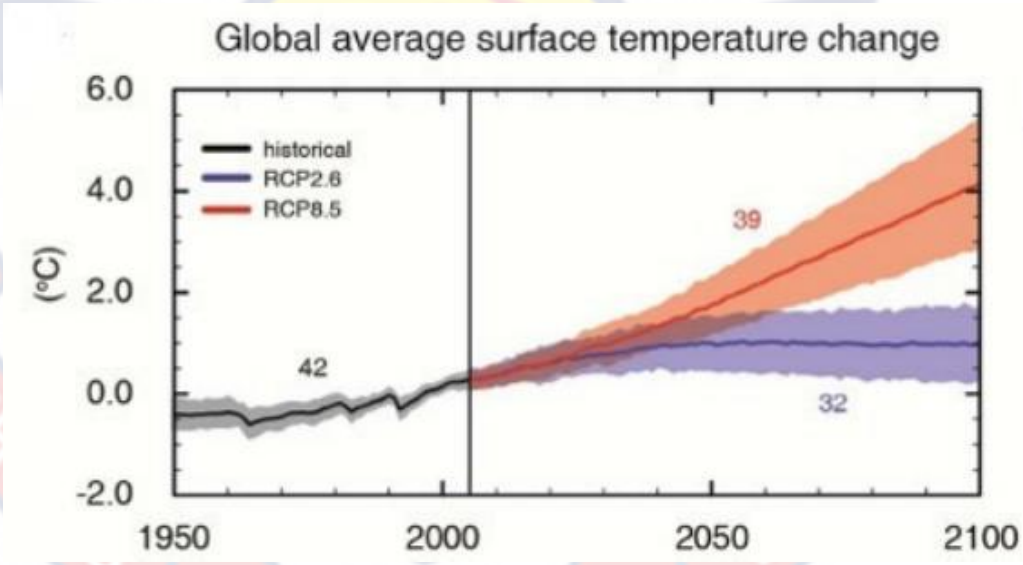

Figure 2: Historical and future projections of changes in annual mean surface temperature. IPCC 2013 report.

Not only will temperature rise as predicted, but also sea level rise, heat waves as well as extreme hydrological oscillations (such as floods and droughts)

will significantly alter the dynamics of salinity, nutrients and pollutants and their interactions with biota in estuarine and ecosystems.

Vulnerability of tropical estuarine ecosystems to global climate change.

Tropical estuaries are highly productive and support a variety of organisms (Bissoli & Bernardino, 2018). They are characterized by relatively mild temperatures with little or no annual variations, and significant precipitation in at least one part of the yearLecher & Goldstein (1997). These ecosystems are believed to be very vulnerable to global change, as they are believed to be living at their optimum.

Previous studies have established that the effects of temperature increase may have severe impacts on tropical coastal ecosystems (IPCC *et al*., 2018) as they are particularly vulnerable to these changes, as many of the species in these systems already live at the edge of their tolerance (Nguyen *et al*., 2011), and many of the tropical areas are already under threats from multiple anthropogenic stressors (Halpern *et al*., 2015). These stressors alter the state of tropical estuaries and therefore making it harder for these systems to produce the natural services and maintain their resilience to global climate change (IPCC, 2014).

Impacts of global climate change on zooplankton biology

According to the National Estuarine Research Reserve [NERR] (1997) report, changes in temperature impacts the composition and abundance of estuarine organisms as well as their functional properties as these parameters greatly impact other parameters like conductivity, salinity, pH and dissolved oxygen. According to Delorenzo (2015), fluctuations in abiotic conditions can cause different levels of

stress in estuarine organisms though they have unique adaptation measures to tolerate a wide range of water conditions (Mora & Ospina, 2001).

Warmer temperatures have been reported to increase metabolic activities in ectothermic organisms, such as zooplankton, according to Brown *et al*. (2004). When zooplankton is exposed to warmer temperatures, it exhibits higher feeding rates (Turner, 2004), increase in respiration (Lehette *et al*., 2016; Zervoudaki *et al*., 2014), and growth (Roman *et al*., 2019). According to Bakhtiyar *et al*. (2021), as the temperature approaches lethality above the thermal optima level, filtering rate also declines. Warmer temperatures decrease viscosity and dissolved oxygen and thus affecting the feeding rate of zooplankton (Vanderploeg, 2020). Again, warming has an inverse association with species growth, and increase in metabolic rate slow down species growth.

Zooplankton is no exception as research has indicated that increased temperatures alters their growth rate. Also, the size of individual zooplankton at maturity is highly affected by higher temperatures (Roman *et al*., 2019). According to Lee *et al*. (2020), the growth and hatching success of zooplankton and the survival of their offspring is generally controlled by temperature, thus affecting their rate of reproduction. Some researchers in their experimental studies discovered that when copepods were exposed to warming stress, their rate of survival and reproduction decreased (Ruiz *et al*., 2020).

Tropical estuaries and zooplankton studies

In almost all pelagic ecosystems around the world, zooplankton serve as vital linkages between producers and higher organisms, transporting nutrients,

organic matter, and energy (Figure 3).

Figure 3: Illustration of a simple aquatic food chain. Image created by Eric A. Krampah

Within the aquatic food web, interactions and processes that occur between producers, primary consumers, and secondary consumers provide the basis for matter and energy transfers throughout the entire ecosystem (Calbet, 2008; Steinberg & Landry, 2017).

Zooplankton are classified into two major distinct groups based on body size; microzooplankton (body size < 200 µm) and mesozooplankton (body size > 200 µm) (Pasternak, 2008). With regards to mesozooplankton in particular, they are the main primary consumers in many aquatic ecosystems as they are actively selected by higher trophic organisms, thus making them key prey items for fish (Meyers, 2020). Their faecal pellet has high concentration of carbon (Urban-Rich, Hansell & Roman, 1998). As pellets sink down the ocean, it buries higher amounts of carbon in a process known as the biological carbon pump (Frangoulis *et al*., 2005).

Mesozooplankton have a relatively short life cycle, as a result, they are inextricably linked to climate change and population dynamics. They have been described as more sensitive indicators of change in environmental factors by Hays, Richardson and Robinson (2005) and Taylor, Allen and Clark (2002). Small

changes in environmental conditions can have a great impact on mesozooplankton community structure, population size, and diversity.

Also, changes in oceanographic parameters such as temperature, salinity and primary production of water masses impact significantly on the functioning of mesozooplankton in coastal ecosystems (Mackas *et al*., 2001; Vidjak *et al*., 2012). Increase in temperature for example have a direct impact on the rate of biological processes - e.g.,feeding, reproduction and migration.- that underlie the productivity of mesozooplankton (Chen & Folt, 2002; Mackas *et al*., 2012). In some species of mesozooplankton (*Temora* and *Acartia*), biological rate increases with increasing temperature (Weissenberg *et al*., 2022); in others, the rate of biological processes does not directly vary with temperature (Hall & Burns, 2001). Damgaard and Davenport (1994) reported that the biological rates of mesozooplankton is significantly influenced by salinity fluctuations. Medina and Barata (2004) reported that the daily rate of mortality of *A. tonsa* increased by approximately 50% at high salinity values but remained constant at 5% in the optimum salinity.

Therefore, the referenced studies above suggest a great relationship between mesozooplankton and their environment. However, studies to quantify the extent of this relationship on mesozooplankton response is very limited especially in the tropics as studies have mostly been concentrated in temperate and polar regions (Bhattacharya, 1988). Among the few studies conducted in tropical waters is a study by Ruiz *et al*. (2020) which demonstrated the vulnerability of tropical copepod to oil pollution and marine heatwave in the Gulf of Guinea. The Guinea Current Large Marine Ecosystem (GCLME) also conducted a study in the Gulf of

Guinea from 2005 to 2006 to determine zooplankton abundance and structure in the region. A report from this study emphasised the seemingly lack of research initiatives on zooplankton in tropical waters which has left more issues on the subject unresolved. Dapaah (2013) in a study within the lower Volta estuary also emphasised the relationship between environmental parameters and variations in zooplankton composition.

However, most of the studies within tropical waters have been limited to zooplankton distribution and community structure with very limited knowledge on abundance, taxonomic composition and species biological response in relation to environmental changes in the event of climate change. This lack of knowledge is a limit on global efforts to predict the impact of climate change in part because organisms in the tropics have reduced adaptation to drastic changes in the environment as they are already living near their tolerance limit (Griffen *et al*., 2016).

Statement of the PROBLEM

Knowledge of zooplankton dynamics in tropical waters is very limited (Colloquium, 2001) as most studies focus on temperate regions. Out of the few works that exist most are centered on marine zooplankton distribution and community structure with very little understanding on estuarine zooplankton abundance and taxonomic composition. Knowledge on zooplankton biological response to environmental changes is also very limited with little or no knowledge on which eco-physiological indicator could best explain their response to environmental factors driven by climate change (Hernandez *et al*., 2021).

Indeed, the impact of multiple environmental factors is a major frontier in ecology. Most studies have been focused on individual stressors with a few on multiple stress impacts in exposure experiments under simulated conditions which may not give a true representation of the system (Crain *et al*., 2008). In general, there is limited knowledge on the cumulative effect of multiple stressors especially on estuarine organisms.

Purpose of the Study

This study focuses on mesozooplankton within the Volta estuary in Ghana. It determines the composition and abundance of mesozooplankton with the aim of providing the necessary baseline information for predicting changes in estuarine pelagic ecosystems. It also highlights the response of mesozooplankton to surface warming and salinity fluctuations expected under global climate change. In addition, the study identifies reasonable eco-physiological indicators for describing the combined effect of warming and salinity fluctuations on estuarine copepods. These indicators are needed for modelling investigations related to the dynamics of estuarine food webs under global climate change (Asiedu, 2020).

Research Objectives and Hypotheses

The overarching objective is to assess the abundance, composition and response of estuarine mesozooplankton to global climate change factors (warming and salinity fluctuations).

Specifically, the study:

1. Characterizes the physico-chemical conditions that determine the mesozooplankton abundance in a selected estuary

2. evaluates functional response of model estuarine copepods to warming and salinity fluctuations
3. identifies reasonable eco-physiological indicators for describing the combined effect of warming and salinity fluctuations on estuarine copepods

The study made and tested the following hypotheses:

1. Physico-chemical parameters influence the abundance and taxonomic composition of mesozooplankton.
2. Copepod functional response is altered by global climate change factors such as warming and salinity fluctuations.
3. Specific physiological rates may indicate the response of copepod species to warming and salinity fluctuations combined.

Statistical Hypotheses

H_{01}: There is significant difference between physico-chemical parameters and mesozooplankton abundance and taxonomic composition.

H_{A1}: There is no significant difference between physico-chemical parameters and mesozooplankton abundance and taxonomic composition.

H_{02}: There is significant difference between copepod functional response and global climate change factors warming and salinity fluctuations.

H_{A2}: There is no significant difference between copepod functional response and global climate change factors warming and salinity fluctuations.

H_{03}: There is significant difference in copepod physiological rates in response to warming and salinity fluctuations combined.

H_{A3}: There is no significant difference in copepod physiological rates in response to warming and salinity fluctuations combined.

Significance of the Study

Achieving the objectives indicated under the above Section will provide the data needed to understand the impact of global climate change factors on estuarine mesozooplankton. This will further provide understanding on the impact of these change factors on the aquatic pelagic food web.

The work will also provide the necessary evidence on the consequences of multiple stressors interactions in tropical coastal systems hence improve scientific understanding of global climate change factors and impacts on tropical estuarine organisms as well as the health of these ecosystems. The study will also lead to the identification of reasonable eco-physiological indicators necessary in understanding the health of the estuarine ecosystem and inform management strategies and actions needed.

Delimitations of the Study

The Volta River estuary at Ada presently serves as a single outlet channel of the Volta River into the sea. Transects were established to cover the entire estuary at Ada Foah from the point where the river enters the estuary to the point where the river finally discharges into the ocean. These transects were established to cover different water masses representing the different salinity ranges of the estuary. The first transect was established close to the surf zone of the estuary, the second and third were established within the middle section of the estuary and

finally the fourth transect was established at the point where the river entered the estuary (salinity less than 0.5ppt).

Physical and chemical parameters including salinity, temperature, conductivity, amount of dissolved oxygen, and acidity (pH) as well as chlorophyll-a concentration were measured. These parameters were measured in order to establish a link between the physico-chemical conditions and the abundance and composition of mesozooplankton as well as the relationship between primary production (chlorophyll-a concentration) and primary consumers (mesozooplankton).

For the microcosm study, the biological rate of fecal pellet production, egg production and mortality were taken into account. The rate of faecal pellet production was used as a proxy in determining the amount of food materials ingested by the copepod, egg production rate was used as a proxy for growth in copepod and the rate of mortality was used as a proxy for determining lethal effects of the combined stressors.

Limitations of the Study

Nutrient analysis was not done in this study as the study was mainly focused on the factors that impacted primary consumers other than primary producers. Mesozooplankton collection was not done at different depths as the estuary was shallow, hence a vertical haul through the entire water column was used to sample mesozooplankton.

In terms of the microcosm experiment, live copepods that were used for the experiment were not residents of the study area but was obtained from the marine

environment at Elmina, this was due to the inability to keep copepods alive from the study area to the laboratory where the experiment was conducted as aeration could not be provided for thermal mixing though organisms were kept on ice pack to prevent thermal stress. However, the copepods sampled for the experiment were allowed 12 hours to acclimatize to the water samples from the study area before the incubation period commenced. Also, the water taken from the estuary for the bioassay experiment might have other parameters that were not considered in the study.

Organisation of the Study

There are six chapters in this thesis. The first chapter provides an overview of the study, a description of the problem, its purpose, aim and objectives, significance in relation to the study as well as limitations and delimitations. Relevant literature relating to this study is examined in Chapter Two. It will focus on pollution, its sources and effects on marine ecosystems.

The study area, the materials and methods as well as the statistical data for the study are dealt with in the third chapter. The results of the study are presented in Chapter 4. Chapter 5 presents the discussion of the study. Conclusions and suggestions are given in Chapter 6. The last section provides a list of references used in the study.

Chapter Summary

There are seven sections in this chapter. The first section provided an overview of the context of the study, the second section concentrated on the problem statement, and the third and fourth sections, respectively, highlighted the aim and objectives of the study. The significance of the study was covered in the fifth section. The delimitations and limitations of the study were the focus of the sixth section. The organization of the study was the primary focus of the final section. Reviewing the relevant study literature is the focus of the next chapter.

CHAPTER TWO

LITERATURE REVIEW

This chapter reviews relevant literature related to the objectives of this study. It discusses the anthropogenic challenges facing estuarine ecosystems. The impact of global climate change has been thoroughly discussed under Chapter one; it is therefore not addressed in this literature review. Instead, the review is focused on the pollution of estuaries.

Anthropogenic Pollution of Estuaries

Pollution in estuarine ecosystems has been of concern in recent studies (Cabrerizo *et al*., 2014; Davies *et al*., 2016; Gillanders *et al*., 2011; Mounieret *et al*., 2020; Schulte, 2007). According to the Group of Experts on Scientific Aspects of Marine Pollution (GESAMP), pollution is the release of harmful substances into the marine environment (including estuaries) directly or indirectly as a result of human activities, resulting in negative effects such as harm to living resources, hazards to human health, and marine activities, including fishing, loss of amenities and deterioration of sea water quality.

Estuaries are the focal points of land-sea interactions, where a wide range of phenomena, including physical, chemical, biological, and geological, are intertwined making these sensitive biological ecosystems more vulnerable. Araújo-castro *et al*. (2009) stated that estuarine and shallow coastal systems have the highest amounts of pollutants because these habitats experience significant anthropogenic impact from both point and non-point sources. According to Chen and Chen (2002) roughly 60% of the population and two-thirds of medium to large-

sized cities are situated along estuaries. Increasingly extensive human activities along estuarine catchments put enormous strain on these systems and have a direct impact on the physico-chemical state as well.

The rapid development of the estuaries and their catchments has led to a deterioration in water quality, eutrophication, degradation and loss of important habitats which have disrupted migratory routes as well as reduced fish stocks (Gabric & Bell, 1993).

The increasing rate of human activities along the Ghanaian coast has been well documented (Olympio & Amos-Abanyie, 2013). These activities have a number of negative consequences that eventually lead to ecosystem degradation as water quality is reduced. Studies by Asare-Donkor *et al*. (2015), Fianko *et al*. (2010) and Klubi *et al*. (2018) indicates the presence of pollutants in some estuaries in Ghana.

The Major Types of Pollutants

According to Pooja *et al*. (2020), pollutants can be grouped into two major types based on how well they are known and the amount of knowledge that exists on these pollutants. These major groups are termed conventional and emerging pollutants.

Conventional pollutants are those pollutants that have been known to society for a long time. As several researchers have shown their sources and detrimental effects on ecosystems, these pollutants are referred to as conventional pollutants (Pooja *et al*., 2020). Since several studies have extensively documented their sources and detrimental impacts on ecosystems, such pollutants have long

been known to society. These pollutants are usually found in domestic, commercial, or industrial wastes, and includes Fluoride, Nitrate and trace metals and metalloids (Ahamad *et al*., 2020).

On the contrary, pollutants with harmful effects on ecosystem health that have not been known to exist yet and which are found at very minimal levels of environmental concentration in waters may also be termed emerging pollutants (Fata-kasinos, Meric & Nikolaou 2011). Pollution from Emerging pollutants can originate from both point and non-point sources, including agricultural, urban and industrial areas. Point sources release these pollutants directly into bodies of water, where their ultimate fate is a major concern because they could move in the aqueous phase, decompose, or adsorb into sediments.

However, different Emerging pollutants with different characteristics, such as their adsorption effectiveness, polarity, persistence, as well as interacting compartments, determine their movement from the source to the sink (Ahamad *et al*., 2020). Over the past several decades, globalization and population growth have led to increased waste generation but also an increase of a number of new water contaminants including pharmaceuticals, cosmetics, personal care items, pesticides, herbicides etc. which are endocrine disrupting substances. Emerging pollutants are constantly being introduced to our environment, and the by-products of our contemporary lifestyle are the primary cause of their development.

Diverse categories of these pollutants are present within most coastal aquatic ecosystems and they pose a severe problem at global level, since they can impact both flora and fauna as well as human health (Vasilachi *et al*., 2021).

Key pollutants and their sources

According to Kennish, 1994, the key pollutants of estuarine and coastal marine ecosystems comprises the following;

a. oxygen-demanding wastes such as sewage and other (mostly carbonaceous organic matter)
b. infectious pathogens and other infectious agents found in sewage waste
c. oil spillages resulting from the influx of river run-off, urban run-off, municipal wastes, and effluents from non-petroleum industries and activities such as oil transportation
d. heavy metals - such as mercury, lead and cadmium - derived from fossil fuel combustion, smelting, sewage-sludge disposal and other anthropogenic activities
e. polycyclic aromatic hydrocarbons from sewage and industrial effluents and petroleum spills
f. chlorinated hydrocarbon compounds (such as organochlorine pesticides and polychlorinated biphenyls) from agricultural and industrial sources
g. radioactive waste from mining, nuclear power plants, and other industries.
h. disposal of condenser cooling water from electric power plants.

Many of the aforementioned pollutants are known to accumulate in estuarine sediments (Chuan & Yunus, 2019) or bioaccumulate in both pelagic and sediment-dwelling species (Meador *et al.*, 1995). Through storm drains, industrial discharges, riverine and surface run-offs, farmlands, sewage treatment and

electrical power plant outputs, and atmospheric deposition, these pollutants are able to enter estuaries and other coastal waters (Duce *et al*., 2008; Howarth, 2008).

Key Polluting Sectors

Agricultural and Land Use

Land-use activities modify chemical loads, watershed hydrology, and sediment loads, all of which have an impact on water quality (Basnyat *et al*., 1999). According to previous studies, agriculture is a significant source of phosphorus (P) and nitrogen (N) pollution, and it is well known that it has a significant impact on water quality (Viessman & Hammer, 1993). Use of excessive fertilizers, outdated methods of irrigation, the use of pesticides and herbicides, and poorly managed animal farming operations are all means by which agricultural activities impact water quality (Moss, 2008). These activities may cause nutrient, chemical, pathogen, and sediment fluxes within adjacent estuaries, thereby affecting estuarine water quality (Hunter & Walton, 2008). According to the UNEP-GEF Volta Project report (2013), Agriculture in the Volta Basin is the primary cause of water contamination (including livestock and fisheries).

Soils are easily eroded into the watercourses during mild floods along the riverbanks, where farming is practiced. Increased nutrient loading is clearly visible, and the usage of pesticides and fertilizers is on the rise. Animal droppings are improperly managed and end up in rivers due to poor livestock management methods, which raises the water's biochemical oxygen demand. In Ghana, phosphates and nitrates have been detected at all water depths within the Volta Basin network. The presence of these contaminants may be attributed to farming

activities particularly along the banks of the Black and White Volta basins, which also contribute to the improper use of chemicals and unregulated fishing activities (UNEP-GEF Volta Project, 2013).

On agricultural lands around the world, farming systems have surpassed natural nutrient cycles in terms of nutrient transport (Powell, Fernandez-Rivera, Williams & Renard, 1995). A net flow of P and N is produced from the fertilizer manufacturing site to fertilizer deposition or manure production sites (Carpenter *et al*., 1998). This has caused an excess of nutrients in farmland, and this is one reason why agriculture contributes to pollutions in estuarine basins and marine ecosystems.

Pathogens such as bacteria and viruses may be carried into water bodies as a result of run-off from agricultural lands, affecting the quality of these waters and the life of aquatic organisms (Lipp *et al*., 2001). The application of pesticide and herbicide in agricultural activities has resulted in chemical deposits into soils; which is eventually carried through run-off and leaching into estuarine ecosystems, impacting the organisms that reside in these estuaries (Cerejeira *et al*., 2003; Guzzella *et al*., 2006).

Urbanisation and Industrialisation

The world's coastlines are changing due to urbanization on time-scales of years to decades. Its impacts on coastal ecosystems have been observed through sediment increase, increased nutrient levels resulting in harmful algal blooms and faecal microbial loading; these lead to subsequent changes in water quality and stream flow (Freeman & Steppe, 2019).

Worldwide, significant coastal habitats have been degraded by human activities (Kennish *et al*., 2007; Viles & Spencer 1995). These anthropogenic effects, which have threatened the biological integrity of many estuaries, are strongly related to increased populations leading to urbanization and infrastructural development as a result of industrialisation within coastal zones. A variety of human activities and competing uses in adjacent estuarine waters, as well as coastal watersheds, have contributed significantly to decreased water quality and loss of habitat, reduced resources, and modification (Kennish *et al*., 2007). Poorly planned residential and industrial construction can increase sources pollution in coastal waters which subsequently causes the destruction of natural habitats in these ecosystems. Anthropogenic activities such as construction in water catchments may speed up the loading of sediment, which may have an effect on benthic communities (Kennish, 2002).

Africa's coastal region is far more urbanized than the inland as a whole, with a percentage of the urban population of 72% compared to only 38% in the continental region according to Neumann, Vafeidis, Zimmermann and Nicholls (2015). In Ghana, about 80% of industrial establishments are located in coastal areas; which is home to about 25% of the population (Boampong, 2020). Most of these industries have been reported to channel their waste directly or indirectly into watersheds.

According to the UNEP-GEF Volta Project (2013), a number of industries in most of the transboundary nations within the Volta River Basin release their waste into the watershed. According to the report, waste water from

slaughterhouses and the Brasserie du Burkina Faso in Burkina Faso contains high levels of fats, proteins, and phosphates. Solid waste from farms, including manure and blood, is also dumped into local rivers. Solid waste and waste water are released by oil refineries and soap companies.

A variety of industrial chemical facilities are also present (mostly in Ouagadougou), including factories that make paint, mattress foam, textiles, cosmetics, medicines, plastics, paint, and tanning plants. According to reports, Ghana's two largest textile manufacturers release their effluent—majority of which is improperly treated—directly into water systems. Industrial pollution has been reported in Togo, where sewage from the Brewery of Benin is dumped into the nearby streams and oil spills from the power plant enter directly into water bodies. Due to urban growth along coastal areas, coastal habitats around the world receive large anthropogenic pollution inputs, dissolved and particulate matter, fluvial inputs as well as anthropogenic trace element fluxes have increased significantly, causing biogeochemical cycles to be disrupted.

Mining

The prevalence and intensity of human activities with respect to mining have caused an increase in heavy metal pollution of the Earth's surface. Mining operations drastically influence the environment and pollute the air, soil, and water (Singh & Steinnes, 2020). Mining removes sediment, uses mercury, produces waste oil, and alters the morphology of riverbeds. Additionally, it is associated with forced migration, biodiversity loss, and the degradation of sensitive ecosystems (Candeias, Ávila, Coelho & Teixeira, 2018). Again, inputs of mercury, cooper, and

silver are produced during ore processing and are released into rivers and coastal ecosystems.

It has been well established that mining for metals significantly impact the quality of downstream water bodies (Candeias, Ávila, Coelho & Teixeira, 2018). Sediments and waste water from mined areas are eventually deposited in fluvial systems, where they finally accumulate in estuaries and coastal waters. Most studies (Lee *et al*., 2001; MacKenzie & Pulford, 2002; Okyere, 2018) show that waste from mining activities is a frequent source of pollution in sediments downstream. Numerous nearby mining and/or ore processing operations have resulted in the pollution of rivers and other coastal waters all over the world (Figure 4) .

Figure 4: A tropical estuary (Pra) polluted as a result of mining activities. Image from Graphic.com.gh

Bioaccumulation and Biomagnification of Pollutants in Aquatic Ecosystems

Bioaccumulation occurs when pollutants get into an organism and accumulate in it, relative to the level of pollutants found in the environment (Borg 2013), Biomagnification may happen when pollutants are transferred in the food

chain, which means that an organism's pollutant concentration increases compared to its prey; therefore, it is caused by atrophic transfer (Borgå, 2013).

According to earlier studies (Naggar *et al*., 2018; Nyarko *et al*., 2014), transfer of chemical contaminants via the food web may have an impact on ecosystem function, biota, and human health. In terms of their potential effects on organisms, three broad groups of pollutants that rate among the most dangerous are PAHs, halogenated hydrocarbons, and heavy metals (Kennish *et al*., 2007). Some of the compounds that make up these classes are ubiquitous, persistent, and toxic to most organisms. They mainly accumulate in aquatic organisms, and for some substances such as the organochlorines, dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) biomagnify in the food chain to reach highest concentrations in top carnivores of the higher trophic levels (Naggar *et al*., 2018).

The concentrations of PAH in estuarine organisms depends on the bioavailability of the pollutant and the ability of the organisms to digest them. In bottom sediments of urbanized systems, PAHs invariably reach high levels and may persist for prolonged periods mostly unaltered. These pollutants have been proven to negatively affect the composition and functioning of organisms (Bennett *et al*., 2000)

According to Yan *et al*. (2016), a major environmental problem is the pollution of aquatic environments with heavy metals. They're one of the worst persistant contaminants in water, sediments and biota (Mucha *et al*., 2003). Heavy metals pose a threat, since they are poisonous to fish species above the threshold of

toxicity; and have been linked to emergence of biotic disorders such as feeding, digestive and respiratory dysfunctions, as well as reproductive functions, tissue inflammation, and degeneration (Kennish, 2002). According to a review by Suedel *et al*. (1994), laboratory studies show that there is the potential for mercury to be transferred across feeding levels in aquatic systems. This has been confirmed by Berk and Colwell (1981) in their food chain study involving *Vibrio sp*., *Pseudomonas sp*., *Uronema nigricans*, and *Eurytemora affinis*. Findings from the study indicated that both bacterial species easily accumulated mercury and, when fed to ciliates, underwent biomagnification.

Some of the most hazardous synthetic organic substances that are present in estuarine environments are the halogenated hydrocarbons, also known as organochlorines. Several insecticides (such as DDT, aldrin, dieldrin, and toxaphene), herbicides (2,4-D and 2,4-T), and industrial chemicals are significant contaminants in this category (Wania *et al*., 1998). They enter estuaries through different sources which include runoffs and atmospheric deposition (Wania *et al*., 1998). Halogenated hydrocarbons readily accumulate in the tissues of aquatic organisms due to their lipophilic nature. They are often the cause of a range of diseases such as altered physiology, developmental patterns and reproductive abnormalities in these organisms (Kennish, 2002).

Chapter Summary

This chapter of the study focused on reviewing the relevant literature that support the aim of the study. It explained the sources of pollution and the key contaminants found in coastal water systems. The chapter further identified the key

sectors contributing to the pollution of coastal ecosystems, it also explained the concept of conventional and emerging pollutants together with bioaccumulation and biomagnification of these pollutants across the food chain.

CHAPTER THREE

MATERIALS AND METHODS

The materials and procedures utilised for the purpose of the study are described in the current chapter. The sampling locations and research area are first described. This part also includes information on field sampling, microcosm experiments and data analysis to quantify the combined impacts of surface warming and salinity fluctuations on calanoid copepods.

Study Area

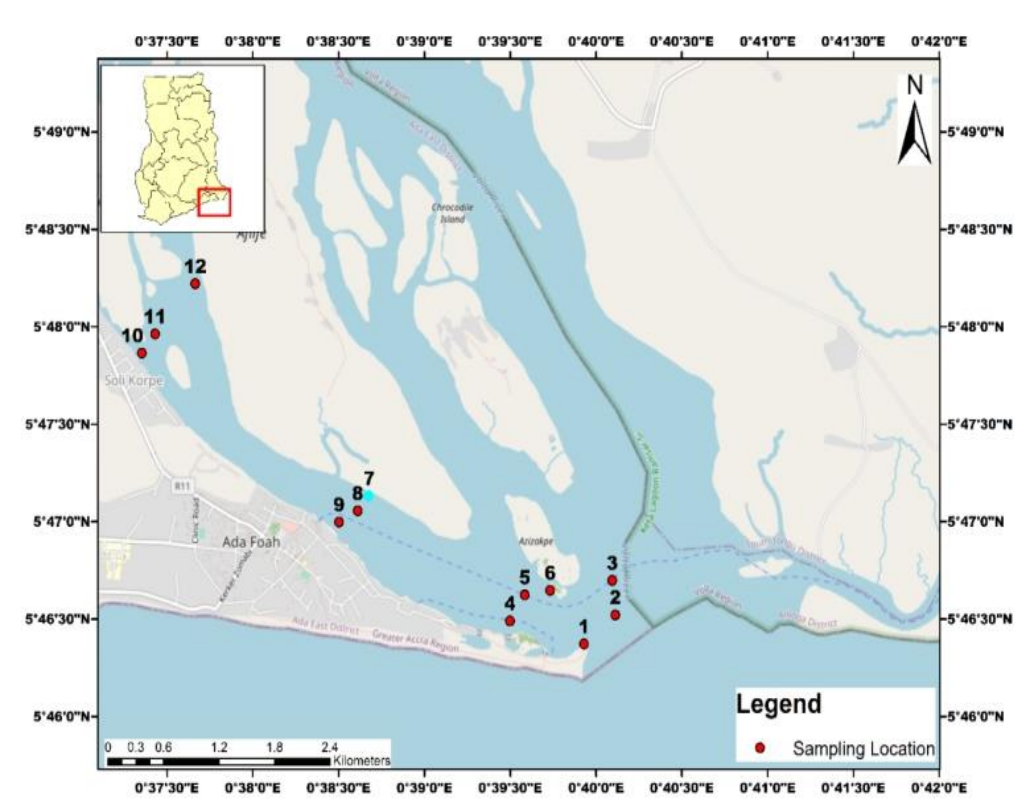

Figure 5: Map of Study Area with legend and numbers showing sampling points on study transects

The study was carried out in the Volta River estuary at Ada Foah (05°49' 18.6" N and 000°38.46' 1"E) in the Greater Accra region of Ghana (Figure 5). The Volta River estuary is the biggest in Ghana. The River basin covering about 400,000 km^2 is shared by six riparian West African countries (Mul *et al*., 2015). The watershed is 40% in Ghana, 42% in Burkina Faso, 6% in Togo, 5% in Mali, 4% in Benin, and 3% in Côte d'Ivoire (Oguntunde *et al*., 2006). Due to the trans-national nature of the river that feeds the estuary, there is the likelihood of transport impact from across sub-regions into the estuary. Agriculture has been reported to be the predominant socio-economic activity within the catchments of the estuary and this has been reported in previous studies to be a key source of pollutants into the estuary (UNEP-GEF Project, 2013). Studies have also reported increase in heavy metal concentration and polycyclic Aromatic Hydrocarbon (PAH) levels in the estuary (Asare-Donkor *et al*., 2015; Nyarko *et al*., 2010). This indicates the possibility of already existing anthropogenic stressors on estuarine organisms, hence the choice of the study area.

Sampling of Mesozooplankton

Mesozooplankton abundance and composition within the Volta River Estuary were assessed on four transects established across the length of the estuary (Figure 5).

Samples of mesozooplankton were collected using a conical zooplankton net (mesh size: 200 µm) in line with previous studies (Asiedu, 2020; Mack, Conroy, Blocksom & Ludsin, 2012). The net was attached to a wench and lowered into the water for a vertical hauling of mesozooplankton. Samples collected were washed

through a 200µm net sieve with a wash bottle into screw cap bottles containing 5ml of 5% formaldehyde; this was used for the preservation of collected mesozooplankton. Samples were kept on ice in an icebox for transportation to the laboratory for identification and enumeration.

In addition, measurements were also taken in order to describe the environment of the mesozooplankton. These measurements were physico-chemical parameters - temperature, conductivity, salinity, pH and dissolved oxygen and chlorophyll-a concentration assumed to represent food availability to the animals (Hirst & Bunker, 2003)

Vertical profiles of temperature, salinity and conductivity were obtained using CTD probe (Idronaut Ocean Seven 310). The probe was lowered into the water to the bottom and pulled out gradually after one minute. Surface water temperature, salinity, conductivity, pH and dissolved oxygen were measured in situ using a multiparametric water quality checker (HORIBA-U52)

Water for chlorophyll-a measurement was sampled using Niskin bottle (volume: 5 litres) into 250 ml dark bottles. To avoid exposure to sunlight, which could encourage the growth of algae cells, bottles were covered with aluminum foil and kept refrigerated on ice. This followed the procedures by Aminot and Rey (2001) and Pápista, Ács and Böddi, (2002).

The sampling procedures were repeated at each sampling station. Sampling was done on three different occasions, one each in March (dry season), May (beginning of wet season) and July (peak of raining season) 2022. These periods were considered because the oceanography of coastal ecosystems in general and

the Volta Estuary in particular changes with the seasons of the year (Gyau-Boakye, 2001).

Laboratory Analysis of Chlorophyll-a and Mesozooplankton

Determination of chlorophyll-a concentration

Chlorophyll-a concentration was measured following the methods of Teira, Serret and Fernández (2001). Water stored in the dark bottles were filtered through 0.7µm GFF Glass micro fibre filter paper using a vacuum pump (storm 3000 with the flow of 20 litres per minute). The filter papers after filtration were gently folded into 50ml capped glass vials containing 5 ml of 95% ethanol for chlorophyll-a extraction. Glass vials containing the filter papers were wrapped in aluminium foil and kept in the fridge for 24 hours. This was done to ensure effective extraction of Chlorophyll-a as recommended by Teira, Serret and Fernández (2001).

Chlorophyll-a concentrations were measured using a calibrated fluorometer by fluorescence. The fluorometer was blanked with 5ml of 95% ethanol before measurement commenced. The ethanol-extracted chlorophyll-a samples were transferred into smaller glass vials and inserted into the cuvette of the fluorometer for reading. Chlorophyll-a measurement was carried out in $\mu g.1^{-1}$ using stored calibrations on the fluorometer.

Mesozooplankton samples were analysed under the binocular microscope (Leica M 50) with a magnification of x4. Organisms were identified to the order level using an identification manual by Mauchline, (1998) and Waife and Frid (2001) on the Gulf of Guinea zooplankton and other identification manuals.

Mesozooplankton were enumerated as well to determine the abundance and composition.

Laboratory Experiments

A bioassay was conducted to investigate the response of calanoid copepod to climate - induced factors of sea surface warming and salinity changes. The focus of the experiment was to capture the response behaviour of the animals in both dry and wet seasons. Two species of calanoid copepods were selected for the experiments. These species were *Temora stylifera* and *Paracalanus parvus* for the dry and wet seasons, respectively (Figure 6).

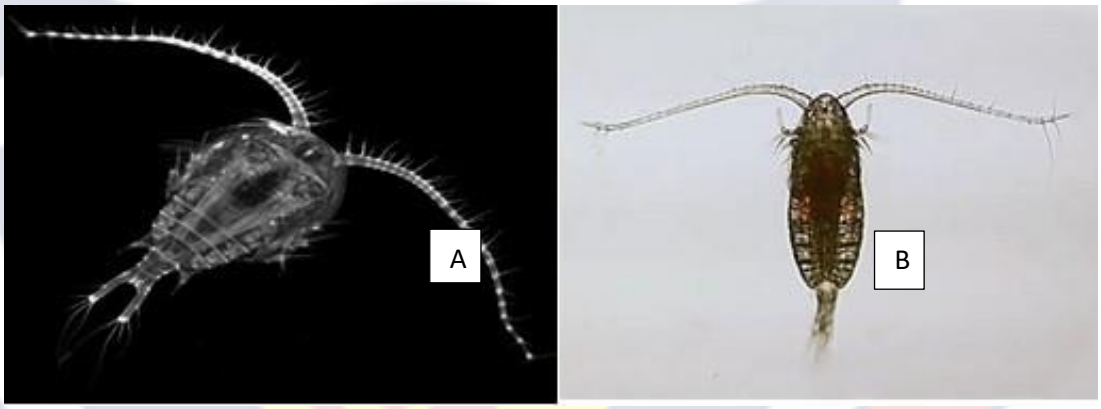

Figure 6: Calanoid copepods Temora stylifera (A) and Paracalanus parvus (B) used for laboratory experiments in this study. Source: Culverhouse et al., (2006)

The justification for the use of these species is that they are cosmopolitan (Razouls *et al*., 2005) and were also observed in the Volta estuary during this research.

Unlike copepods collected from the study sites for identification and enumeration, copepods collected for the experiment were sampled from the marine environment off the coast of Elmina (5° 03'N, 1° 20'W) within the Central Coast of Ghana. The sampling was done on board a semi-industrial outboard motor fishing vessel. A 200µm zooplankton net was towed slowly through the top 2 m of

the water for about 30 minutes along a distance of about 1 NM in line with previous studies (Asiedu. 2020). The copepods were kept alive by preventing heat stress as animals were kept on ice packs an icebox following the procedures used by Asiedu (2020). After sampling, the animals were quickly transported to the laboratory for incubation. Target copepods for the experiments were separated from other species with the aid of a sterile glass Pasteur pipette under a binocular microscope (Leica M 50).

The incubation was done using water collected from the estuary. Water was sampled from the bottom on each transect. The water samples were collected using Niskin sampling bottle (volume: 5 litres). The bottle has two openings with each opening covered with a stopper attached with an elastic cord. The stopper of the outlet is first opened and hooked onto the clamp with the elastic cord followed by that of the inlet where the release mechanism is attached. The bottle is then lowered into the water until it is submerged. A messenger is then sent through the rope to activate the release of the stoppers to close the two openings. By this mechanism, the sampler prevents the mixing of water at different depths. Water collected was kept in 5 litre gallons and immediately kept on ice to prevent decay. The procedure was repeated on two more transects representing distinct water masses with different salinities. Samples were transported to the lab on ice for treatment and use.

In the laboratory, water was filtered through 30µm mesh sieves to remove debris and larger organisms while maintaining a fair number of food items for experimental animals. This followed the procedures of Bess *et al*. (2021).

Incubation

The animals were accessed under two different warming scenarios (+2ºC and +4ºC) above the average sea surface temperature (28ºC) of coastal marine waters in Ghana during the dry, stable hydrographic period when the impact of warming is expected to be significant (Acheampong *et al*., 2021). This means that the control temperature in this experiment was 28ºC, whereas the test temperatures were 30 and 32ºC. These warming scenarios were set based on the expected changes in sea temperature projected by Copernicus Ocean State Report (2022) and IPCC Report (2014).

They were established in plastic water baths (volume: 80 L) using thermostatic heaters (EHEIM Thermo control 200) as demonstrated in Figure 7. The baths were constantly aerated to ensure even distribution of temperature in the water bath. With the aid of temperature loggers (HOBO), the experimental temperatures were monitored every 15 minutes in line with previous studies (Asiedu, 2020).

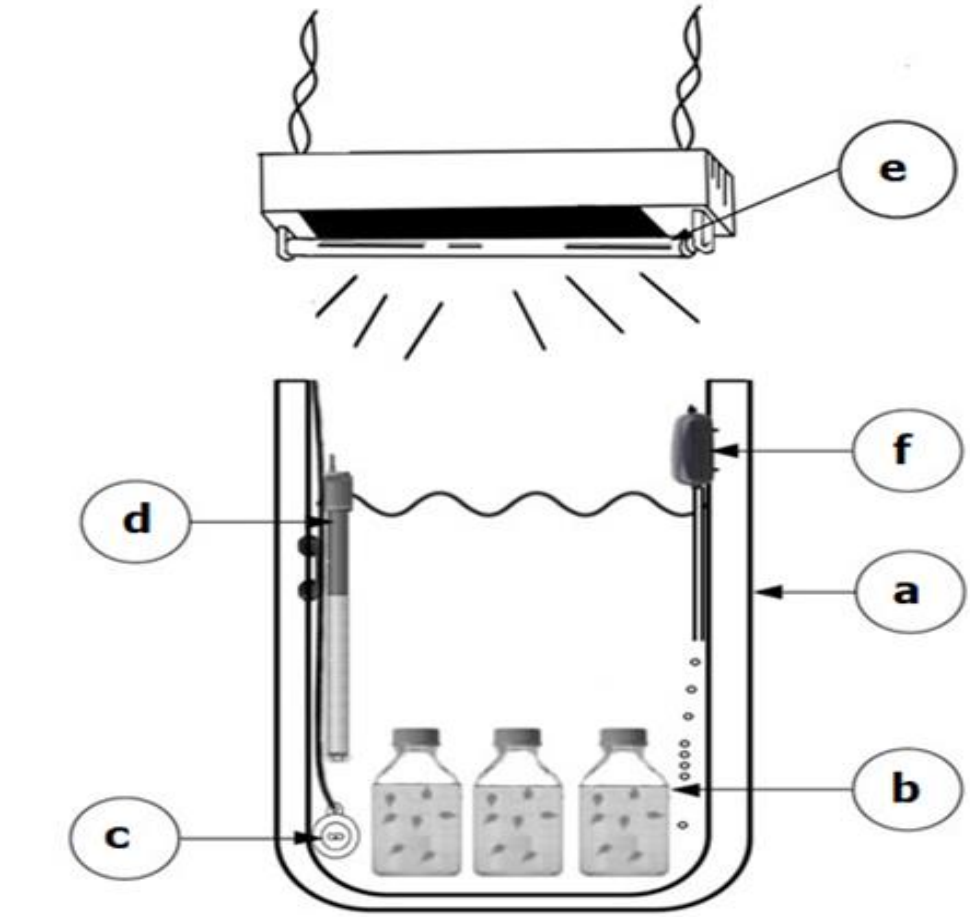

Figure 7: Illustration of experimental treatments established using water baths. Specific treatments were established in different baths using plastic barrel (a), Duran bottles (b), temperature logger (c), thermostatic heater (d), illumination source (e) and aeration source (f). Image credit: Emmanuel Acheampong (PhD)

Salinity levels used in experiments were based on the salinity of the different water masses identified in the estuary. These salinities were 0.5, 22 and 30 ppt for the dry season and 21, 25 and 29 ppt for the wet season. The different scenarios of warming and salinity conditions were combined in the experiment as shown in Table 1.

Table 1: Experimental design showing the levels of stressors investigated. T_1 refers to the control temperature (28ºC), T_2 and T_3 represent the control temperature +2ºC and +4ºC respectively. S_1, S_2 and S_3 represents the different salinity levels considered for each of the seasons

	Warming Scenario (T)		
Salinity (S)	T_1	T_2	T_3
S_1	S_1T_1	S_1T_2	S_1T_3
S_2	S_2T_1	S_2T_2	S_2T_3
S_3	S_3T_1	S_3T_2	S_3T_3

For the running of the experiment, nine pairs of 1000 ml Duran bottles were filled with the treated estuarine water to about 970ml for each salinity treatment. This was to allow for thermal expansion as recommended by Asiedu (2020). These represented three replicates of each temperature treatment. Seven females of the experimental animals (Figure 5) were put in each of the experimental bottles for acclimatization. This sex preference reflects personal observations of the species from field samples.

Before incubation, animals were acclimatised to each environmental condition for a period of 12 hours. This procedure was in line with previous studies (Punnarak, Jarayabhand, & Piumsomboon, 2017).

Animals were transferred into incubation bottles immediately after the acclimatisation period. This was done by carefully sieving acclimatisation bottles through a 200µm mesh size sieve and immediately washing the animals onto petri dishes. The filtrates were observed under the microscope to determine if the animals

were alive before transfer into incubation bottles. After acclimation, the animals were incubated for 24 hours and maintained on a 12-hour dark and 12-hour light cycle. At the end of the incubation, the rates of egg production, faecal pellet production and mortality were measured as described in the following section.

Determination of Biological Rates

The biological response (faecal pellet production, egg production and mortality rate) of experimental organisms were quantified following the approaches of previous studies (Carlotti, Rey, Javanshir, & Nival, 1997).

After the 24-hour incubation period, incubation bottles were removed from the water bath. Each bottle was inverted 3 times and the contents gently filtered through a 200µm mesh size to remove the copepods. The filtrates were washed onto a petri dish and viewed under a binocular microscope (Leica M 50) with a magnification of x4 to determine mortalities. Faecal pellet and egg production were estimated by sieving the content further through a 20µm mesh sieve to trap the faecal pellets as well as the eggs. Filtrates were again washed onto a petri dish and stained with 10% Lugol's solution for counting under the binocular microscope (Leica M 50) with a magnification of x4 in line with previous studies (Carlotti, Rey, Javanshir, & Nival, 1997).

Data Analysis

Determination of Mesozooplankton Species Abundance and Composition

Mesozooplankton abundance at different sampling stations and months were determined by dividing the total number of copepods by the litres of water filtered following the procedures in previous studies (Webber, Edwards-Myers,

Campbell & Webber, 2005). The volume of water filtered was deduced from the formula below :

$$V = \pi r^2 d \quad \textbf{1}$$

Where *r* is the net radius and d is the depth of water sampled (Zakaria *et al*., 2016).

The percentage composition (%) of each taxonomic group was calculated using the equation below:

$$(\%) = \frac{\text{No.of copepods in each taxonomic group}}{\text{Total No.of copepods in all taxonomic groups}} \times 100 \quad \textbf{2}$$

Estimation of Copepod Biological Rates under Warming and Salinity Fluctuations

Egg production rate (EPR), faecal pellet production (FPR) and mortality rate (MR) of the copepod was estimated using the Equations below as used in previous studies (Carlotti, Rey, Javanshir, & Nival, 1997; Hack et al., 2008; Kiørboe & Sabatini, 1994).

$$FPR = \frac{Number\ of\ feacal\ pellets\ produced\ per\ day}{Individual\ number\ of\ copepods} \quad \textbf{3}$$

$$EPR = \frac{\text{Number of eggs produced per day}}{\text{Individual number of female Copepod}} \quad \textbf{4}$$

$$MR = \text{Total experimental copepods} - \text{Number alive per day} \times 100 \quad \textbf{5}$$

Statistics Used

Each of the individual physico-chemical parameters, chlorophyll-a concentrations, mesozooplankton abundance and species composition observed on different months at different sampling stations were compared using a two-way ANOVA using the minitab statistical software version 21.1. The abundance measures, expressed as percentages (Equation 2) were arcsine transformed to achieve normality prior to the ANOVA. The same analysis was used to compare each of the individual biological rates observed when different warming and salinity conditions were combined. Where there were differences, a post hoc test (Tukey pairwise comparison) was conducted at an alpha value of $p < 0.05$ to determine the source(s) of the difference.

The possible relationship(s) between physico-chemical conditions and mesozooplankton abundance of the estuary was/were evaluated using linear regression, assuming a linear relationship is the simplest function for describing interaction between environmental variables (Bazdaric *et al*., 2021). Prior to this analysis, the environment of the animal was reduced to its defining parameter(s) using a Principal Component Analysis (PCA), following previous studies (Banda & Kumarasamy, 2020). Therefore, for the evaluation of the relationship, the environment was represented using the variables determined by the PCA to be most significant (variables with eigen value ≥ 1).

The analysis was done for the different groups of mesozooplankton observed in this study. An environmental variable was considered to be significantly related to mesozooplankton abundance when the coefficient of

determination for the relationship (R^2) was ≥ 0.5 (Chicco, Warrens & Jurman, 2021).

The relationship between measured biological rates and the multiple stressors (salinity and temperature) was determined using multivariate regression analysis at an alpha value of $p < 0.05$. This was done following previous studies (Chuku *et al*., 2020) in order to determine the biological rate(s) that could best explain the combined impacts of temperature and salinity. A biological rate was considered to significantly explain the effects when the coefficient of determination for the relationship is greater than or equal to 0.5 (Chicco *et al*., 2021).

Chapter Summary

The chapter presented the materials and methods that were used in the study, it also described in detail the area within which the study was conducted. The statistical and analytical tools that were used to make inferences have also been described in this chapter.

CHAPTER FOUR

RESULTS

Results from field campaigns as well as microcosm experiments are addressed in the present chapter. Tables and graphs are presented to illustrate the findings of this study. Standard errors (± SE) around average estimates have also been indicated as bars on graphs. Findings from the study are presented in tables and graphs. Standard errors (± SE) around average estimates have also been indicated as bars on graphs. The Chapter also presents the discussions to the results. Results on mesozooplankton abundance and composition is first discussed, followed by the relationship between physico-chemical parameters and mesozooplankton, and finally the biological rates that explains the combined impact of warming and changes in salinity is discussed.

Physico-chemical Parameters

Temperature

On average, temperature ranged from ≈ 27 - 30 ^{o}C. Highest temperature was recorded in May at 1 NM away from the surf zone of the estuary. The lowest temperature (27.7 ± 0.1 ^{o}C) was in July at 2 NM away from the surf zone. Average temperature in March was 29.8 ± 0.1 ^{o}C. In May, an average temperature of 30.4 ± 0.1 ^{o}C was recorded and in the month of July, the average temperature was 27.7 ± 0.1 ^{o}C (Figure 8).

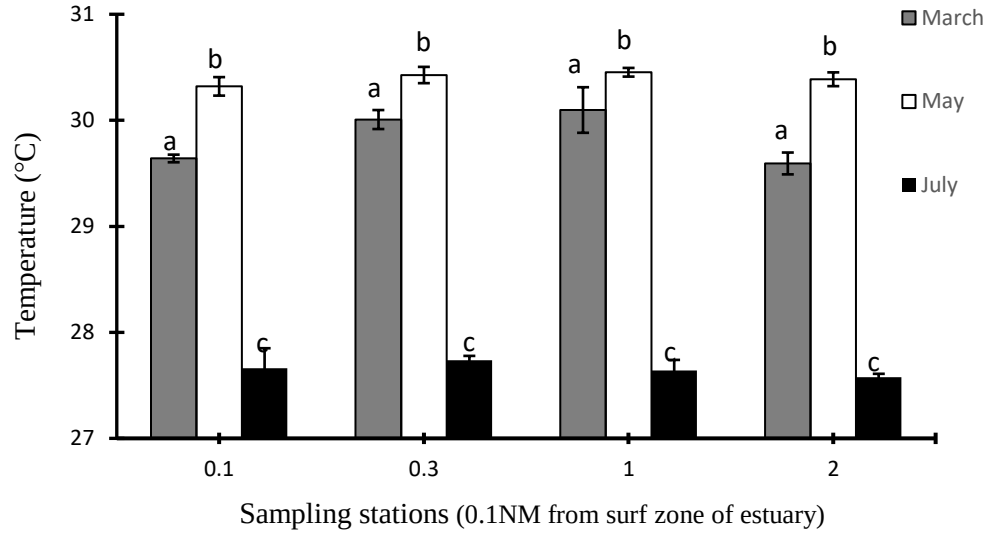

Figure 8*:* Temperature (average ± standard error) at different stations (NM from surf zone) on the Volta River Estuary of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) within stations are shown using different alphabets

The temperatures observed on different months at different sampling locations were significantly different. However, differences in temperature between the different sampling stations were not significant. (Table 2). Similarly, combining sampling period and location showed no significant difference in temperature.

Table 2: Two-way ANOVA comparing water temperature at different stations of the Volta Estuary on different months (March, May and July 2022)

Source of Variance	DF	Adj SS	Adj MS	F	P
Sampling month	2	50.3933	25.1966	754.83	0.000
Sampling station	3	0.3523	0.1174	3.52	0.052
Month*Station	6	0.3002	0.05	1.5	0.221
Error	24	0.8011	0.0334		
Total	35	51.8469			

Temperature in May was ≈1^{o}C warmer than the temperature in March. The temperature was ≈ 2^{o}C colder in July (27.7 ± 0.1 ^{o}C) than in March (29.8 ± 0.1 ^{o}C). Similarly, the July temperature was ≈3^{o}C colder than the temperature observed in May (30.4 ± 0.1 ^{o}C).

Considering the distribution of temperature along the depth of the estuary, the surface waters were on average markedly warmer than the waters at lower depth (Figure 9). However, the differences were not statistically significant.

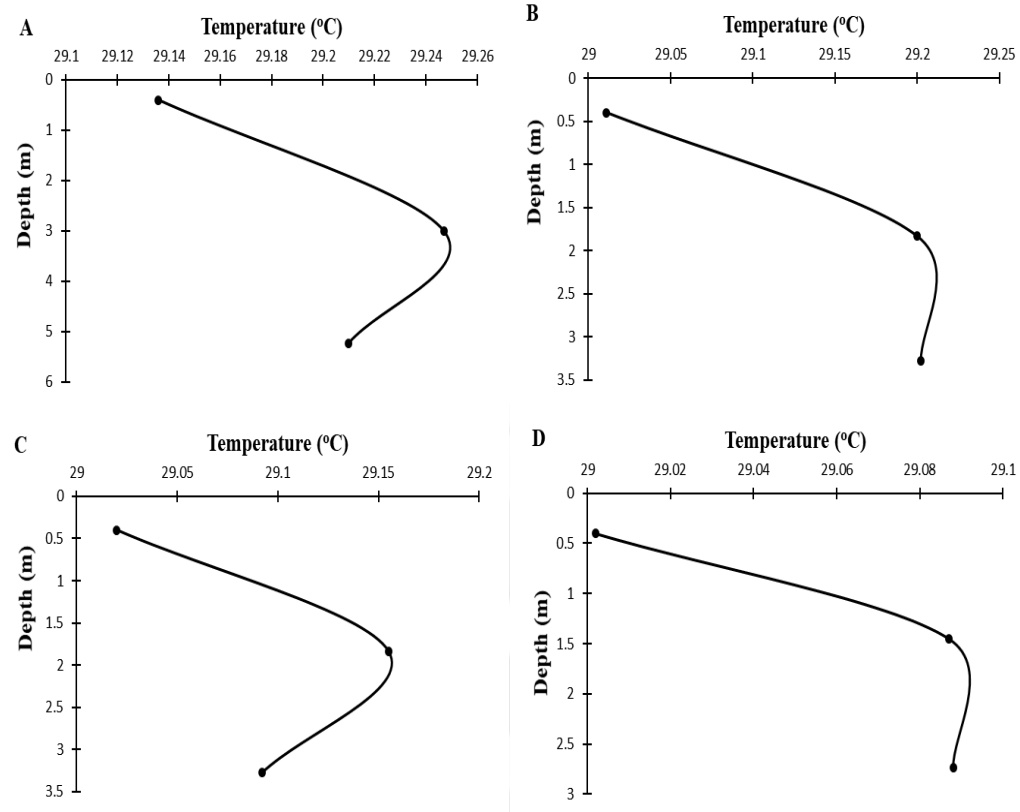

Figure 9: Vertical profile of average dry season temperature of the Volta Estuary. Sub plots A, B, C and D represent sampling sites located at different locations {0.1NM(A), 0.3NM (B), 1.0NM (C) and 2.0 NM (D) from surf zone} of the estuary

Salinity

Average surface salinity ranged from 0.04 –7 ppt. The highest salinity was recorded in July at 0.1 NM away from the surf zone of the estuary. The lowest was recorded in May; this occurred on each month of the sampling at the same location (2NM away from the surf zone) (Figure 10). The average salinities recorded in March, May and July were 1.5 ± 0.8 ppt, 0.6 ± 0.2ppt and 3.2 ± 1.2ppt respectively.

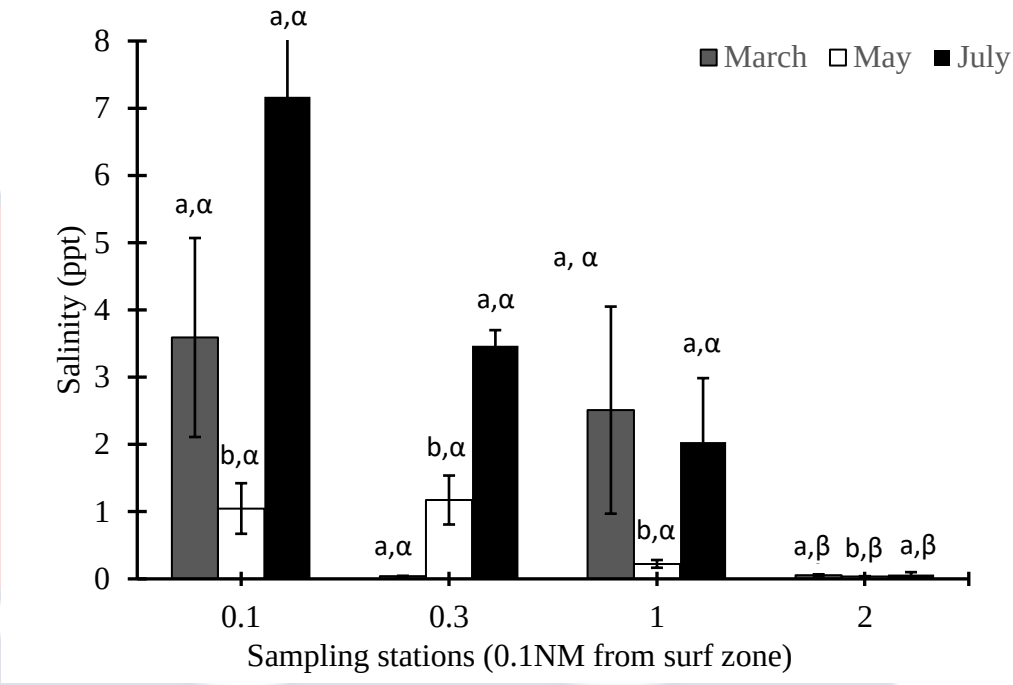

Figure 10*:* Surface salinity (average ± standard error) at different sampling locations and months (March, May and July, 2022) on the Volta Estuary. Significantly different findings (Tukey post hoc test) are shown using different alphabets: English alphabets (a, b) compare observations within stations for different sampling months while Greek alphabets (α, β) compare variations between the sampling stations.

Surface salinities recorded on the different sampling months were significantly different; the salinities were also different at the different sampling stations (Table 3).

Table 3*: Two-way ANOVA comparing surface water salinity at different stations of the Volta Estuary on different months (March, May and July 2022)*

Source of Variance	DF	Adj SS	Adj MS	F	P
Month	2	40.428	20.2138	4.43	0.023
Station	3	69.409	23.1364	5.07	0.007
Month*Station	6	43.289	7.2148	1.58	0.196
Error	24	109.498	4.5624		
Total	35	262.624			

Salinity was ≈ 1 ppt lower in May than in March. In contrast, salinity was higher (≈ 2 - 3 ppt) in July than in March and May.

With respect to sampling locations, salinity was higher closer to the sea: it was 2 ppt higher at the station located 0.1 NM near the surf zone of the estuary than the sampling areas located 0.3 and 1.0 NM further away; the salinity was ≈ 4 ppt lower in areas located 2 NM away from the surf zone. This difference was shown to be significant (post hoc test; $p < 0.05$).

Water column profile of salinity in the estuary showed that surface waters were less saline than bottom waters. (Figure 11).

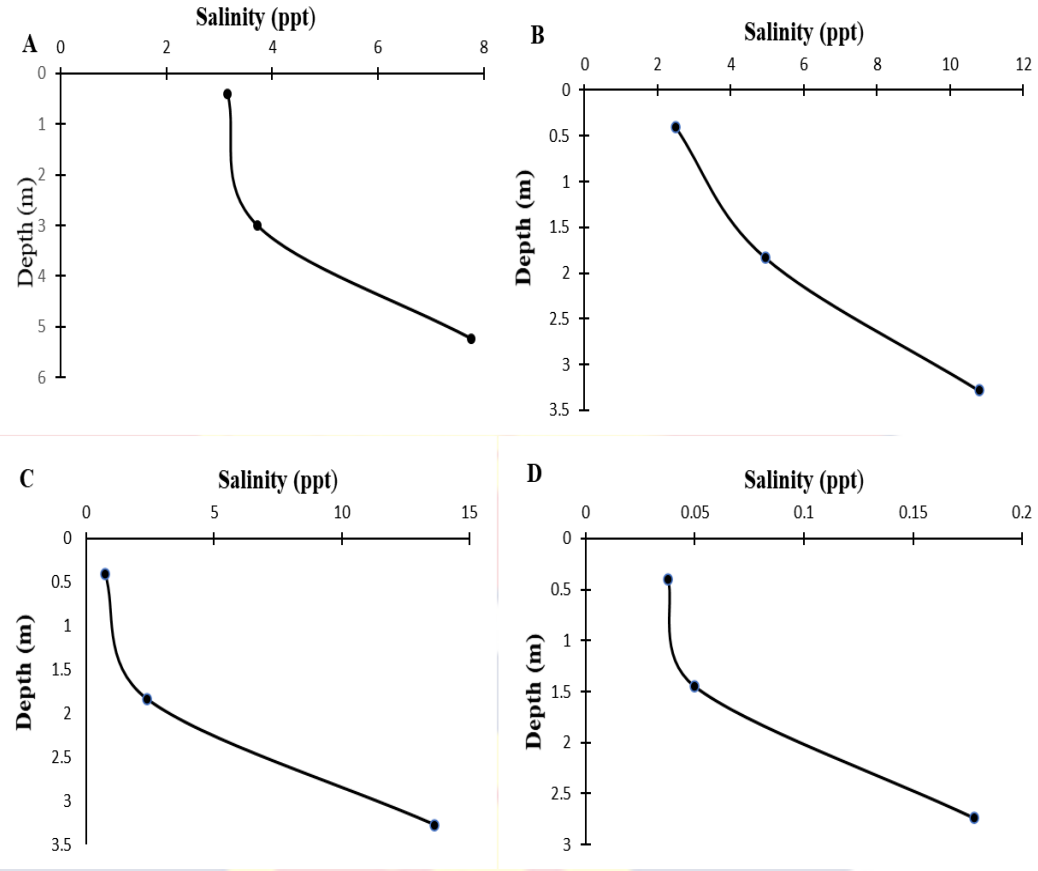

Figure 11: Vertical profile of average dry season salinity of the Volta Estuary. Sub plots A, B, C and D represent sampling sites located 0.1, 0.3, 1.0 and 2.0 NM from surf zone of the estuary respectively.

Conductivity

Figure 12 shows the conductivity observed during the study period on the Volta estuary. On average, surface conductivity ranged from 0.1 - 12.3 mS/cm. Highest conductivity was recorded in July at 0.1 NM away from the surf zone. The Lowest was also recorded in May at 2 NM away from the surf zone of the estuary (Figure 12).

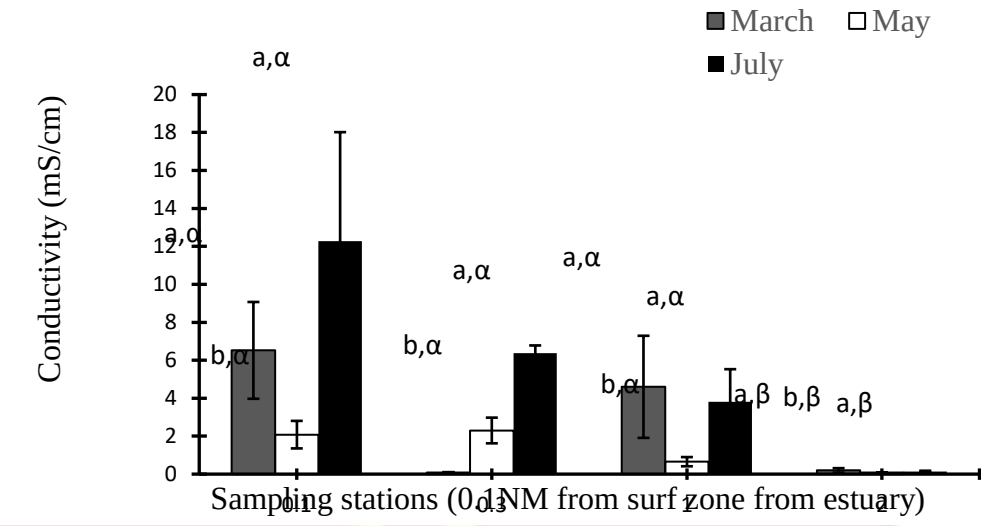

Figure 12*:* Surface water conductivity (average ± standard error) at different sampling locations and months (March, May and July, 2022) on the Volta Estuary. Significantly different findings (Tukey post hoc test) are shown using different alphabets: English alphabets compare observations within stations at different sampling months while Greek alphabets compare variations between sampling stations

Conductivities were significantly different on different sampling months and stations (Table 4)

Table 4: Two-way ANOVA comparing surface water conductivity at different stations of the Volta Estuary on different months (March, May and July 2022)

Source of Variance	DF	Adj SS	Adj MS	F	P
Month	2	117.414	58.707	4.6	0.020
Stations	3	212.107	70.7025	5.54	0.005
Months*Stations	6	126.035	21.0058	1.65	0.178
Error	24	306.144	12.756		
Total	35	761.7			

The post hoc analysis of the results showed that conductivity was lower (≈2 mS/cm) in May compared to the observation in March and July; the observations in March and July were not significantly different.

With regards to sampling station, conductivity values were higher (3 - 4.0 mS/cm) at locations closer (0.1 NM) to the surf zone of the estuary. It was about 89% lower at sampling stations located farthest away from the surf zone (Figure 12).

Figure 13 show the conductivity observed in the dry season on the Volta estuary. Generally, however, the conductivity increased with the depth of the estuary.

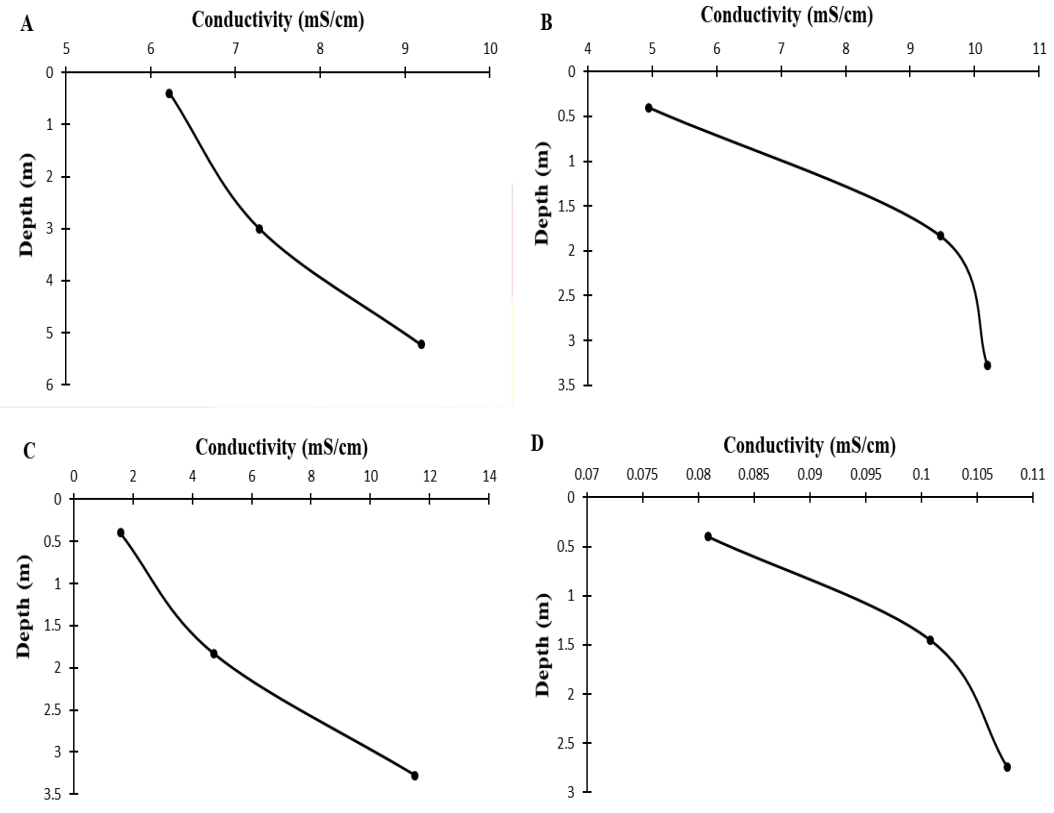

Figure 13: Vertical profile of average dry season conductivity of the Volta Estuary. Sub plots A, B, C and D represent sampling sites located 0.1, 0.3, 1.0 and 2.0 NM from surf zone of the estuary respective

Dissolved Oxygen (DO)

Amount of dissolved oxygen in the water ranged from ≈ 2 – 7 mgl^{-1}. Highest DO was recorded in May at 0.1 NM away from the surf zone. The Lowest was recorded in July at sampling stations located 2 NM away from the surf zone of the estuary (Figure 14).

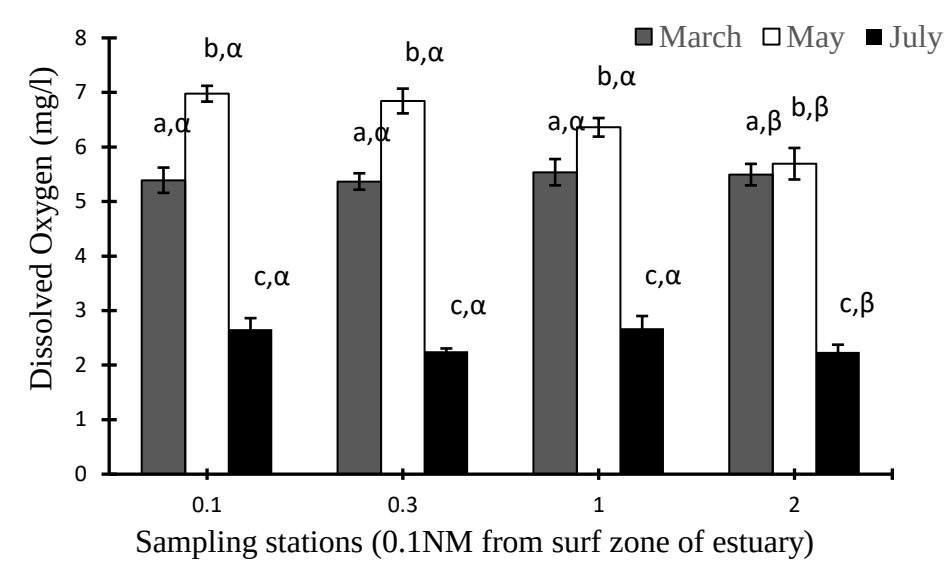

Figure 14*:* Dissolved oxygen (average ± standard error) at different sampling locations and months (March, May and July, 2022) on the Volta Estuary. Significantly different findings (Tukey post hoc test) are shown using different alphabets: English alphabets compare observations within different sampling months while Greek alphabets compare variations between the sampling stations

The oxygen content of the water varied significantly with both sampling months and sampling locations (Table 5).

Table 5: Two-way ANOVA dissolved oxygen at different stations of the Volta Estuary on different months (March, May and July 2022)

Source of Variance	DF	Adj SS	Adj MS	F	P
Month	2	104.17	52.0848	445.23	0.000
Station	3	1.365	0.4551	3.89	0.021
Month*Station	6	2.254	0.3757	3.21	0.019
Error	24	2.808	0.117		
Total	35	110.597			

Dissolve oxygen was highest (≈ 3 - 4 mgl^{-1}) in May compared to the concentrations recorded in March and July. In terms of sampling location, the concentration of the oxygen in the estuary was higher (≈ 0.2- 0.5 mgl^{-1}) closer to the surf zone; it was significantly reduced at sampling stations located farther away. On average, the DO measured closer to the surf zone of the estuary was 5 ± 0.2 mgl^{-1}. Average DO at the farthest transect away from the surf zone of the estuary was 4.6 ± 0.2 mgl^{-1} (post hoc test $p < 0.05$).

pH

Figure 15 shows the acidity of the water of the Volta River estuary during the study period. The range of pH was ≈ 7 – 8: the pH was highest at sampling stations located 0.3NM from the surf zone of the estuary; it was lowest at stations located 2NM away. The highest and the lowest values were recorded in March and May respectively. Average pH in March was ≈ 8 ± 0.1. May recorded an average pH of ≈ 7 ± 0.1. Also, the average pH in July was ≈ 8 ± 0.1.

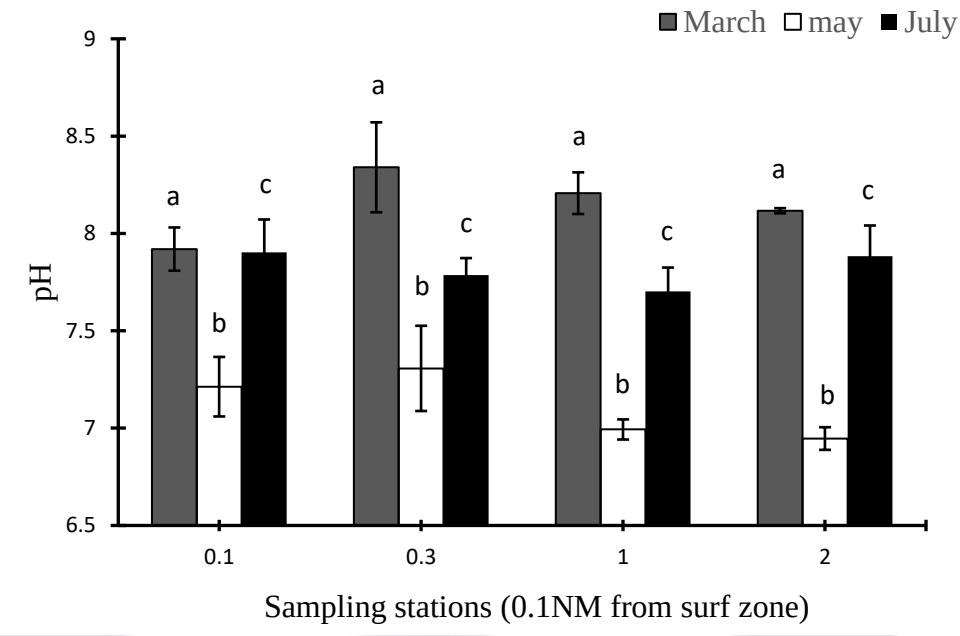

Figure 15*:* pH (average ± standard error) recorded at different stations on the Volta River Estuary of Ghana; findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets

[[

The acidity of the water in the estuary changed significantly only based on the sampling month (Table 6). The acidity at the different sampling stations were similar, at 7.7 ± 0.12.

Table 6: Two-way ANOVA comparing surface water pH at different stations of the Volta Estuary on different months (March, May and July 2022)

Source of Variance	DF	Adj SS	Adj MS	F	P
Month	2	6.66072	3.33036	57.66	**0.000**
Station	3	0.17571	0.05857	1.01	0.404
Month*Station	6	0.44971	0.07495	1.3	0.296
Error	24	1.38627	0.05776		
Total	35	8.6724			

For all the sampling stations, the acidity of the water was ≈1.0 pH unit lower in May (pH = 7 ± 0.1) when compared to the observations in March and July; pH in March and July were not significantly different (pH = 8 ± 0.1)

Chlorophyll-a concentrations

Chlorophyll-a concentration of the estuary is shown in the Figure 16. The concentration of chlorophyll-a ranged from 0.6 – 1.7µg.l^{-1}. The highest concentration was recorded in May at sampling station located 0.1 NM from the surf zone. Lowest chlorophyll-a concentration was recorded in March the at sampling location 0.3 NM from the surf zone of the estuary.

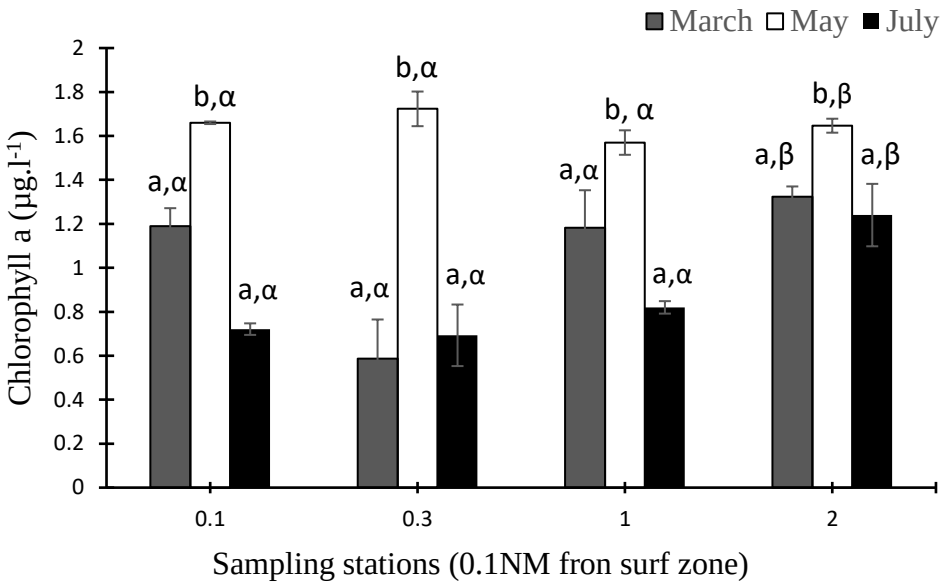

Figure 16*:* Chlorophyll-a concentrations at different stations (NM from surf zone) on the Volta River Estuary of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets: English alphabets compare monthly observations within different sampling stations and Greek alphabets compare variations between the sampling stations

Changes observed in chlorophyll-a concentrations were significantly dependent on sampling months as well as the sampling locations (Table 7).

Table 7: Two-way ANOVA comparing chlorophyll-a concentration at different locations (0.1, 0.3, 1.0 and 2.0 NM from surf zone) of the Volta Estuary on different sampling months (March, May and July 2022)

Source of Variance	DF	Adj SS	Adj MS	F	P
Sampling month	2	3.94758	1.97379	65.25	0.000
Sampling station	3	0.72922	0.24307	8.04	0.001
Month*Station	6	0.85987	0.14331	4.74	0.003
Error	24	0.72603	0.03025		
Total	35	6.26269			

Chlorophyll-a concentrations were highest in May (≈ 16 - 22%) than in March and July. Concentrations measured in March and July were not significantly different for all the sampling stations (post hoc test $p< 0.05$).

With regards to sampling locations, concentration of chlorophyll-a was about 4 – 8% higher at the sampling station 2 NM farthest from the surf zone of the estuary compared with the locations closest to the surf zone. Concentration at sampling stations closer to the surf zone (0.1. 0.3 and 1.0 NM) were observed to be statistically similar at 1.1 ± 0.1 $\mu g.l^{-1}$ (post hoc test; $p< 0.05$).

Abundance of Mesozooplankton

On average, the abundance of mesozooplankton collected in the estuary for the study duration was 10 ± 4 $Ind.l^{-1}$. Maximum mesozooplankton abundance (14 Ind.l-^{1}) was recorded in July at the sampling station located 2NM from surf zone of the estuary. Minimum abundance (3 $Ind.l^{-1}$) was recorded in the same month at the station located 0.1 NM from the estuarine surf zone (Figure 17).

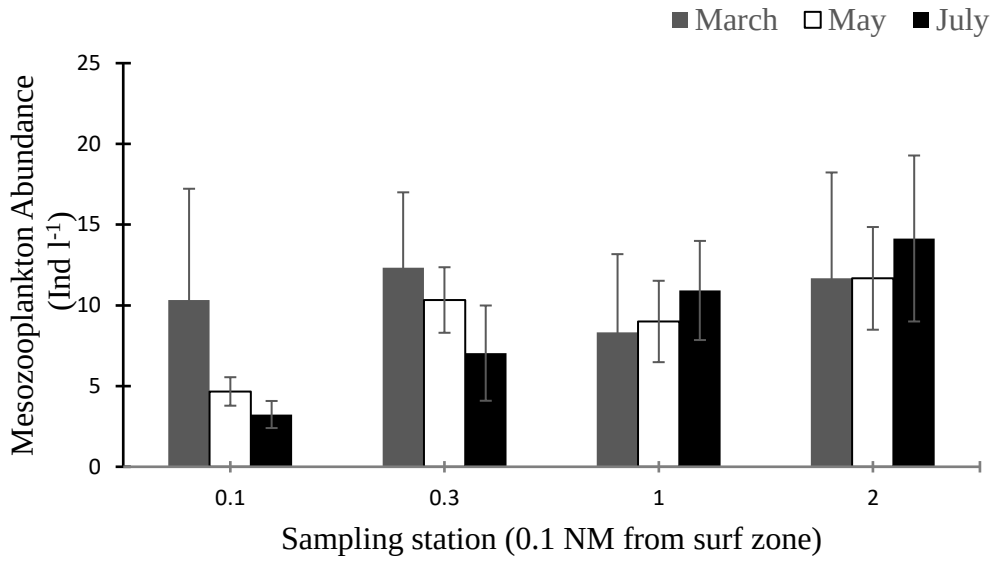

Figure 17*:* Mesozooplankton Abundance (average ± standard error) recorded at different stations (NM from surf zone) of the Volta Estuary of Ghana for the different sampling months

Marked differences were observed in the mesozooplankton abundance on the different sampling months and stations. Abundance of mesozooplankton was highest in March (≈ 6 – 8%) as compared to May and July; which recorded average abundance of 9 ± 3 Ind.l-^{1}. With respect to sampling locations, lower mesozooplankton abundance (7 - 10%) was recorded at sampling stations closer to the surf zone of the estuary. However, ANOVA comparison of the results showed that the differences were not statistically significant (Table 8).

Table 8: Two-way ANOVA comparing Mesozooplankton Abundance at different locations of the Volta Estuary on different sampling months (March, May and July 2022)

Source of variance	DF	Adj SS	Adj MS	F	P
Sampling month	2	28.15	14.0728	0.28	0.759
Sampling station	3	193.38	64.4608	0.28	0.306
Month*Station	6	120.73	20.1210	0.40	0.873
Error	24	1213.72	50.5716		
Total	35	1555.97			

Taxonomic Composition of Mesozooplankton in the Volta River Estuary

Figure 18 shows the taxonomic composition of mesozooplankton in the estuary. Four different mesozooplankton orders were identified in this study. These were the Copepoda, Decapoda, Cladocera and Rotifera. The bulk (68%) of the mesozooplankton was made up of copepods. This was followed by the decapods (25%), the cladocerans (4.5%) and the rotifers (0.5%) in decreasing order. A fraction (2%) of the mesozooplankton could not be identified in this study (Figure 18).

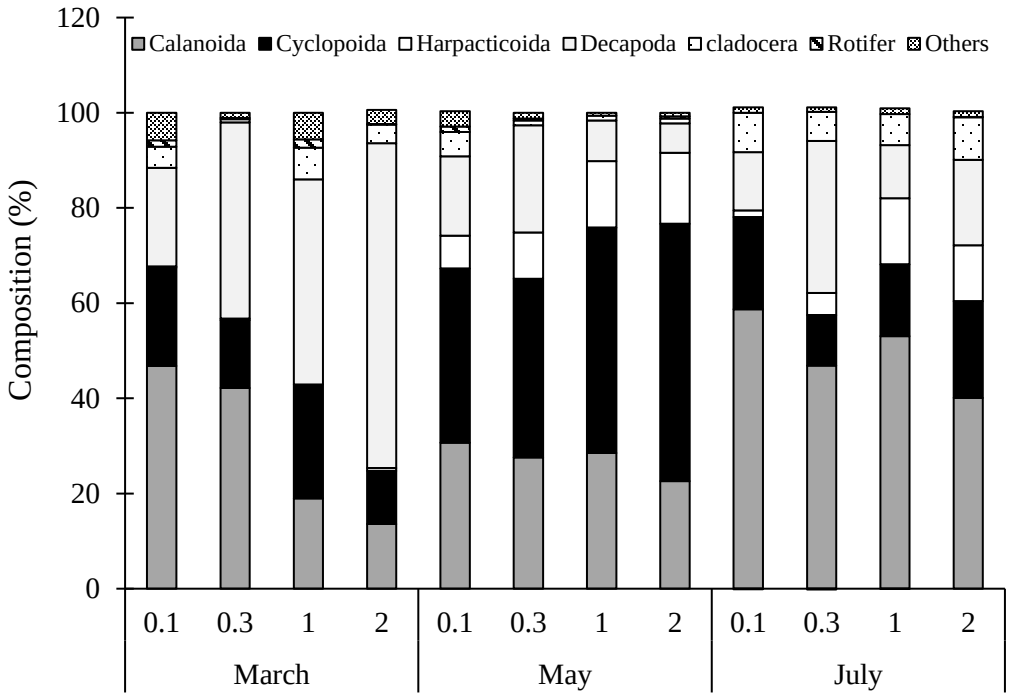

Figure 18: Contribution of the different taxonomic groups to the total number of mesozooplankton observed at different stations (0.1,0.3, 1 and 2 NM) for the different sampling months on the Volta River Estuary of Ghana

The copepods which constituted the bulk of the mesozooplankton were dominated by individuals belonging to the Order Calanoida (52%), followed by Cyclopoida (38%) and Harpacticoida (10%) (Figure 19).

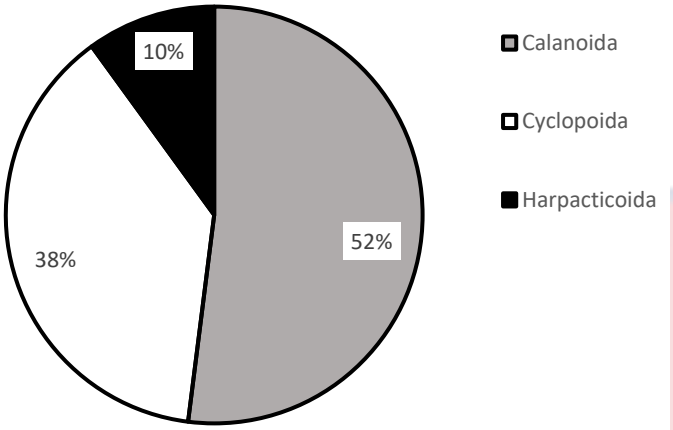

Figure 19: Composition of Copepod Orders observed at different stations and sampling months combined

The contribution of the different groups to the total mesozooplankton abundance was significantly different (Table 9). The abundance of the different taxonomic groups was different on the different sampling months. With regards to the sampling stations, only the abundance of harpacticoid was different.

Table 9: Two-way ANOVA comparison of the composition of different taxonomic groups to the total number of mesozooplankton recorded in March to July 2022 on the Volta River Estuary of Ghana

Variables	Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Calanoida	Month	2	3526	1763.1	6.18	0.007
	Transect	3	1930	643.5	2.26	0.108
	Month*Transect	6	1216	202.7	0.71	0.644
	Error	24	6847	285.3		
	Total	35	13520			
Cyclopoida	Month	2	5722.2	2861.12	33.59	0.000
	Transect	3	405.9	135.29	1.59	0.218
	Month*Transect	6	642.2	107.04	1.26	0.314
	Error	24	2044.4	85.18		
	Total	35	8814.8			
Harpacticoida	Month	2	771.0	385.476	56.30	0.000
	Transect	3	275.2	91.722	13.40	0.000
	Month*Transect	6	163.3	27.225	3.98	0.007
	Error	24	164.3	6.846		
	Total	35	1373.8			
Decapoda	Month	2	6148	3074.2	9.45	0.001
	Transect	3	1517	505.7	1.56	0.226
	Month*Transect	6	3216	535.9	1.65	0.177
	Error	24	7804	325.2		
	Total	35	18685			
Cladocera	Month	2	200.40	100.20	4.91	0.016
	Transect	3	52.95	17.65	0.87	0.473
	Month*Transect	6	66.56	11.09	0.54	0.770
	Error	24	489.72	20.40		
	Total	35	809.62			
Rotifera	Month	2	5.422	2.7109	3.45	0.048
	Transect	3	2.060	0.6868	0.87	0.469
	Month*Transect	6	5.451	0.9084	1.16	0.362
	Error	24	18.868	0.7862		
	Total	35	31.801			
Others	Month	2	53.43	26.716	3.60	0.043
	Transect	3	27.87	9.290	1.25	0.313
	Month*Transect	6	32.28	5.379	0.72	0.634
	Error	24	178.27	7.428		
	Total	35	291.85			

The abundance (30 ± 6) of the Calanoida was about 19 – 22% higher in July when compared with abundance in March and May. Observations made in March and May were statistically similar, at 39 ± 4. Abundance of the Cyclopoida (44 ± 3) was ≈ 30% higher in May than in March and July; abundance in March and July were statistically similar. The abundance (7 ± 1) of the individuals belonging to Harpacticoida was about 8 – 11% higher in May than the abundance in March and July. The Harpacticoida abundance observed in March and July were not statistically different (post hoc test; $p < 0.05$); the average was 6 ± 1, the abundance (25 ± 5) of the Decapoda was highest in March. This observation was about 25 - 30% higher than the abundance in May and July; which were not statistically different. The average abundance of decapod in May and July was 16 ± 5. Rotifera recorded higher abundance ≈1% in July than in March and May (post hoc test; $p < 0.05$).

Variations in the abundance of the different taxonomic groups were not significantly different when the different stations were compared: except for the Harpacticoida group where the observations made at the different sampling stations were statistically significant.

Relationship between Environmental Parameters and Mesozooplankton Abundance

The environment in this study was described using six different physico-chemical parameters (pH, temperature, conductivity, salinity, DO and Chlorophyll-a). Therefore, Principal Component Analysis (PCA) was used to determine environmental variable(s) that may drive mesozooplankton abundance in the

estuary using the minitab statistical software version 21.1. Results showing the principal components are shown in Table 10.

Table 10: Loading coefficients of variables and their contribution to the variations in data for First Four Principal Components (PC) with Eigenvalues >1.0

Variable	PC1 34%	PC2 21%	PC3 17%	PC4 8%
Chlorophyll-a concentration	0.41	0.07	-0.03	0.14
Total mesozooplankton	0.00	0.11	0.45	0.07
Calanoida abundance	-0.30	0.27	-0.13	-0.46
Cyclopoida abundance	0.40	0.18	-0.20	0.09
Harpacticoida abundance	0.17	0.48	-0.02	0.29
Decapoda abundance	-0.04	-0.43	0.39	0.15
Cladocera abundance	-0.20	0.02	-0.35	0.53
Rotifer abundance	0.16	-0.34	-0.39	0.04
Unidentified zooplankton abundance	0.08	-0.39	-0.40	0.08
pH	-0.29	-0.38	0.15	0.09
Temperature	0.41	-0.16	0.10	-0.31
salinity	-0.25	0.03	-0.34	-0.40
Dissolved oxygen	0.41	-0.16	0.02	-0.32

The first four components together explained 84% of the variations in the data. The first principal component (PC1) explained 34% of the variations, with chlorophyll-a concentration, abundance of Calanoida, Cyclopoida, sea surface temperature and dissolved oxygen being the key variables of the component. The second principal component (PC2) explained 21% of the variations in the data; the key contributors to this component were the abundance of the zooplankton groups Harpacticoida, Decapoda, Unidentified zooplankton and Rotifera and pH. The

third principal component contributed 17% to the variations in the data. The major determinants of this component were the abundance of total mesozooplankton, Decapoda Unidentified zooplankton and salinity. Principal component four contributed only 8% to the variations. The main contributors to this component were Calanoida, Cladocera, temperature, salinity and dissolved oxygen.

Variables that co-vary were grouped together on the loading plot (Figure 20); three different groups were detected. The variables for the first group were sea surface temperature, amount of dissolved oxygen, chlorophyll-a concentration and Cyclopoida abundance. The second group was made up of. pH, salinity, Cladocera and Calanoida and the third group constituted Decapoda, unidentified zooplankton and Rotifera.

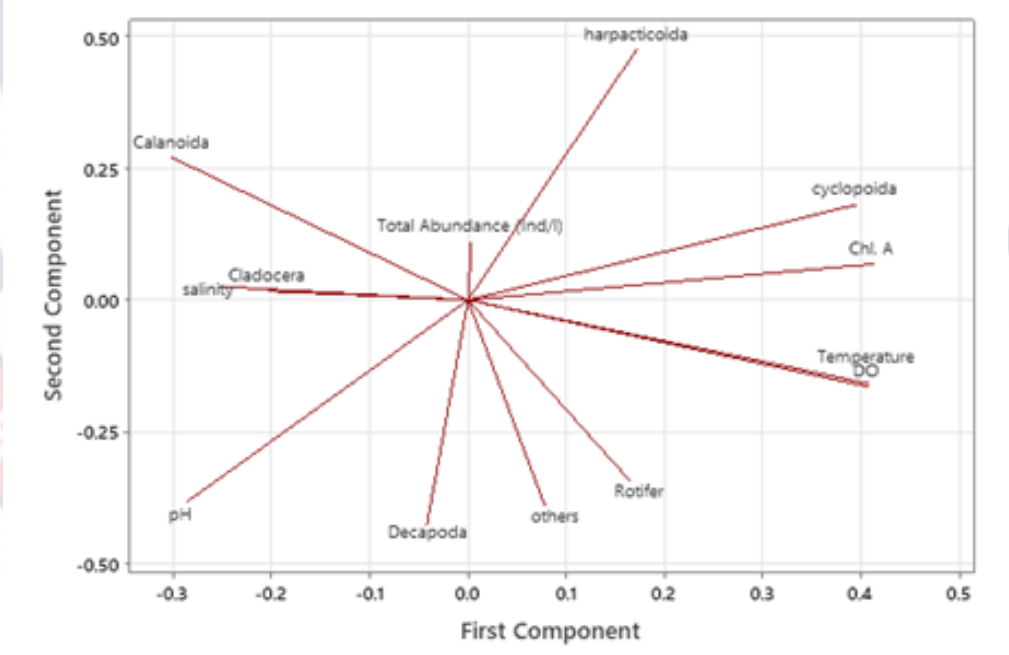

Figure 20*: Loading plot for environmental variables and mesozooplankton abundance collected from the Volta River Estuary in March, May and July, 2022*

Environmental variables determined as major contributors to the first principal component were analysed for possible relationship(s) using a linear regression analysis. Results are presented in Table 11.

Table 11: Summary of linear regression describing the relationship between environmental variables and mesozooplankton abundance from the Volta Estuary

Mesozooplankton	**Variable**	**p-value**	**R^2**
Calanoida abundance	Chlorophyll-a concentration	0.012	0.487
	Temperature	0.008	0.517
	Dissolved oxygen	0.014	0.469
Cyclopoida abundance	Chlorophyll-a concentration	0.002	0.648
	Temperature	0.024	0.415
	Dissolved oxygen	0.033	0.381

The abundance of the Order Calanoida was significantly related to chlorophyll-a concentration, sea surface temperature and amount of dissolved oxygen. Respectively, chlorophyll-a concentration, sea surface temperature and amount of dissolved oxygen accounted for 49%, 52% and 47% of the variations in the abundance of the calanoid copepod ($p < 0.05$).

Cyclopoida abundance showed significant relationship with all the variables as well; with chlorophyll-a accounting for 65%, temperature 42% and dissolved oxygen 38% in the variations observed in Cyclopoid.

Mesozooplankton Biological Rates

Biological rates - faecal pellet, egg production and mortality - of mesozooplankton under different conditions of temperature and salinity were investigated using microcosm experiments (Chapter 3). Two different species of calanoid copepod (*Temora Stylifera* and *Paracalanus parvus)* were used for the experiments. The justification for this was that the two species are cosmopolitan (Razouls et al., 2005) and were also observed in the Volta estuary during this research The results of the experiments are presented in the following sections.

Faecal Pellet Production

Figure 21 shows the rate of faecal pellet production (FPP) by *Temora stylifera and Paracalanus parvus* under different conditions of temperature and salinity. Highest rate of faecal pellet production (≈ 45 ± 7 pellets.cop^{-1}.day^{-1}) by *T. stylifera* was recorded when the animal was cultured at the control temperature (28 ^{o}C) when there was no warming; the lowest FPP (≈ 3 ± 1 pellets.cop^{-1}.day^{-1}) was recorded when the culture temperature was 4 ^{o}C warmer (Figure 21A). These observations were made irrespective of the salinity of the cultures (Table 6.0). Similar observations were made on *P. parvus.* The highest rate of faecal pellet production (≈ 21 ± 5 pellets.cop^{-1}.day^{-1}) by the species occurred at the control temperature; and the lowest rate of FPP (≈ 3 ± 1 pellets.cop^{-1}.day^{-1}) was observed when the culture was 4 ^{o}C warmer (Figure 21B; Table 12). Therefore, for both species, the combination of salinity and temperature had no significant impact on the rate of faecal pellet production

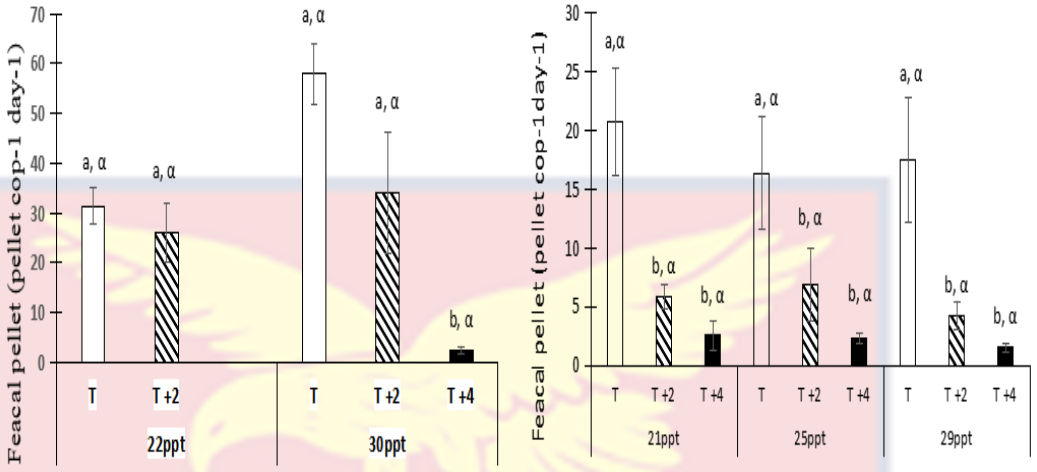

Figure 21: Combined effect of warming and salinity on the rates (mean ± SE) of faecal pellet production by Temora stylifera (A) and Paracalanus parvus (B). T, T+2 and T+4 represent the levels of thermal stress. Mean values that are significantly different are indicated by different letters (post hoc test at $p < 0.05$ after two – way ANOVA); Roman and Greek letters represent a comparison of observations within temperatures and between salinities respectively

Table 12: Two-way ANOVA comparing faecal pellet production under different temperature and salinity conditions *by Temora stylifera and Paracalanus parvus*

Copepod species	Source of variance	DF	SS	MS	F- Value	P-Value
Temora stylifera	Salinity	1	893.9	893.9	5.90	0.053
	Temperature	2	4436.0	2218.0	14.65	0.001
	Salinity*Temperature	2	468.1	234.0	1.98	0.180
	Error	11	1665.4	151.4		
	Total	14	6128.6			
Paracalanus parvus	Salinity	2	17.09	8.546	0.29	0.749
	Temperature	2	1280.37	640.184	22.05	0.000
	Temperature*Salinity	4	26.00	6.499	0.22	0.922
	Error	18	522.64	29.036		
	Total	26	1846.10			

Egg Production

Egg production rate (EPR) by *Temora stylifera and Paracalanus parvus* under different conditions of temperature and salinity is presented in Figure 22. Highest EPR (≈ 3 ± 0.2 females^{-1}.day^{-1}) of *T. stylifera* was recorded when cultures were exposed to a no warming scenario at 28 ^{o}C. The lowest egg production rate (≈ 1 ± 0.2 females^{-1}.day^{-1}) was recorded at the scenario where the culture temperature was 4 ^{o}C warmer (Figure 22A). These effects were independent of the salinity of the cultures (Table 13).

The behaviour of egg production rate by *P. parvus* was similar to the rate exhibited by Temora: highest rate of egg production (≈ 2 ± 0.4 females^{-1}.day^{-1}) by the species was observed at the control temperature; lowest EPR by *P. parvus* (≈ 0.2± 0.1 females^{-1}.day^{-1}) occurred when the culture was 4 ^{o}C warmer (Figure 22B; Table 13). Therefore, the combination of salinity and temperature had no significant effect on the rate of egg production by both species.

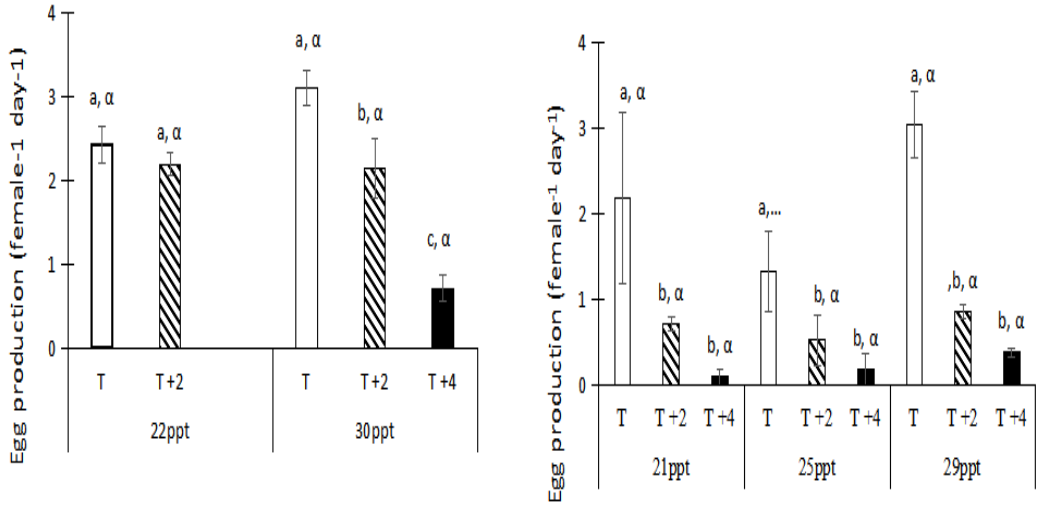

Figure 22: Combined effect of warming and salinity on the rates (mean ± SE) of egg production by *Temora stylifera* (A) and *Paracalanus parvus* (B). T, T+2 and T+4 represent the levels of thermal stress. Mean values that are significantly different are indicated by different letters (post hoc test at $p < 0.05$ after two – way ANOVA); Roman and Greek letters represent a comparison of observations within temperatures and between salinities respectively\

Table 13: Two-way ANOVA comparing egg production under different temperature and salinity conditions by Temora stylifera and Paracalanus parvus

Species	Source of Variation	df	SS	MS	F	P-value
T. stylifera	Salinity	1	0.29	0.29	1.61	0.230
	Temperature	2	8.32	4.16	23.33	0.000
	Salinity*Temperature	2	0.55	0.27	2.08	0.168
	Error	11	1.96	0.18		
	Total	14	10.66			
P. parvus	Salinity	2	2.52	1.26	2.52	0.108
	Temperature	2	18.98	9.49	19	0.000
	Temperature*Salinity	4	2.18	0.54	1.09	0.391
	Error	18	8.99	0.50		
	Total	26	32.68			

Post hoc analysis of results showed that the rate of egg production by *Temora* at the control temperature (28 ^{o}C) was about 33% higher than the rate measured when the temperature of the culture was warmest (32 ^{o}C). The rate of the decline in egg production by the copepod under warming was ≈ 1 egg per each degree of warming (linear regression analysis; R^2 = 0.94). The response of *Paracalanus* was similar to these results; the difference between the rate of egg production by the copepod at the control and warmest temperatures was about 63%, at a rate of 2 eggs per day.

Mortality

Figure 23 shows the mortality rate (MR) in *Temora stylifera* and *Paracalanus parvus* under different temperature and salinity conditions. *For Temora,* the highest rate of mortality (≈ 71 ± 17 %) was observed when temperature was 4 ^{o}C warmer. The MR of the copepod was lowest (≈ 24 ± 7 %) when the copepod was exposed to no warming scenario. (Figure 23A). These observations were significantly related to the salinity of the cultures (Table 7.0). However, the analysis of the results indicates the impact of salinity was particularly significant when there was no warming (Figure 23). Mortality rate of *Paracalanus* was highest (≈ 57 ± 6 %) at the warmest temperature (+4^{o}C); the mortality of the animal was lowest, at ≈ 27 ± 3% when there was no warming (Figure 23B). This response to warming was significantly related to salinity (Table 14).

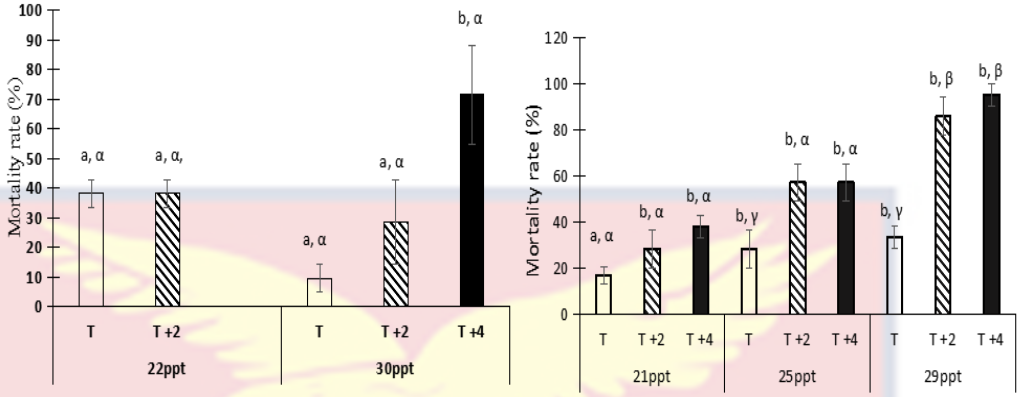

Figure 23: Combined effect of warming and salinity on the rates (mean ± SE) of mortality by *Temora stylifera* (A) and *Paracalanus parvus* (B). T, T+2 and T+4 represent the levels of thermal stress. Mean values that are significantly different are indicated by different letters (post hoc test at $p < 0.05$ after two – way ANOVA); Roman and Greek letters represent a comparison of observations within temperatures and between salinities respectively.

From the ANOVA analysis of results, the mortality rate of *Temora stylifera* and *Paracalanus parvus* was similar at the different salinities (Table 14).

Table 14: Two-way ANOVA comparing faecal mortality rates under different temperature and salinity conditions by Temora stylifera and Paracalanus parvus

Species	Source of Variation	SS	df	MS	F	P-value
	Salinity	1	1088.4	1088.4	3.38	0.093
T. stylifera	Temperature	2	5759.6	2879.8	8.96	0.005
	Salinity*Temperature	2	8458.0	4229.0	15.54	0.000
	Error	11	3537.4	321.6		
	Total	14	9306.1			
P. parvus	Temperature	2	4731.7	2365.84	10.79	0.001
	Salinity	2	5457.3	2728.65	12.45	0.000
	Temperature*Salinity	4	1798.9	449.74	2.05	0.130
	Error	18	3945.6	219.20		
	Total	26	15933.5			

Eco-physiological Indicators for Salinity and Temperature Combined

In order to determine suitable indicators for the impact of temperature and salinity on the copepods (*Temora stylifera* and *Paracalanus parvus)*, a multivariate regression analysis was performed. Results are presented in Table 15.

Table 15: Summary of multivariate regression analysis indicating the relationship between biological rates of copepods exposed to sea surface warming and salinity fluctuations combined

	Temora stylifera		*Paracalanus parvus*	
	P-value	R^2	P-value	R^2
Faecal pellet production	0.001	0.667	0.000	0.637
Egg production	0.000	0.739	0.000	0.559
Mortality	0.017	0.495	0.012	0.308

In both species the relationship between each of the biological rates and the combination of sea surface warming and changes in salinity was statistically significant. However, the biological rate that could highly be related to sea surface warming and changes in salinity in *Temora* was egg production. For this species ≈74% of the variations in egg production rate could be explained by the combination of the two stress factors. In contrast, the species *Paracalanus* the biological rate that could highly explain the combined stressors (sea surface warming and changes in salinity) was faecal pellet production rate. The two

stressors together could explain 64% of the variations in this biological rate. This result suggests that different biological rates may indicate the response of *Temora* and *Paracalanus* to the combination of sea surface warming and changes in salinity.

CHAPTER FIVE

DISCUSSION

The objective of the study was to evaluate the abundance, composition and response of mesoplankton in Ghana's Volta River estuary relative to physicochemical conditions and climate induced temperature and salinity factors. Therefore, the effect of physicochemical conditions on the abundance of mesozooplankton within the catchment is discussed in this section. This is followed by discussion on the eco-physiological indicator that best relates common copepod species (*Temora stylifera* and *Paracalanus parvus*) to the combined impact of the two climate-induced factors.

Mesozooplankton Composition in the Volta River Estuary

Total mesozooplankton abundance in the Volta Estuary comprised four major groups (Figure 18). These groups of zooplankton were also observed in a previous study in the same study area (Darpaah, 2013). The bulk of this composition was the group copepoda. Their composition in coastal waters has been well documented (Meremo *et al*., 2022; Saidin, 2010; Weinstock *et al*., 2022). Their abundance has been attributed to their sexually reproducing ability which help them to delete "mutations and promote good genes" (Kiørboe, 2011). They are also known to have a wide range of adaptation to unfavourable environmental conditions; thus, they have the ability to reduce their metabolic rate in responding to stressors (Mayor, Sommer, Cook & Viant, 2015).

In contrast to this observation, Abdul et al. (2016) identified Copepoda as the least abundant group in a study conducted in a tropical estuary at the Bight of

Benin South-west, Nigeria. However, the study explained that predatory pressure by planktivorous fishes might have caused the drastic reduction in these larger zooplankton groups.

According to Wetzel (1983) copepods are divided into three suborders; Calanoida, Cyclopoida, and Harpacticoida. All three orders were identified in the Volta Estuary in the current study and in previous works as well (Darpaah, 2013) with the order Calanoida being the most dominant. This finding is in agreement with other works which have reported similar observations in Ghanaian coastal waters (Asiedu, 2020; Wiafe & Freid, 2001). The dominance of this order may be attributed to their torpedo shape and presence of sensory organs, including antennule which promote their ability to escape predators (Wiafe & Freid, 2001). The least abundant order was the Harpacticoida (Figure 18). Their least occurrence may be due to their benthic nature which makes them less dominant in pelagic waters (Lee & Lee, 2019).

According to Weinstock *et al*. (2022) different groups of mesozooplankton show different response to environmental conditions. This is reflected in the response of the different groups of mesozooplankton recorded in this study to the different physico-chemical parameters shown in the principal component analysis (Table 10)

Relationship between physico-chemical factors and mesozooplankton

Generally, the structure, abundance and composition of planktonic communities are regulated by the physical and chemical conditions of the environment (Dirican, 2014; Richardson & Schoeman, 2004). This is because

organisms require specific environmental conditions in order to grow and function at optimum levels (Nyekodzi *et al*., 2017). According to Chen (2020), a key environmental factor affecting the growth and development of mesozooplankton is temperature. This is because the ectothermic nature of these organisms makes them exceptionally vulnerable to changes in ambient temperature.

From the results of this research, temperature contributed significantly to mesozooplankton variations occurring in the estuary (Table 10). A regression analysis of temperature and the two most abundant mesozooplankton which constituted about 65% to the total mesozooplankton shows the extent of the influence of temperature. Temperature accounted for 52% and 42% in the differences in both Calanoida and Cyclopoida respectively. Therefore, temperature is a key variable that determines mesozooplankton composition in the estuary.

In this study, temperature had significant impact on the rates of faecal pellet production and egg production (Table 12 and 13) of copepods. Both processes decreased with increasing temperature (Figure 21 and 22). This observation compares with results from experiments conducted by Choi *et al*. (2021) and Majid *et al*. (2016).

Biological processes, such as food intake, digestion and absorption, generally influence the production of faecal pellets in copepods. According to Wotton (1994), faecal pellets are produced after ingested food items has been digested and assimilated. Previous studies e. g. Hu *et al*. (2018), Tirelli and Mayzaud, (2005) have shown that food ingestion by copepods, including *Temora stylifera* and *Paracalanus parvus* increases as temperature increase. However,

Boscolo-Galazzo *et al*. (2018) observed that the rates of digestion and assimilation increases only to an optimum temperature level and then decrease when temperature increases due to temperature-related constraint on the reactivity of digestive enzymes. Also, according to (Martin & Båmstedt, 2016) the period for food digestion increases as temperature increases.

From the above reports, an assertion can be made that the results of the current study showing a decline in the production of faecal pellet as temperature increased (Figure 21A and Figure 21B) may be attributed to one of two of the following reasons;

1. *Temora stylifera* and *Paracalanus parvus* might have not ingested enough food items for digestion or
2. they might have ingested enough food items but digestion may have not completed before their death due to long digestion periods or
3. the animals might have digested but have over-assimilated to meet energy demands for high metabolic activities under higher temperatures.

The reduction in faecal pellet production in copepods could affect the transfer of carbon from surface waters into deeper parts of coastal systems for burial in a process known as biological carbon pump.

In effect this the process which helps to regulate carbon dioxide concentrations in the atmosphere will be limited (Coppock et al., 2019; Steinberg & Landry 2017). Eventually, the role of coastal ecosystems in carbon sequestration will be negatively impacted.

Choi et al. (2021), reported that egg production rate increases with

increasing temperature to optimum levels but starts to decrease when temperatures increase exceeding the optimum. The decline in egg production when temperature was increased by +2 and +4 °C above the control (28 °C) is in agreement with previous studies. The decrease may be as a result of the fact that organisms when exposed to warmer temperatures invest most of the energy assimilated into their metabolic maintenance including physiological processes such as thermoregulation in order to ensure their survival (Acheampong, Hense & John 2014). Once maintenance costs are covered, the remainder of the substrates can be made available for egg production (Acheampong, Nielsen, Mitra, & John, 2012).

The effect of warming on the rate of egg production observed in this study (Figure 22A and 22B) suggests that it is likely for the growth of the copepod to be negatively impacted by the increase of sea surface temperatures projected under global climate change (IPCC, 2018). These occurrences will lead to a direct decrease in the production of eggs, and this is followed up by nauplii's decline and subsequently their recruitment among plankton populations. This will result in negative effects on the aquatic food web as copepods act as the major primary consumers in these ecosystems.

Contrary to faecal pellet production and egg production rates, the rate of mortality in each copepod species used in the present study increased significantly as temperature increased across the different salinity levels (Figure 23A and 23B). Hall and Burns (2001) made a similar observation in a calanoid copepod as temperatures increased from 10 to 20 ^{o}C. Pörtner and Peck (2010) reported that mortality rates provide basic information on the tolerance of organisms to

environmental conditions. Therefore, the findings from this study provide an insight to the influence of temperature on tropical calanoid copepods under climate change conditions and its implication on aquatic food webs.

Temperature range (27 – 30°C) recorded in this study corresponds to the ranges reported by other studies conducted in the study area (Obirikorang, Amisah & Adjei-Boateng, 2013). This temperature range has been reported (Dzakpasu & Yankson, 2015; Okyere, 2010) to be a typical characteristics of shallow tropical coastal water bodies. However, the temperature recorded in this study was higher compared to previous studies in the same area (Adjei-Boateng & Wilson, 2013; Madkour *et al*., 2011). Higher temperatures recorded in March and May and colder temperatures in July (Figure 8) reflect the climate conditions of the coastal zone of the study area based on the climatic zonation of Ghana (Bessah & Amekudzi, 2022). Colder temperatures in July may also be as a result of the upwelling conditions during this period.

With respect to the vertical profiles in temperature, a marked decrease was observed as depth increased (Figure 9). This variation was marginal, rarely greater than 1°C from that recorded at the surface.

Similar observation was made by (Geawhari *et al*., 2014) in a tropical estuary. This observation indicates a homogenous temperature across the water column of the estuary.

According to Cloern *et al*. (2017) and Havens (2015), salinity in estuaries decreases through the dilution of the riverine source or rainfall and increases through evaporation and saltwater intrusion. In this study, higher salinities were

recorded at stations closer to the surf zone of the estuary as compared to those farther away. Similar trends have been reported in works done in the same study (Adjei-Boateng & Wilson, 2013; Madkour *et al*., 2011; Nyekodzi *et al*., 2017). Higher salinities closer to the surf zone may be attributed to the influence of sea water as this station is closer to the ocean. As one moves farther away from the surf zone, the rate of salt water intrusion reduces and the influence of freshwater increases. This may also explain the less saline conditions at these stations.

Salinity was also observed to increase with depth (Figure 11). This agrees with the observations made by Nyekodzi *et al*. (2017); it may be attributed to the density of salt water to freshwater. Saline water is denser than freshwater hence in estuaries where both freshwater and saline water come together, the surface water is mostly less saline than the bottom water.

The impact of salinity on mesozooplankton in this study was not obviously shown. However, a general trend could be observed between salinity and the total mesozooplankton abundance; where higher abundance was recorded in areas of lower salinity as compared to areas where salinity was higher. This relationship has been reported in similar works done in estuarine environments (Yu *et al*., 2020).

With regards to mesozooplankton biological rates, only mortality rate related significantly with salinity. Hall and Burns (2001) made a similar observation in a study involving a calanoid copepod across different salinity levels. Again, Medina and Barata (2004) reported that the daily copepod mortality rate of *A. tonsa* was constant at 5% in optimal salinity gradient, but it increased by about 50% in high salinity with values above 25 psu. From the above reports, an assertion

can be made that salinity at increasing levels subject copepods to high levels of stress as the organism will be investing much energy in dealing with osmoregulation.

The close relation between salinity and conductivity has been well noted. In this study conductivity was observed to increase with increasing salinity. Egbi et al., (2018) also reported a similar trend in the same area as this study. Elevated conductivity values were observed at the locations closer to the surf zone. Values ranged between 2.08mS/cm and 12.27mS/cm (2080µS/cm and 12270µS/cm respectively). Lower values ranging from 79.33µS/cm to 195µS/cm were recorded upstream (2.0NM). These findings correspond to some conductivity values reported in earlier works. Example Amoah and Koranteng (2006), Anornu *et al*. (2017) and Gampson *et al*. (2014) respectively, reported 99–287 µS/cm, 152–231 µS/cm and 62.5–83.6 µS/cm for surface water at sections of the study area. However, some works are in contrast with the current study. For instance, Madkour *et al*. (2011) reported 52 – 70 µS/cm. However, the conductivity measures in the current study were found to be in optimal ranges.

The amount of dissolved oxygen has also been reported in many studies to be a key determinant of abundance in most aquatic organisms (Ismail & Adnan, 2016; Meremo *et al*., 2022). In the current study, DO ranged between 2.24 and 6.98 mgl^{-1}. This falls within the optimal range between 2.0 – 11.0 mgl^{-1} (Behar, 1997) which is tolerable to aquatic organisms including mesozooplankton. This range also corresponds to that which was reported by (Madkour *et al*., 2011) in a study conducted in the same area as the current study. The high DO recorded in May,

may be attributed to the rate of photosynthetic activities during this period considering the number of chlorophyll-a concentrations at the time.

Dissolved oxygen also contributed significantly to the changes occurring in the estuary (Figure 10). It contributed 47% to the changes in the most abundant mesozooplankton group (Table 11) and 38% to the second largest group of mesozooplankton observed in the estuary. This finding was in agreement with the works of Ismail and Adnan (2016) and Meremo *et al*., (2022) conducted in Lake Victoria, Kenya and two man-made Lakes (Harapan and Aman Lakes) in Malaysia respectively.

According to Fondriest Environmental Inc. (2013b), majority of aquatic organisms prefer a pH range of 6.5 to 9.0. Also, Ekubo and Abowei, (2011) emphasized that pH between 7 to 8.5 is ideal for biological productivity. In the current study, pH values recorded throughout the study period was within these ranges as well as within the range reported by Egbi *et al*. (2018) and Madkour *et al*. (2011) in their works conducted in the same study area as the current research. Hence the above assertion suggests that the pH ranges (7 - 8) recorded in the present study are within the range required for the survival of mesozooplankton.
However, statistical analysis showed no significant relationship between pH and the mesozooplankton encountered in the estuary.

Relationship between chlorophyll-a and mesozooplankton abundance has been reported over the years (Gołdyn & Kowalczewska-madura, 2008; Vallina *et al*., 2014). Most of these studies have reported significantly positive relationships, however, findings from the current study suggest that different groups of

mesozooplankton respond differently to chlorophyll-a concentrations. According to (Meyers, 2020) different mesozooplankton groups may not entirely be dependent on phytoplankton abundance to sustain their biomass. Thus, some groups may use alternative food sources such as microzooplankton to meet their energy needs. This may explain the reason why mesozooplankton total abundance in the present study showed no significant relationship with chlorophyll-a concentration. Individually, some groups showed strong significant correlation with chlorophyll-a.

The strong positive correlation between copepods and chlorophyll-a was reported by Asiedu (2020) as well as many other works. This strong correlation is explained based on the assertion that copepods are the major consumers of phytoplankton in coastal waters (Jones, Flynn, & Anderson, 2002; Richardson, 2008). The current study somehow agrees with these findings as the copepod orders Cyclopoida showed significantly strong relationship (65%) with chlorophyll-a. In contrast, the copepod order Calanoida exhibited a negative relationship (Table 10) with chlorophyll-a. Jenkins and Black (2018) made a similar observation in a study involving the calanoid copepod *Paracalanus* and diatoms. Vargas *et al*. (2006) also made similar observations in *Paracalannus parvus* in a coastal upwelling system off central Chile. Taylor *et al*. (2012) reported that non-calanoid copepod harpacticoid is resistant to toxic effects of a diatom diet. Bouley and Kimmerer (2006) and Nishibe, Kobari and Ota. (2010) also found that cyclopoid copepods prefer larger motile prey such as ciliates and dinoflagellates over diatoms. Inferring from these findings, the observations made in this study may be attributed to the presence of phytoplankton groups that may be toxic to Calanoida like diatoms but

have limited effect on Cyclopoida and Harpacticoida.

In this study the highest monthly mean concentration ($1.65\mu gl^{-1}$) was attained in the month of May. This significant increase from the previous sampling month may be attributed to the fact that the rains set off the previous month and might have resulted in much freshwater inflow into the estuary which might have brought in more nutrients during this period. Also, lower chlorophyll-a levels in July might have been as a result of the increase rains at this period which might have increased suspended particles hence decreasing light transparency in the estuary.

Biological Rates for Explaining the Combined effect of Warming and Salinity

Warming as a result of global climate change together with changes in salinity resulting from variations in freshwater inflows are causing radical changes in estuarine ecosystems. Organisms who reside in these ecosystems are already experiencing osmotic stress, this together with thermal stress from warming may have severe impacts on these ecosystems.

Warming and salinity changes combined may not only affect the distribution of mesozooplankton, but also their physiological responses (Peck *et al*., 2015). However, the extent of impact on different physiological responses may be dependent on individual species. According to Weissenberg *et al*. (2022), combining temperature and salinity significantly affect copepod biological rates. The findings of this study agree with the above statement as the effect of warming and changes in salinity combined was significant ($p < 0.05$) in all the biological rates measured in this study (Table 15). However, the coefficient of determination

(R^2) from the regression analysis indicates that the effects of the combined stressors is more pronounced in different biological rates in the different species. In this study, egg production highly related with temperature and salinity combined in the species *Temora*; in the copepod species *Paracalanus*, faecal pellet production related significantly with the stressors in combination.

Studies have shown that combining salinity and temperature results in a significant reduction in the rate of egg and faecal pellet production (Choi *et al*., 2021; Peck *et al*., 2015). Weissenberg *et al*. (2022) reported similar observation in egg production as the current study when *Acartia sp* was exposed to the combination of salinity and temperature. Also, Peck *et al*. (2015) reported higher rate of egg production at low salinity under optimal temperatures, however, egg production was observed to decrease as temperature increased. Some studies have also shown that copepods adapt to stress by investing energy into their survival other than reproductive activities (Chew & Chong, 2016; Milione & Zeng, 2008). This may explain the high significant relation between egg production in *Temora* and the combined stressors in this study.

Faecal pellet production in copepods is determined by the rate of food ingestion (Hu *et al*., 2018) and the rate of digestion and assimilation of ingested food (Wotton, 1994). Therefore, the high relation between faecal pellet production in Paracalanus and the combined stressors may be an indication of the higher rate of energy requirement during stress conditions. The animal may be shifting higher energy sources for osmoregulation at the expense of faecal pellet production. Also, the possibility of organisms not ingesting enough food items in stress conditions

may also be a reason for the pronounced impact observed in faecal pellet production and Paracalanus in this study.

The study therefore suggests that different biological rates may indicate the response of different mesozooplankton species to the combination of sea surface warming and changes in salinity. The findings from this study also provide insight into the influence of salinity and temperature on tropical calanoid copepods under climate change conditions and its implication on aquatic food webs.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study sought to determine the abundance, composition and sensitivity of mesozooplankton to global change factors (temperature and salinity fluctuations). The study provided critical information that can be used for predicting future changes in mesozooplankton abundance and composition in the Volta estuary of Ghana. It also provided important information for the development of useful parameters which would be useful in modelling the dynamics of estuarine food chain under global climate change.

The study established a significant relationship between environmental conditions and abundance of mesozooplankton in the Volta River estuary. Mesozooplankton abundance were highest in the dry season (March) and lowest during the peak of the raining season (July). Also, the most abundant group of mesozooplankton encountered were copepod with the Order Calanoida being the bulk constituent. Microcosm experiments showed that temperature significantly affects the rate of faecal pellet production and egg production regardless of the salinity when they act in combination. However, the combination of the stressors had a synergistic impact on the mortality rate of both *Temora stylifera* and *Paracalanus parvus*.

Conclusions

The study made the following conclusions:

In general, the environmental conditions of the estuary are within optimal ranges to support the abundance of composition of Mesozooplankton. The major environmental variables determining the state of the estuary as determined by this study are chlorophyll-a, temperature and dissolved oxygen.

Mesozooplankton composition in the Volta estuary of Ghana is dominated by Copepods with the Order Calanoida being the most abundant and the least being the Order Harpacticoida. Calanoida showed a negative relationship with chlorophyll-a in the study and this may indicate the presence of phytoplankton groups that are not selected by organisms.

The biology of *Temora stylifera and Paracalanus parvus* is affected by temperature and salinity fluctuations. Increasing temperature and salinity combine to affect the survival of calanoid copepods. However, no significant impact on egg production and faecal pellet production. The combined effect of temperature and salinity can best be explained with the egg production rate and faecal pellet production rate of the organism.

Recommendation

The study recommend that investigations should consider species level differences when quantifying the response of zooplankton to global change factors. Again, Calanoid copepods were identified to dominate the mesozooplankton groups in the estuary and can therefore serve as a good proxy in mesozooplankton assessment.

Also, further studies are recommended to:

a. identify copepod taxonomic groups in the Volta estuary to the highest (species) level.
b. Assess the impact of climate change factors on copepods over generations to quantify phenotypic plasticity
c. ascertain the combined effect of temperature, salinity and pollution on key mesozooplankton in the estuary
d. collect social data from fisher folks on fish catches over a period to determine the impact of the decline in mesozooplankton abundance on fish species

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