UNIVERSITY OF CAPE COAST

COPEPOD ABUNDANCE AND IMPACT OF SELECTED HUMAN-INDUCED STRESSORS ON CALANOID COPEPOD IN THE COASTAL SEA OF GHANA

DELOVE ABRAHAM ASIEDU

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BY

DELOVE ABRAHAM ASIEDU

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

JUNE 2020

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 the 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 guidelines on supervision of thesis laid down by the University of Cape Coast

Principal Supervisors Signature..... Date .....

Name .....

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

Name .....Dr. Emmanuel Acheampong.....

ABSTRACT

According to the UN, sea surface temperatures (SST) are increasing at alarming rates and the oceans are increasingly polluted by anthropogenic activities. This thesis sought to determine how two pollutants – cadmium (Cd) and pyrene (PY) interacting with sea surface warming affect copepod egg production (EP), mortality and recycling of ingested food through faecal pellet (FP) production. A field study was conducted on transects established on the Western, Central and Eastern Coast Transects of Ghana during the dry season to evaluate copepod taxonomic composition as well as the supportive environment of the animals. The copepods were dominated by the Order Calanoida (51-53 %), followed by Cyclopoida (18-30 %), Poecilostomatoida (10-22 %) and Harpacticoida (8-10 %). The abundance of these copepods was highest (16-1276 ind. l^{-1}) on the Western Coast Transect. The combined effects of the pollutants and warming were assessed on *Temora Stylifera*, a dominant copepod found in Ghana, using a microcosm experiment involving different concentration levels of Cd (0.1-100 µg/l), PY (1-100 nM) and warming (1-3 °C) above Ghana's average dry season SST of 28 °C. Recorded EP (average: 1.73 ± 0.05 $female^{-1}.day^{-1}$) increased by ≈ 62 % with Cd concentration but had insignificant change with PY. FP (average: 54.14 ± 8.02 $copepod^{-1}.day^{-1}$) declined only under PY at concentration > 5.41 ± 0.61 nM. These effects worsened under warming. At the end, the Cd level at which 50 % mortality of the copepods were recorded (LC_{50}) decreased by ≈ 117 µg. l^{-1} per every degree of warming. In contrast, LC50 for PY increased by ≈ 6 nM per every degree of warming. These observations agree with previous studies, suggesting that warming may alter the toxicity of Cd and PY with harmful effects on marine copepods.

KEY WORDS

Phytoplankton

Zooplankton

Copepod

LIST OF ACRONYMS

IPCC	Intergovernmental panel on climate change
UN	United Nations
FAO	Food and Agriculture Organisation
APHA	American Public Health Association
GCLME	Guinea Current Large Marine Ecosystem
GMA	Ghana Meteorological Association
USAID	United States Agency for International Development
UCC	University of Cape Coast
PAHs	Polycyclic Aromatic Hydrocarbons
CCMP	Culture Collection of Marine Phytoplankton
DTU-Aqua	National Institute of Aquatic Resources-Danish Technical University
PY	Pyrene
EPR	Egg production rate
FPR	Faecal pellet production rate
MR	Mortality rate
NM	Nautical mile

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DEDICATION

To Fredrick Ekow Jonah of Blessed Memory

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CHAPTER ONE

INTRODUCTION

The coastal seas of Ghana are richly endowed with abundant marine resources such as fisheries and petroleum (Guinea Current Large Marine Ecosystem [GCLME], 2010). However, recent reports suggest that they are being exposed to elevated levels of environmental stressors such as sea surface warming, heavy metals, sewage and petroleum pollutants (Mahu, Nyarko, Hulme, & Coale, 2015; Wiafe, Yaqub, Mensah, & Frid, 2008). Combinations of the stressors mentioned above are capable of altering the functions of marine organisms, especially in tropical marine ecosystems, where the organisms are almost living near their ultimate tolerance (Bhattacharya, 1988). Consequences of these stressors are not fully understood. This study, therefore, aimed to investigate the individual and combined effects of multiple stressors on calanoid copepods in the coastal sea of Ghana.

Determining the impact of these stressors on copepods requires an in-depth knowledge of the copepod composition and abundance within Ghana's coastal seas. However, there is limited information on the copepod abundance and composition in the coastal seas of Ghana. This study also sought to provide relevant information on copepod taxonomic composition as well as the supporting environments of the animal in coastal waters of Ghana *vis-à-vis* the combined impact of sea surface warming and pollution.

1.1 Background to the Study

Marine zooplankton, inclusive of phylogenetically and functionally distinct assemblages of protists and metazoans, are significant constituents of the marine pelagic food web (Steinberg & Landry, 2017). These organisms, according to Gibbons (1997), are often classified into two different size classes: microzooplankton (size class < 200 µm) and mesozooplankton (size class > 200 µm). Copepods, particularly those belonging to the Order Calanoida, have been found to constitute a substantial part (≈ 70-90 %) of mesozooplankton biomass in the Ocean (Turner, 2004; Verity & Smetacek, 1996; Wilson, 1987). They are the major secondary consumers in the oceanic food web: consuming approximately 40 % of phytoplankton biomass in the ocean (Gréve, 2014; Frost, 1991). As a consequence, calanoid copepods provide the principal pathway of energy from primary producers to higher trophic level consumers such as fish (Figure 1.1), marine mammals and turtles (Jones, Flynn, & Anderson, 2002; Richardson, 2008). Juvenile stages of most marine fishes and some large marine animals such as whales depend solely on copepods for nutrition (Richardson, 2008). This makes copepods just one or two trophic levels away from species consumed by humans (Buskey, White, & Esbaugh, 2016).

Figure 1.1: *Illustration of the food web in marine ecosystems. (source: Kirby, 2011; Randall, n.d; Wilson,n.d)*

As critical components of the oceanic food web, calanoid copepods have the potential to mediate environmental effects with consequences for energy transfer (Acheampong, Hense, & St. John, 2014).

According to Richardson (2008), copepods are the most definite indicators of stressors in the marine ecosystem. This is because:

1. they are poikilothermic, as a result, their physiological processes such as ingestion, respiration and reproductive biology are much dependent on temperature, with rates doubling or tripling with 1^{o}C temperature rise
2. they have a relatively short life cycle. Hence, they have strong links with climate change and population dynamics. Hays, Richardson, & Robinson, (2005) and Taylor, Allen, & Clark, (2002) described zooplankton as more sensitive indicators of change than environmental variables themselves, and,
3. copepods are less exploited compared to other marine groups. Therefore, studies of long-term trends in response to environmental change are generally not confounded with trends in exploitation.

In view of the abovementioned characteristics, slight variations in environmental conditions of the ocean can significantly affect the community structure, population size and diversity of copepods.

Research suggests that global warming is one of the significant stressors influencing the abundance (Figure 1.2), biogeography, size structure and timing of important life cycles of copepods (Beaugrand & Reid, 2003; Edwards & Richardson, 2004; Richardson, 2008; Wiafe et al., 2008)

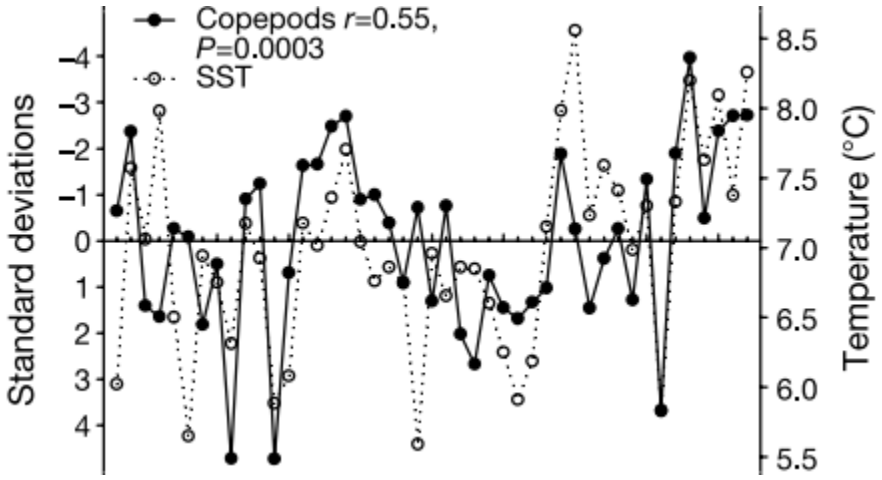

Figure 1.2: The relationship between sea surface temperature (SST) and copepod abundance (Adopted from Edwards & Richardson, 2004)

Anthropogenic activities have been identified as the primary drivers of global warming (National Research Council, 2012; National Academy of Sciences, 2014; Australian Academy of Sciences, 2015). Since the start of the industrial revolution, anthropogenic perturbations, among other things, burning of fossil fuels, cement production and changes in land use (deforestation) have increased atmospheric concentrations of greenhouse gases (Figure 1.3; Forster et al ., 2007). These gases, including carbon dioxide, methane, nitrous oxide and chlorofluoro-carbons use global warming by obstructing outgoing thermal radiation. Hence, altering the earth energy balance.

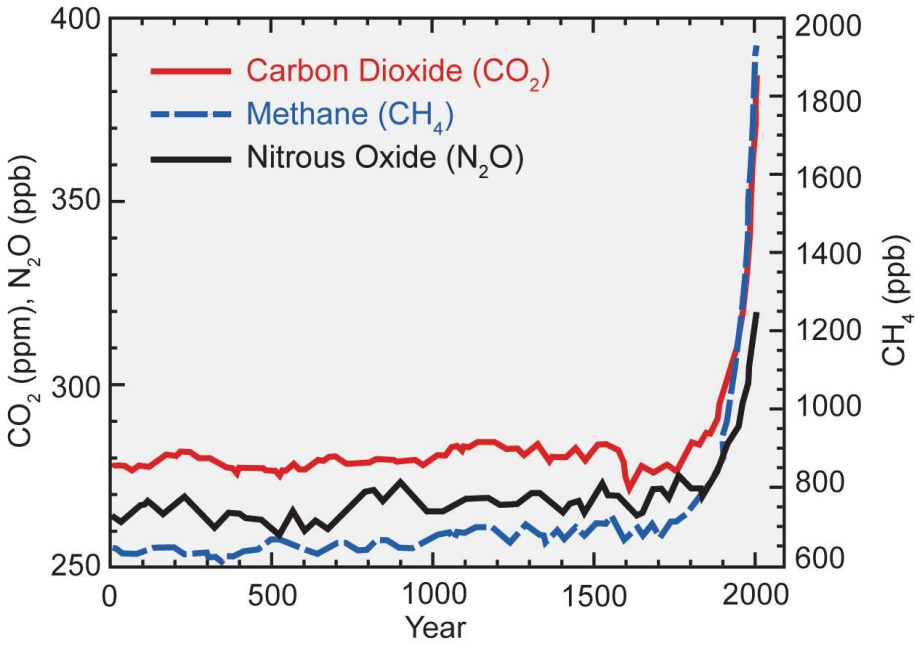

Figure 1.3: Atmospheric concentrations of important long-lived greenhouse gases over the last 2000 years (Adopted from Forster et al., 2007).

In addition to climate change, pollution from domestic and industrial sources is another human activity that can be harmful to the growth and development of marine copepods. Pollutants of significant concern include petroleum, excess nutrients from agricultural source, microplastics, polychlorinated hydrocarbons (PCBs) and heavy metals from industrial sources (National Academy of Sciences, 2007). Majority of these pollutants are sourced from land and are discharged directly or indirectly via rivers and atmospheric dropouts into the oceans. Most researchers have reported on their direct toxic effect on survival, egg production efficiency and other endocrine activities of marine copepods (Cole, 2014; Kadiene, Bialais, Ouddane, & Souissi, 2017; Kurihara, Shimode, & Shirayama, 2004; Toudal & Riisgard, 1987). The population diversity and richness of the copepods are, in turn, affected.

1.2 Statement of Problem

Globally, the stressors, as mentioned above, do not act in isolation; instead, they co-occur within marine ecosystems. As a result, they act together to impact on marine biota (Crain, Kroeker, & Halpern, 2008). However, efforts to address their combined effects are hampered by several challenges. This is because there are no general patterns for predicting individual species' responses to multiple and simultaneous changes in environmental stressors (Griffen, Belgrad, Cannizzo, Knotts, & Hancock, 2016). This limits efforts to categorise individual environmental stressors according to their relative importance for management actions. In particular, there is little knowledge of the cumulative effects of the stressors on tropical species (Crain et al., 2008; Griffen et al., 2016). These reasons, provide strong justifications for this study.

1.3 Purpose of the Study

The present study is focused on providing information on the copepod community structure in coastal seas of Ghana. The study also aims to highlight the responses of calanoid copepods to both individual and combined effects of sea surface warming and pollution by petroleum hydrocarbons and heavy metals. Information provided by this study would be critical for predicting future changes in copepod community structure, culturing of copepods as well as other mariculture activities. It would also provide vital information for the development of useful parameters for modelling the dynamics of marine food webs under global climate change.

1.4 Research Objectives

The main objective of this study was to identify the spatio-temporal dynamics of copepod communities and their supporting environments in coastal seas of Ghana. The study also sought to investigate the combined impact of sea surface warming and pollution on the selected vital rates (faecal pellet production, growth [via egg production], and mortality) of *Temora stylifera*, the most abundant calanoid copepod in Ghana's coastal waters.

The specific objectives were to:

1. evaluate spatio-temporal changes in the concentrations of inorganic nutrients, phytoplankton and the abundance of copepods along the Coasts of Ghana,
2. investigate the relationship between (a) the concentrations of inorganic nutrients and phytoplankton, (b) phytoplankton concentration and copepod abundance,
3. identify the different copepod taxonomic groups along the coastal seas of Ghana, and,
4. quantify the combined effect of sea surface warming and pollution by heavy metal (cadmium) and petroleum (pyrene) on the biology of *Temora stylifera*

1.5 Significance of the Study

Coastal marine waters of Ghana constitute part of the Guinea Current Large Marine Ecosystems (GCLME), an area regarded as one of the most productive coastal and offshore ecosystems in the world (GCLME, 2010). It is endowed with

marine resources, including commercially important fish species, essential element minerals, and oil and gas reserves. It also serves as an essential reservoir of marine biological diversity and habitats for endangered species such as marine mammals and turtles (Amlalo, 2006). Despite its enormous ecological and socio-economic benefits, coastal marine waters of Ghana and other areas of the GCLME have been exposed to elevated levels of environmental stressors. Research indicates that there are high levels of pollution (heavy metals, polyaromatic hydrocarbons compounds, plastics, general marine litter) in these areas (Botwe et al., 2017a,b; Darpaah, 2013; Mahu et al. 2015; Van Dyck, Nunoo, & Lawson, 2016; Wiafe et al., 2008). The temperature of Ghana's coastal waters is also increasing as a result of global war ming (Figure 1.4).

However, there is a paucity of knowledge on how these stressors either interact individually or collectively to shape the community structure, population abundance, and diversity of the copepod biomass in these regions. An investigation by Wiafe et al. (2008) on the impact of sea surface temperature on the zooplankton biomass in the upwelling regions of the Gulf of Guinea is the only documented study found that relates temperature to marine zooplankton biomass in Ghanaian waters.

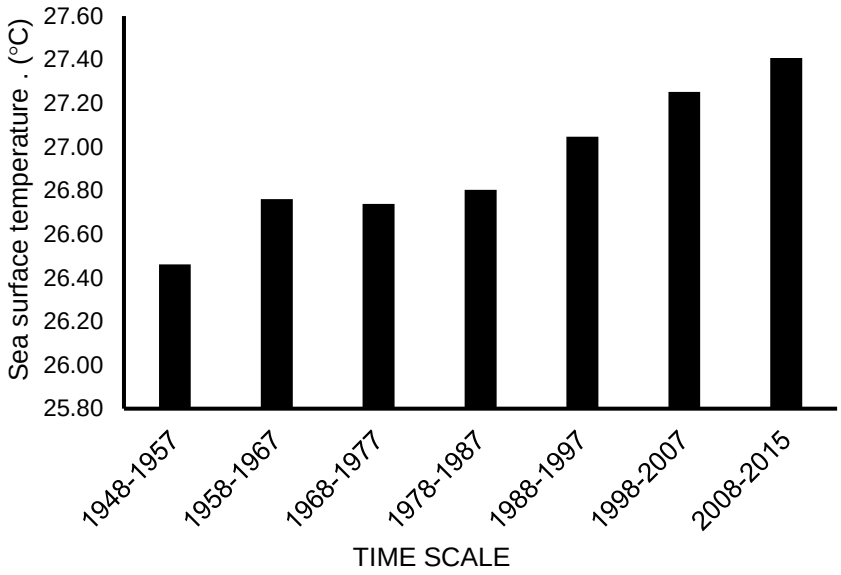

Figure 1.4: Decadal trends in the sea surface temperature of Ghanaian marine coastal waters (stable period). Source: Ghana Meteorological Agency (GMA, 2016).

This research is therefore relevant to the fisheries sector of Ghana because subsequent scientific research works attributed the seasonal abundance of commercially important fish stocks, including sardinella, chub mackerel and shrimps to the seasonal abundance of copepod populations (Castro, Skrobe, Asare, & Kankam, 2017; Quaatey & Maravelias, 1999).

Determining the impact of these stressors on copepods requires in-depth knowledge of the copepod composition and abundance within the tropical regions. Nonetheless, the Fisheries and Scientific Survey Division (FSSD) of the Fisheries Commission of Ghana is the only institution to have collected and reported on copepod communities in the coastal waters of Ghana (1969–1992). The research was conducted on a single transect (Tema) along the Ghanaian coastline. As a result, findings from the research by FSSD do not give an accurate representation

of the copepod population structure in the tropical region. Also, the report is quite outdated; hence, undocumented shifts in the community structure of copepods may have occurred over this period. It is, therefore, prudent to investigate the copepod dynamics as well as their supporting environment within nearshore waters of the country. This research aimed to provide current information on the copepod community structure as well as their supporting environment. The environment of the copepod is based on inorganic nutrients (nitrate and phosphate) and phytoplankton fluorescence. It also aims to highlight the responses of selected calanoid copepods to both the individual and combined effect of sea surface warming and pollution by petroleum hydrocarbons (pyrene) and heavy metal (cadmium). This information is critical for the development of useful parameters for modelling the dynamics of marine food webs under global climate change. It would also provide biologically reasonable parameters for predicting the responses of planktonic communities (e.g., zoo- and phytoplankton) to the combined effects of the stressors.

1.6 Delimitations of the Study

Geologically, the 550 km coastline of Ghana has been categorised into three zones as described by Armah (2005) and Dickson and Benneh (1970). There is the Western Coast Transect, which is between Newtown in the Western region and Axim, the Central Coast Transect extending from Axim to Apam, and the Eastern Coast Transect located between Apam and Keta. Transects were established along each of these three coasts. On the Western Coast Transect, transects were established at Axim. Along the Central Coast Transect, the transect

was established at Elmina and on the Eastern Coast Transect the transect was established at Keta. Axim and Elmina were selected because they are hotspots for fisheries issues in Ghana (Food and Agriculture Organization [FAO], 2016). Keta was selected for its geographical diversity being farther away from the Eastern and Central Coast Transects. This gives a fair representation of Ghana's coastline. Apart from inorganic nutrients, other physico-chemical parameters such as sea surface temperature and salinity were not measured. This was because the study was focused on establishing a link between inorganic nutrients, primary producers and primary consumers.

In terms of the microcosm experiment, selected vital rates considered were the rates of faecal pellet and egg production and mortality. The rate of faecal pellet production was used as a proxy for determining the transfer of ingested food back into the water by the copepod, egg production rate was used as a proxy for growth of the copepod, and the rate of mortality was used as a proxy for determining lethal effects of the stressors.

1.7 Limitations of the Study

During the field campaign in May 2019, on the Central Coast Transect, samples were taken only at Station 1. This was because the vessel developed technical issues after sampling at Station 1. However, data analysis was not adversely affected since subsections of the Station 1 data were used for the various analyses. In this study, primary production was intended to be estimated by converting phytoplankton concentration values in relative fluorescence units to Chlorophyll-a value. However, the Fluorometer malfunctioned after a few measurements. Consequently,

the correlation between chlorophyll-a and phytoplankton fluorescence could not be established.

1.8 Organisation of the study

This thesis is divided into six chapters. Chapter One highlights the concept of the study, including the background, statement of the problem and the purpose, objectives and significance of the study, and a summary of the study. Chapter Two is a review of literature related to the study. It includes marine heatwaves, their causes, their effects on marine ecosystems, and ways to mitigate these effects. Chapter Three deals with the study area, materials and methods and the statistical approaches used for analysing the data. Chapters Four and Five present the results and discussions, respectively. Chapters Four and Five are divided into two sections; the first section focused on the results and discussion of the field sampling, whereas the second section focused on the results and discussion of the microcosm experiments. Conclusions and recommendations are given in Chapter Six. Other sections presented in this thesis include a list of references and appendices.

1.9 Chapter Summary

This chapter is made up of seven sections. The first section gave a general background to the study whiles the second section focused on the statement of the problem. The third and fourth sections highlighted the purpose of the study and the study objectives, respectively. The fifth section addressed the significance of the study. The sixth section was dedicated to the delimitations and limitations of the study. The last section focused on the organisation of the study. The next chapter is dedicated to reviewing the literature and conceptual frameworks of the study.

CHAPTER TWO

LITERATURE REVIEW

This chapter reviews the literature relevant to the objectives of the study. It entails the sources of heatwaves and pollutants in marine ecosystems whiles highlighting their effects and mitigation strategies to control these effects.

2.1 Marine Heatwaves, their Causes and Ecological Effects

Marine heatwaves (MHWs) refer to the extreme warming of the sea surface temperature in a particular location over time (from days to months) Frölicher, Fischer, & Gruber (2018); Hobday et al. (2016). MHWs occur due to two independent processes driven by ocean currents and the atmosphere. The primary process involves ocean currents transporting warm water via thermohaline circulation to colder regions of the world's oceans. The build-up of warm water in a particular region, over time, causes MHWs. The secondary process occurs when the atmosphere becomes warm, especially when humidity and wind speed are reduced. This leads to the rapid warming of the upper layers of the ocean and subsequently, MHWs (Oliver et al., 2018). Recent findings point to an exponential increase in global resilience and frequency of MHWs. According to Oliver et al. (2018), MHWs are 34 % more likely to occur and last 17 % longer since the industrial era. Globally, the daily occurrence of marine heatwaves increased by 50 % per year between 1987 to 2016 compared to 1925-54 (Smale et al., 2019; Figure 2.1).

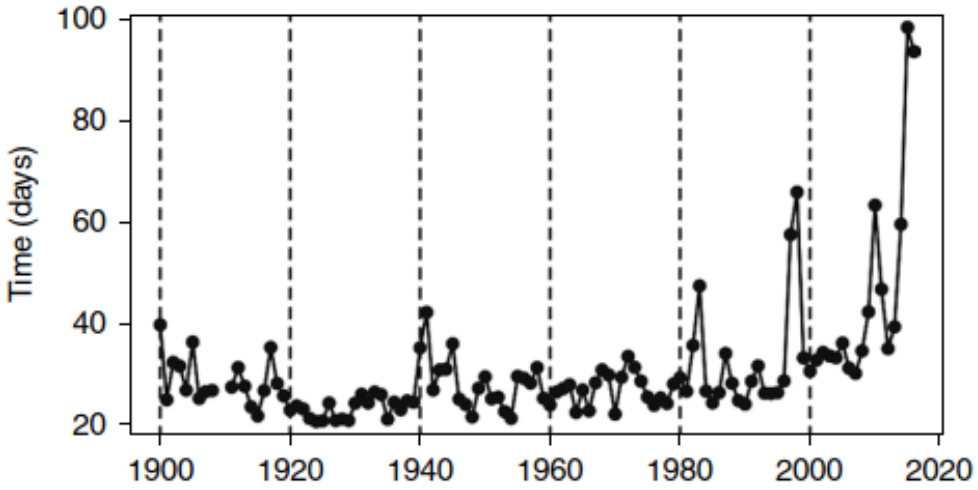

Figure 2.1: Globally averaged time series of the annual number of MHW days and trends in the annual number of MHW days (in the periods 1900–2020) across the global ocean (Adopted from Smale et al., 2019)

This anomaly is a result of increasing concentrations of greenhouse gasses released into the atmosphere through human activities. These gasses trap refracted heat from the earth's surface, therefore, causing an imbalance in the earth's energy. This leads to an increase in the input of the ocean's energy budget because the ocean absorbs more than 90 % of the earth's residual thermal energy (Cheng, Wang, Abraham, & Huang, 2018).

Marine heatwaves have enormous ecological and biological effects. Effects, according to Oliver et al. (2018)and Smale et al. (2019); Wernberg et al. (2013), range from coral bleaching, reduction in the chlorophyll levels due to increased surface stratification, mass mortality of marine invertebrates due to heat stress and shifts in species composition and distribution. Bhattacharya (1988) noted that a slight rise in temperature could affect marine copepods in different ways and

may even result in mortalities, particularly in tropical waters, since these animals live very near their upper-temperature tolerance limits.

2.2 Sources and Ecological Effects of Heavy Metal and Petroleum Pollution

Petroleum and heavy metal pollutants in marine ecosystems have been a significant concern since the start of the industrial era (Gerlach, 1981; Gupta 2005; Goudie, 2006). Naturally, these pollutants were present in trace quantities within marine environments (Forstner & Wittmann, 2012; Tait & Dipper, 1998). They occur naturally through volcanic eruptions, escape of gases or fluids on major fractures on the earth's crust and weathering of metal-enriched rocks (Garrett, 2010). Most marine organisms require these metals for their physiological functions and the regulation of many of their biochemical activities (Ansari, Marr, & Tariq, 2004). However, since the start of the industrial revolution, anthropogenic activities, including metalliferous mining and smelting, combustion of fossil fuels and fuel application of pesticides has exacerbated the concentrations of petroleum and heavy metals in marine environments (Fatima, Muzammal, Rehman, & Rustam, 2020).

Heavy metals are regarded as one of the most dangerous pollutants due to their ability to persist and accumulate in the marine environment (Ensibi, Nejib, & Yahia, 2017). Most research works have reported on their lethal and sub-lethal effects on marine organisms. These effects include reduction of the respiration rate resulting in oxidative damage (Ensibi et al., 2017; Moraïtou-Apostolopoulou & Verriopoulos, 1982), feeding, digestion rates, sexual maturity, egg production rates, and morphological arbitrations (Gentile, Gentile, Walker, & Heltshe,1982;

Moraïtou-Apostolopoulou & Verriopoulos, 1982 and references therein). These, in turn, make the organisms susceptible to diseases, predators and other stressors, with consequent negative effects on marine biota.

Hydrocarbons or petroleum occurs naturally in marine environments from the decomposition (at high temperatures and pressures) of organic matter (mainly dead plants and animals) that gradually accumulated and buried at great depths over millions of years (Blumberg & Bruno, 2018). Occasionally, natural seepages of the oil deposited beneath the ocean floor are observed. These seepages are minimal with benign effect on marine organisms (Kennicutt II, 2017). However, due to the world's growing industries, demand for petroleum products has intensified the exploration, production, refinery and transportation of petroleum in the sea (Figure 2.2). This has exacerbated the exposure and threats of petroleum pollution in aquatic environments (National Research Council, 2003).

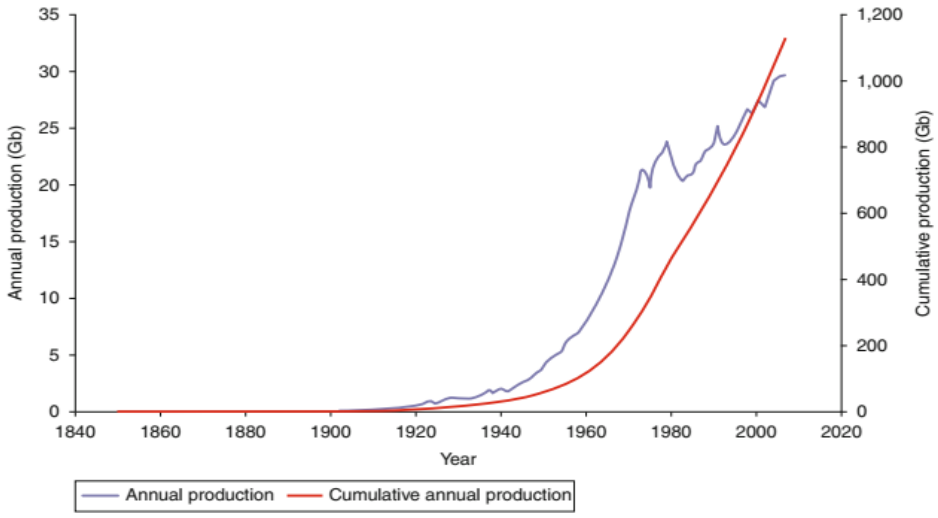

Figure 2.2: Global trends in oil production (Adopted from Miller & Sorrell, 2013)

In the marine environment, oil pollution occurs mainly through spillage, which can directly or indirectly be linked to human error (Saadoun, 2015; Wilhelmsson, Thompson, & Eriksson-Hägg, 2013). The deleterious effect and fate of petroleum in marine environments is dependent on its physical, chemical and biological transformation processes (Wilhelmsson et al., 2013). Almeda, Connelly, & Buskey (2016) outlined two forms of injury associated with the toxicity of petroleum pollutants. These are physical and biochemical injuries. Physical injuries are associated with the absorption, inhalation, and ingestion of the oil. This affects the daily functions of marine organisms. Biochemical injuries occur when chemical-specific compounds in the oil interact and cause damage to an organism's cellular metabolism. Although oil pollution affects a myriad of marine organisms, planktonic communities are the most vulnerable (Almeda, Baca, Hyatt, & Buskey, 2014; Walsh, 1978).

Most studies have reported on the adverse effects of petroleum pollution on marine copepods, the most abundant heterotrophic organism in the marine environment. Effects include impaired swimming, reduction in the population abundance, reduced feeding and egg production rate, and decreased mating success (Almeda et al., 2016; Bhattacharya, 1988; Pringault, 2015). Other effects in marine environments include the inhibition of mixing between atmospheric oxygen and surface waters, therefore, impeding the respiration of all aerobic organisms in the water body, weathered oil sticking quickly to the skin, fur, and feathers of marine animals that disrupt their normal structure and obliterate the ability to trap oxygen bubbles between the hairs and feathers (Saadoun, 2015; Yuewen & Adzigbli,

2018). This leads to hypothermia and subsequent death of the affected organisms due to the inability to regulate their body temperatures.

2.3 Strategies for Mitigating Heatwaves Arising from Climate Change and Pollution.

Mitigation of Climate change and marine pollution involves strategies for reducing the forcing factors and impacts associated with climate change and marine pollution. Since the pre-industrial era, emissions of anthropogenic greenhouse gases are considered the single most crucial forcing factor of climate change. In light of this, the Intergovernmental Panel on Climate Change (IPCC) in 2014 reported that mitigation of climate change would require the stabilisation or lowering of the source of greenhouse gases as well as improving the sinks that accommodate or store these gases. Gerlach (1981) also hinted that manufacturing processes resulting from industrialisation and urbanisation are the be-all and end-all of pollution on earth.

Richie and Roser (2020), highlighted that the sources of anthropogenic greenhouse gases could be classified variously based on the sector of origin. These sectors include the energy sector, agricultural sector, transport sector, industrial sector, residential and commercial sector, and the waste sector. These sectors also contribute significantly to pollution in marine environments. However, this write-up would focus on mitigation processes in the three major sectors (energy, transportation and agriculture, including forestry) responsible for anthropogenic greenhouse gas emissions and marine pollution.

2.3.1 Energy sector

The energy sector has been identified as the most significant contributor to Greenhouse gases in the atmosphere (IPCC, 2014). Data from Ritchie and Roser (2020), indicated that this sector is responsible for approximately 47 % of the total global greenhouse gas emissions. This could be linked to the ever-increasing demand for energy due to global economic and population growth, and a growing share of coal in the global fuel mix (IPCC, 2014). According to Ritchie and Roser (2020) and IPCC (2014), these gases result mainly from the burning of fossil fuels (coal, oil and natural gases) for electricity and heat generation. It is proposed that emissions from this sector would continue to increase, especially in developing countries, as they continue to invest in industrialisation.

Mitigation processes in this sector demand the modification of techniques as well as finding alternative energy sources for electricity and heat generation. Established mitigation processes include:

1. generating electricity and heat from renewable energy sources such as wind, solar, hydropower, and geothermal energy, which have proven to be more efficient in the control of greenhouse gas emissions. Renewable energy sources, furthermore, reduce thermal pollution in aquatic ecosystems. Generating electricity from nuclear power plants has been found to also emit fewer greenhouse gases compared with fossil fuel-powered plants. However, these nuclear-powered plants have the potential to cause thermal pollution if the water used as coolants are released directly into the environment.

2. increasing the efficiency of existing fossil fuel-powered plants through advanced technologies such as the conversion of coal-fired boiler to the use of natural gas and conversion of a single-cycle gas turbine into a combined-cycle turbine would shift the dispatch of electric generators to lower-emitting units. This, in turn, lowers greenhouse gas emissions from fossil fuel-powered plants.
3. reducing electricity use and peak demand by increasing its efficiency and conservation in homes, businesses and industries. An example of this is the use of energy-saving devices such as bulbs, fridges rather than its energy-consuming counterparts.
4. capturing by-products from fossil fuel combustions at its point source before it enters the atmosphere. Capturing could be done by transporting the by-products through pipelines and injecting them deep underground where it is securely stored.

2.3.2 Agriculture, forestry and other land use

According to Reyer, Guericke, & Ibisch (2009); Ritchie and Roser (2020), agriculture, land use, and forestry sector combined are the second-highest global contributor of anthropogenic greenhouse gases. This is due to the continuous conversion of forest covers into agricultural lands as a result of the growing demand for food due to population growth. The forest cover acts as sinks for carbon dioxide. Hence destroying them eliminates or reduces the extent of carbon sinks on earth. Also, carbon dioxide stored within them is released back into the atmosphere. Application of synthetic and organic fertilisers on agricultural lands and the

drainage of organic soil and irrigational processes also contribute to the emission of greenhouse gases and nutrient pollution in marine ecosystems. Rearing of livestock, especially ruminants, release much methane into the atmosphere through their by-products. This significantly contributes to the greenhouse gasses emitted from this sector. The agricultural sector also contributes to heavy metal pollution through biocide application.

Mitigation processes in this sector include:

1. increasing carbon sinks by practising afforestation where non-forested or abandoned agricultural lands are converted to forest lands. This could be achieved by planting trees such as timber and other fast-growing trees as well as the afforestation of degraded mangrove forests. These trees and forest cover absorb carbon dioxide through their photosynthetic activities, hence reducing CO_2 concentrations in the atmosphere. Mangroves tend to maintain aquatic water quality and clarity by filtering pollutants and trapping sediments originating from land. Protection of existing forests and peatlands should as well be intensified since they also act as carbon pools. These can be achieved through the avoidance of deforestation and control of other anthropogenic outbreaks such as fire and pest outbreaks (Bustamante et al., 2014; Niles, Brown, Pretty, Ball, & Fay, 2002)
2. management of croplands in order to reduce greenhouse gas emissions and marine pollution. This could be done by applying the appropriate concentrations of fertilisers. Timing and mode of application should also

be considered. This would reduce the emission of nitrous oxide into the atmosphere and marine ecosystems and prevent fertiliser wastage.

3. management of livestock by supplementing the feed of ruminants through the addition of dietary supplements such as bioactive compounds, fats, and nitrate and phosphate supplements. This would prevent or lower enteric fermentation in the hindgut of most ruminants. Therefore, lowering methane emissions. Changes in dietary compositions to focus more on the consumption of plants (vegetables) and seafood (fishes and shellfishes) instead of ruminants would help reduce greenhouse emissions (Delgado et al., 2011; Sejian, Gaughan, Baumgard, & Prasad, 2015; Smith et al., 2014)

2.3.3 Transportation

Movement of humans and goods via automobiles, trains, ships, aircraft, among others, is the third-highest contributor to global greenhouse gas emissions (Ritchie and Roser, 2020). This sector also contributes to heavy metal pollution in marine ecosystems. Emissions of greenhouse gasses and heavy metals in this sector are mainly as a result of the combustion of petroleum-based products, including gasoline and diesel in internal combustion engines. Cheremisinoff (2001) highlighted that half of the heavy metals released into the atmosphere is from the combustion of fossil fuel. Road traffics are considered the most significant contributor to transport-related emissions (Ritchie and Roser, 2020). Urgent mitigation strategies are needed in the sector as it continues to grow exponentially (especially in developing countries) as a result of population growth (Fuglestvedt, Berntsen, Myhre, Rypdal, & Skeie, 2008)

Heavy metal and greenhouse gas emissions from this sector can mainly be reduced by:

1. replacing gasoline and other petroleum-based fuels with alternative fuel such as biofuels generated from sugar and starch crops, or cellulosic material which have been found to emit minimal to zero greenhouse gases and heavy metals as by-products. Ethanol from corn, used as a blending agent in gasoline, is so far the most successful alternative transportation fuel. However, current scientific findings suggest that marine algae have a more significant advantage of producing oils that can be converted into diesel, bio-gasoline or jet fuel (Pierce, 1996; Delgado et al., 2011). Electrically powered automobiles (with power source from lithium-air batteries) and solar energy could substitute fossil fuel-powered automobiles. This would help reduce greenhouse gas emissions from the transport sector.
2. reducing the distance travelled by vehicles by planning communities in a way that everyday destinations of people would require minimum driving. This can be achieved by providing multiple cheap transportation systems (buses, light rails, and trains) in every community. This would, in turn, reduce the number of single-occupancy vehicles. Reduced single-occupancy vehicles reduce road traffics and in turn, reduces greenhouse gas emissions as well as air pollution. Also, communities, especially in developing countries, should incorporate residential and commercial buildings and centralise jobs and services in one location. This would

encourage individuals to walk or cycle to shorter distances. Accessible transit services should also link these areas.

2.4 Biology of Calanoida Copepods and their Variability to Climatic and Human-Induced Stressors.

Calanoida copepods are the most dominant group of copepod in marine ecosystems (Abo-taleb, Ashour, El-shafei, Alataway, & Maaty, 2020). They occupy critical positions in marine pelagic food webs as they mediate energy transfer from lower to higher trophic level organisms (Richardson, 2008). This section of the thesis is focused on aspects of the biology; feeding and reproduction of calanoid copepods as well as the impacts of climatic and anthropogenic stressors on the organism.

2.4.1 Feeding

Generally, and as in all living organisms, feeding is vital for the survival, growth and reproduction of calanoid copepods (Persson, 2007). Some species of calanoid copepods such as *Temora stylifera* and *Centropages furcatus* are primarily considered as omnivorous, feeding on both phytoplankton and other zooplankton species (Paffenhöfer & Knowles, 1980). Others are herbivores (e.g., *Eucalanus crassus* and *E. Pileatus*) Paffenhöfer, Strickler, & Alcaraz (1982) or carnivores (e.g., *N. cristatus*) Greene & Landry (1988). Feeding is done either passively by filtering prey items from the water column or actively via capturing of prey items (Prowe, Visser, Andersen, Chiba, & Kiørboe, 2019). The mode of feeding is highly dependent on the size of the prey item (Price & Paffenhöfer, 1986). Smaller prey items are captured passively, whereas larger prey items are targeted actively (Mauchline, 1998).

A variable fraction of the food ingested by copepods are assimilated for use in growth, respiration and reproduction while the fraction which is not assimilated is egested as faecal pellets (Wotton, 1994). Faecal pellets produced are much denser than the single-cell phytoplankton prey item; hence they sink faster to the bottom of the water compared with their prey items (Dagg, Jackson, & Checkley, 2014). This, therefore, contributes to increasing the carbon sequestration capability of the ocean.

The two most important environmental factors affecting feeding of calanoid copepods are light and temperature (Mauchline, 1998). In relation to light, copepods undergo a diel vertical migration. Most of them migrate to phytoplankton rich surface columns at night and descend to the lower depths during the day (Cohen & Forward, 2005). This migrational pattern also helps them avoid predation (Liu, Sun, & Han, 2003). Grazing by copepods is also highly dependent on temperature. This is mainly because temperature modulates the metabolic activities of many organisms (Abram, Boivin, Moiroux, & Brodeur, 2017; Alcaraz, Felipe, Grote, Arashkevich, & Nikishina, 2014; Heinle, 1969). Primarily, grazing by copepods increases linearly with temperature (Dam & Peterson, 1988). Food digestion and assimilation, on the other hand, increases only to an optimum level and decline (Acheampong, 2010). Hence increasing ocean warming in response to climate change would affect grazing by copepods, especially in tropical waters where organisms are living near their thermal optima (Miller and Stillman, 2013). This will, in turn, affect the biological carbon pump of the ocean as well as the transfer of energy across the different trophic levels in the marine ecosystem.

Anthropogenic stressors such petroleum pollutant have also been found to affect the ingestion rate of calanoid copepods (Almeda et al., 2016). This affects the amount of ingested materials that are released as faecal pellet after digestion (Nørregaard et al., 2014; Toxværd, Dinh, Henriksen, Hjorth, & Nielsen, 2019). Such an effect may limit the availability of energy for the growth and survival of the organism.

2.4.2 Reproduction

Reproduction in calanoid copepods begins when females release sex attracting pheromones that are detected by the chemosensors of males (Uchimaand Murano, 1988). After attraction, the males grab the females using their claspers and transfer their sperm by attaching their spermatophores to the female's genital orifice. Mating takes place from a few seconds to hours (Mauchline, 1998). Most calanoid copepods species (e.g., *T. stylifera* and *Acartia* spp) broadcast their eggs into the sea (Dhont et al., 2013); however, a few of them (e.g., *Eurytemora americana)* carry their eggs attached to their urosome until the egg hatches (Berasategui, Hoffmeyer, Dutto, & Biancalana, 2012). Free-spawning species lay successive clutches of eggs often at intervals of roughly 24 hours. The number of eggs laid by these spawners varies between 3–50, whereas the number laid by those who carry their eggs until hatching varies between 5–60 eggs (Mauchline).

Temperature, food availability and food quality are the three most important factors affecting egg production by copepods (Bi & Sommer, 2020; Holste & Peck, 2006). The rates of egg production increase with temperature until the optimum temperature limit of the animal is reached; after this, the rate of egg production

reduces with increasing temperature (Mauchline, 1998). Egg production in calanoid copepod has also been found to increase with increasing blooms of phytoplankton (Debes, Eliasen, & Gaard, 2008).

Food quality; particle size, morphology, taxonomic composition, nutritional value palatability and toxicity has significant effects on egg production of copepods (Ambler, 1985; Gifford, Bohrer, & Boyd, 1981; Huntley, Sykes, Rohan, & Marin, 1986; Kleppel, Holliday, & Pieper, 1991; Paffenhöfer, 1984). Findings by (Kleppel & Burkart, 1995) suggest that bigger particle sizes result in higher egg production as it is easier for copepods to capture these food particles. Diets with higher biochemical compositions, including protein, amino acid and fatty acids also resulted in higher egg production. Dietary diversity also had a positive impact on egg production. This is because it increases the likelihood of a nutritionally complete ration; however, it was established that similar results would be obtained when single food has all the nutritional requirements.

In addition to the above, other environmental factors such as heavy metal pollution have been found to affect the reproduction of calanoid copepods by causing morphological damages during hatching (Gentile et al., 1982; Moraïtou-Apostolopoulou & Verriopoulos, 1982 and references therein).

2.5 Copepod Community Structure in the Coastal Sea of Ghana

Coastal sea of Ghana is defined by Guinea Current Large Marine Ecosystem (GCLME), one of the most productive marine areas of the world (GCLME, 2010). Its hydrography is characterized by four regimes; i) a minor upwelling that occurs from December to January, ii) a warm long developed thermocline from February

to June, iii) a cold major upwelling season from July to September, and iv) a minor thermocline from October to November (Dovlo, 2016). These hydrographic regimes are responsible for the high productivity in Ghana's sea. During the cold major upwelling seasons, nutrient-rich bottom waters are brought to the surface. These nutrients fuel biological activities by increasing primary production. The availability of primary producers enhances zooplankton (especially copepod) production (Wiafe and Frid, 2001).

Similar to other marine ecosystems of the world, zooplankton community structure in the coastal sea of Ghana is dominated by copepods (GCLME, 2010). Among these copepods, calanoides were the most diverse and abundant. Other copepod Orders identified were the cyclopoids and harpacticoids. Spatial variations in the distribution pattern of copepods are driven mainly by phytoplankton abundance. However, during the major upwelling season, temperature controls the distribution of the plankton community (Wiafe and Frid, 2001).

CHAPTER THREE

MATERIALS AND METHOD

This chapter gives an account of the materials and methods used in this study. The study area and sampling stations are first described in detail. Field sampling, laboratory analyses and microcosm experiments carried out were also described in this section. Statistical analytical tools and software employed to make inferences are also indicated.

3.1 Study Area

Data collection for this study was categorised into three main components: (i) field sampling; ii) laboratory analysis of field samples; and (iii) laboratory experiments. Field sampling was conducted within the coastal seas of Ghana, which is part of the country's continental shelf area of 24,000 km^2 (Nunoo et al., 2014). Coastal seas of Ghana forms part of the Guinea Current Large Marine Ecosystem (GCLME) classified among the most productive regions of the world oceans (Chukwuone, Ukwe, Onugu, Ibe, 2009). For this research, the coastal seas of Ghana with a coastline about 550 km long, was divided into three zones; the Western, Central and Eastern Zones as described by Armah (2005) and Dickson and Benneh (1970). Sampling was conducted along a 4 km transect established on each of these zones (Figure 3.1).

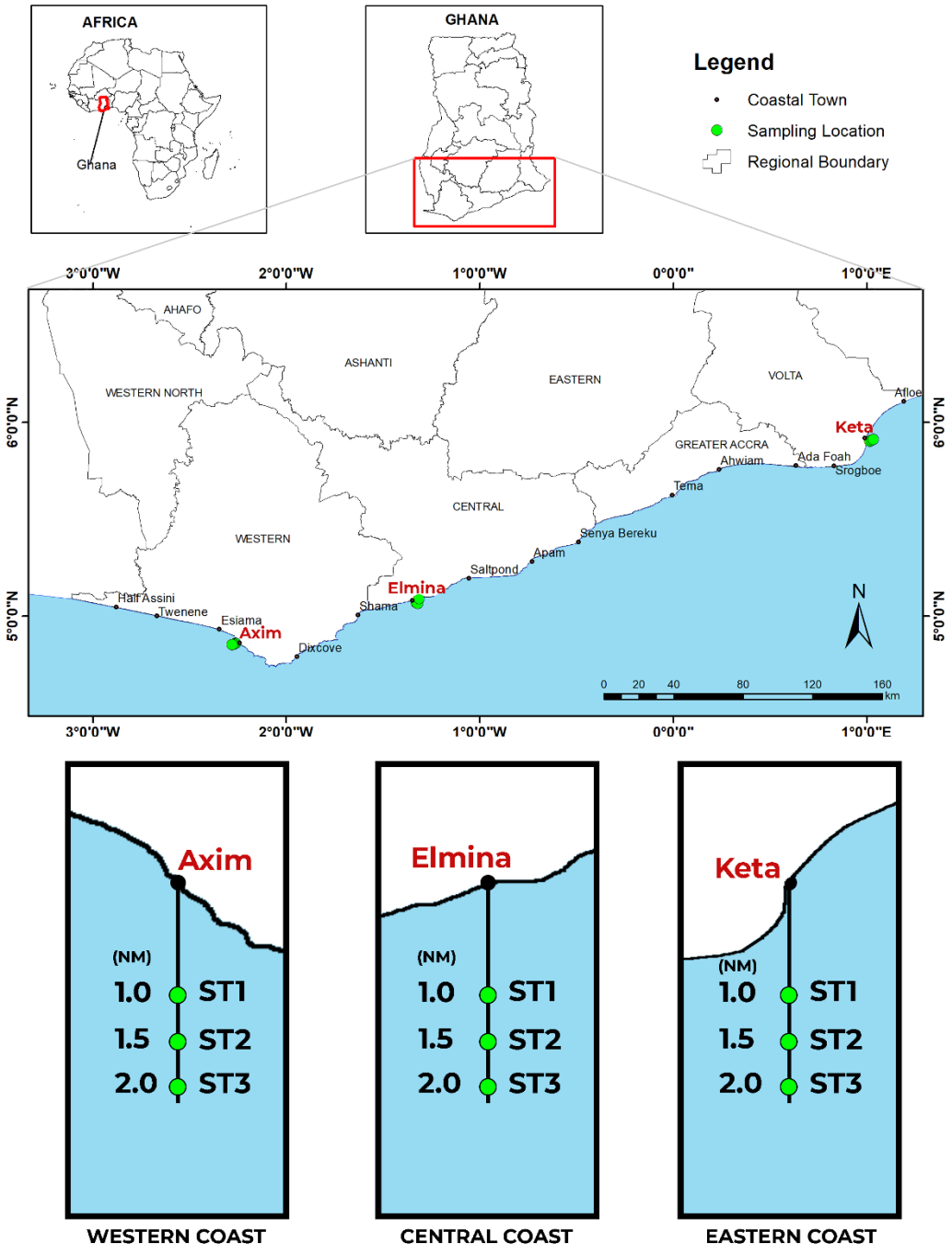

Figure 3.1: Map of the study area showing transects; Western, Central and Eastern and sampling stations (ST) in Nautical Miles (NM).

On the Western, Central and Eastern Coast, transects were established in coastal towns of Axim (coordinates; 4.8665° N, 2.2409° W), Elmina (coordinate; 5.1053° N, 1.3421° W) and Keta (coordinate; 5.9031° N, 0.9868° E), respectively. Three sampling stations, 0.5 nautical miles apart, were located on each transect. The first of these stations (station 1) was sited at a distance of about 1 NM from the shoreline of the selected coastal communities. The second station (Station 2) was located 1.5 NM away from the shoreline and the third station (station 3) was set at 2 NM from the shoreline. At each station, sampling was conducted at three different depths: 5 m, 10 m and 15 m water depth. A GPS device (Garmin etrex 10) was used to collect coordinates of the sampling sites in each station

On the Western Coast Transect, the coordinates were as follows: 4°51’35. 5"N 2°15’52.6"W (Station 1), 4°51’18.6"N 2°16’24.3"W (Station 2) and 4°51’08. 3"N 2°16’51.8"W (station 3). On the Central Coast Transect the coordinates f or stations 1, 2, and 3 were 5° 04’07.6 "N 1°19'49.9"W, 5°03'50.3"N 1°19'21.4" W and 5°05'18.6"N 1°18'56.3"W, respectively. On the Eastern Coast Transect coordinates sampled on were 5°54'06.4" N1°00'51.9"E, 5°54'29.2"N 1°01'22.4"E for stations 1, 2 and 3, respectively.

3.2 Field Sampling

Field sampling was carried out in April and May 2019 onboard a semi-industrial fishing vessel (Figure 3.2). During these cruises, samples of mesozooplankton, as well as seawater for inorganic nutrients (nitrate and phosphate) and chlorophyll-a analyses, were collected.

Figure 3. 2: Sampling onboard a semi-industrial vessel.

3.2.1 Sampling of copepods

Copepods were collected at different depths along each transect using a Van Dorn water sampler (2.5 L) in line with previous studies (Varghese, Thomas, & Susan, 2015). The Van Dorn bottle was used because it has a closing entrapment to prevent mixing of samples from different depths. Samples were collected from depths of 5, 10 and 15 m at each sampling station. Sampling was done by opening the end seals of the Van Dorn water sampler and corking it in order to set the trap mechanism (Figure 3.3 a & b). The free end of the messenger line was attached to the boat. The sampler was then lowered to the desired depth at each sampling station (Figure 3.3 c). The messenger of the sampler activated the mechanism that closes the end seals of the sampler (Figure 3.3 d & e).

Figure 3. 3: Procedure for the vertical sampling of marine copepod; (a-b) corking and (c) lowering of water sampler, (d) releasing messenger, (e) drawing, (f) emptying sampler and (g) filtering sampled water.

The sampler was then retrieved, and the capped water was transferred into a cleaned 5-litre measuring can (Figure 3.3. f). This was done by opening one end of the closed seals. The water was then filtered through a 200 µm mesh sieve (Figure 3.3 h), washed into labelled screw cap bottles containing 5 % formaldehyde solution for preservation. This procedure was repeated two more times at each depth. The labelled samples were then transported to USAID/UCC Fisheries and Coastal Research laboratory for identification and enumeration. Copepods were identified to the Order level under a binocular microscope (Leica M50). Identification was done using manuals by Mauchline (1998) & Wiafe and Frid (2001).

3.2.2 Water collection for nutrient and chlorophyll-a analyses

Water samples for nutrient and chlorophyll-a analyses were collected using Van Dorn sampling bottle, as described in section 3.2.1. Two hundred and fifty millimeters of water collected for determination of chlorophyll-a concentration was 250 ml; these samples were kept in amber bottles wrapped with aluminium foil to prevent sunlight exposure and subsequent growth of the algal cells (Bartram & Ress, 1999). The bottles were kept cool on ice in an icebox in order to prevent de-gradation of the cells, as recommended by Throdsen (1978). Water samples for the determination of nutrient concentrations were collected following the approach described by American Public Health Association- APHA (1992). The nutrients considered were nitrate (NO_3^-) and phosphate (PO_4^{3-}), as this is vital for the growth of marine phytoplankton. For each of these nutrients, the total volume of water collected was 250 ml; the water was transferred into high-density polyethylene

bottles and preserved on ice. All samples were transported to USAID/UCC Fisheries and Coastal Research Laboratory for analysis.

3.2.3 Determination of nitrate and phosphate concentration

Nitrate and phosphate concentrations were determined following the procedure described by the American Public Health Association (1992). The multiparametric calorimeter-Hach DR 900 with powder pillows (Nitraver 5 for nitrate and phosphate RGT for phosphate) was used for the analyses. For phosphate analysis, the program 490 P React PP was selected on the DR 900. Because the samples were preserved on ice during transportation, the temperature was increased to a room temperature of 23 $^{\circ}$C in a temperature-controlled laboratory before the analysis. The sample cell or vial of the calorimeter was filled with 10 ml of the sample water. The contents of one phosphate reaction powder pillow were then added to the cell. A blue colour then developed. This is because the powdered phosphate pillows contain phosphorous, sodium molybdate and potassium pyrosulfate that reacts with the soluble reactive phosphates to form a phospho-molybdate complex. The complex is subsequently reduced by ascorbic acid to form a molybdenum blue colour. Colour intensity of the compound is directly proportional to the phosphate concentration of the water sample and can be quantified by using a wavelength of 610 nm for colorimetric determination. The sample cell was immediately inverted vigorously for 25 seconds and then allowed to settle. The instrument timer was then set for a 2 minutes' reaction time. A blank was then prepared by filling a new sample cell with 10 ml of the water sample. After the expiration of the reaction time, the blank was cleaned with a laboratory

tissue paper and inserted into the cell holder of the calorimeter and then zeroed. The prepared sample was then cleaned and inserted into the sample holder for analysis. The read button was then pressed and the results showing in mg. l^{-1} PO_4^{3-} were read and noted.

For Nitrate analysis, the sample cell was filled with 10 ml of the sample water. The contents of one Nitra Ver 5 reagent powder pillow was added. The instrument timer with a one-minute reaction time was then started. The cell was inverted vigorously until the timer expired. The cell was then allowed to settle. The instrument timer was set for a 5 minutes' reaction time. An amber colour shows the presence of Nitrate. This is because cadmium contained in the Nitraver 5 powdered pillows reduces nitrate (NO_3^-) in the water sample to nitrite (NO_2^-). Nitrite ions then react with sulfanilic acid (in an acidic medium contained in the Nitra Ver 5 powder) to form an intermediate diazonium salt. When coupled with gentisic acid (also contained in the Nitra Ver 5), an amber-coloured solution is formed. Colour intensity of this compound is directly proportional to the nitrate concentration of the water sample and quantified by using a wavelength of 520 nm for calorimetric determination. The blank was then prepared and used as earlier described. The prepared sample cell was cleaned and inserted into the cell holder for analysis. The read bottom was then pressed and the results were recorded.

3.2.4 Determination of chlorophyll-a concentration

Chlorophyll-a concentration was determined according to Teira, Serret, & Fernández (2001). The samples in the amber bottles were filtered through 2.5 µm micron Whatman (GE) filter paper with the aid of a vacuum pump (storm 3000

with the flow of 20 litres per minute). Immediately after filtering, the filter papers were folded gently with forceps into a 50 ml centrifuge bottle for chlorophyll-a extraction in 5 ml of 90 % acetone. The acetone was prepared by diluting 900 ml of analytical grade acetone with 100 ml of deionised water. Centrifuge bottles were then capped and kept in the fridge at 5 $^{\circ}$C overnight to ensure effective soaking and extraction of Chlorophyll-a as recommended by (Smith et al., 2007). Chlorophyll-a concentrations were measured by fluorescence using a calibrated fluorometer. The calibration was done using pure chlorophyll-a samples. The acetone extracted sample (2 ml) was transferred into the cuvette of the fluorometer and the determination of chlorophyll-a was carried in µg. l^{-1} using stored calibrations on the fluorometer.

3.3 Experiments Investigating Effect of Multiple Stressors on Copepods

Microcosm experiments were conducted to ascertain the effect of three environmental stressors, namely pollution by heavy metal, petroleum product and sea surface warming, on a marine copepod. The type of heavy metal used was cadmium because it is found in high concentration in surface waters of mining districts in Ghana (Armah et al., 2010) and occurs in high concentration in seawaters along the coast of Ghana (Ibe & Sherman, 2002and references therein). The petroleum product investigated was pyrene, a polycyclic aromatic hydrocarbon. The justification is that pyrene is one of the most distributed polycyclic aromatic hydrocarbons occurring in coastal waters of Ghana (Essumang, 2010). Three different scenarios of sea surface warming ranging from temperatures of 1-3 $^{\circ}$C above the average sea surface temperature during the stable hydrographic

period off the coast of Ghana were investigated through laboratory simulations. These temperature scenarios are expected to be attained by 2100 if the emission of greenhouse gases continue at their current rate (IPCC, 2014). The investigations were focused on calanoid copepod *Temora stylifera* (Figure 3.4). This copepod occurs throughout the year in Ghana's coastal waters (E. Acheampong, Personal communication, October 17, 2018), in addition, at the time (stable hydrographic period) of this study, it was determined in a preliminary investigation to be the most abundant copepod constituting 37 % of all copepods sampled.

Figure 3. 4: The experimental copepod, *Temora stylifera*

3.3.1 Copepod stock culture

Unlike copepods collected at different depths using Van Dorn bottles for identification, copepods collected from the field for the experiment were kept alive. These live copepods were collected off the coast of Elmina (5° 03'N, 1° 20'W) near Cape Coast. This was done by towing a plankton net (mesh size: 200 µm; diameter:

60 cm) slowly through the top 2 m of the water for about 30 minutes along a distance of about 1 NM on board of a semi-industrial fishing vessel. The copepods were kept alive by keeping the samples on ice in an icebox in order to prevent them from dying of heat stress. Copepods used in the experiments were separated from other species using a sterile glass Pasteur pipette under a binocular microscope (Leica M50). They were identified using a manual on the Gulf of Guinea zooplankton published by Mauchline (1998); Wiafe and Frid (2001).

In the laboratory, the copepods were kept in fresh seawater that was filtered through an 8 µm mesh sieve and maintained on diatom-*Thalassiosira weissflogii* - diet provided at a saturation level of 280 µgC per litre. The choice of food concentration was informed by previous studies (Gréve, 2014); it was determined assuming that each cell of the *T. weissflogii* contains 131 pg of carbon as suggested by Jorg, Koski, & Jonasdottir (2008). The diatom (strain: CCMP 1050) was obtained from the Danish Institute of Aquatic Resources (DTU-Aqua). For the experiment, it was grown in a continuous culture using artificial seawater at a salinity of 33 ppt. The artificial seawater was strengthened with nutrient (B1 media) prepared using recipe provided by (Hansen, 1989) and silicate solution. It was prepared by dissolving laboratory formulated salt with deionised freshwater. For each litre of deionised freshwater 33 grams of the laboratory formulated salt was dissolved. The salinity of the solution was measured using a refractometer. Local salts were not used in the preparation of the medium because it may contain bacteria and other external pollutant infections that may collapse the phytoplankton culture (Harrison and Berges, 2005). For each litre of the artificial seawater, 1.1 ml and 1

ml of the B1 media and silicate solution, respectively, were added. The culture of diatom maintained at a photoperiod of 12 hours and a constant temperature of 19 °C. Illumination was provided by four cool-white fluorescent lamps, each with a light intensity of 28 watts. The culture was continually aerated to prevent self-shading and ensure mixing. Growth of the algal cells was monitored every 24 hours using the cell count described by Hötzel & Croome (1999) and Suthers, Bowling, Kobayashi & Rissik (2009). The cell count was done in a Sedgwick rafter chamber under a compound microscope (AmScope T360B) at a magnification of 10x.

3.3.2 Experimental investigation of the effect of sea surface warming and pollution

The impact of sea surface warming and pollution by heavy metal (cadmium) and pyrene on *Temora stylifera* were measured based on their effect on the rates of egg and faecal pellet production as well as the mortality of the copepod. Each of these stressors was presented individually as well as in combination with others for the experiment (Table 1).

Table 1: *Experimental design indicating the levels of environmental stress investigated. T_0 equals the control temperature of 28 ºC. T_1, T_2 and T_3 represent the control temperature + 1, 2 and 3 ºC, respectively. P_0 equals the nominal concentration of pollutants in seawater. Respectively, P_1, P_2 and P_3 represent the different concentrations of pollutants above the nominal level*

Level of pollution (Concentration in sea)	**Level of Thermal Stress**			
	T_0	T_1	T_2	T_3
P_0	P_0*T_0	P_0*T_1	P_0*T_2	P_0*T_3
P_1	P_1*T_0	P_1*T_1	P_1*T_2	P_1*T_3
P_2	P_2*T_0	P_2*T_1	P_2*T_2	P_2*T_3
P_3	P_3*T_0	P_3*T_1	P_3*T_2	P_3*T_3

Three warming scenarios were investigated. This was 28 °C + 1, 2 and 3 °C. The 28 °C is the average sea surface temperature off Ghana's coast during the stable hydrographic period (GMA, 2016); it was used as the control temperature. Each of these temperature treatments was established in a water bath (volume: 80 litres) fitted with a thermostatic heater (EHEIM Thermo control 200). The baths were aerated continuously with an air pump to ensure even distribution of the temperature (Figure 3.5). The temperatures were monitored every 15 minutes using temperature loggers (UTBI-001) for 24 hours.

Cadmium (Cd) has been described as the heavy metal with highest concentration in seawaters along the coast of Ghana (Acquah 1988). As a result, the effect of cadmium was assessed in this experiment. The metal was presented in the form of cadmium chloride ($CdCl_2$). Stock solution with a concentration of 100

mg/L of the metal was prepared with a litre of deionized water. The salt was weighed using an analytical balance (Adventurer AX124, Ohaus, USA, readability 0.1 mg). The stock solution was kept in a refrigerator for use in the experiment. According to Acquah (1988), the concentration of Cd in Ghana's coastal waters ranges 2-240 µg/l. The levels of Cd pollution investigated in this study were therefore selected to be within this range. The levels used were 0.1, 1, 10 and 100 µg/l. The 0.1 µg/l was assumed to be the nominal concentration of the chemical in line with previous studies. Each of the concentrations was prepared by simply diluting a subsample of the stock solution with deionized water.

Regarding experiments involving pyrene pollution, the concentrations of the chemical were set at 1.0, 10.0 and 100.0 nM, in line with previous experiments investigating effects of petroleum pollution on other copepods in Ghana's coastal waters (Ruiz, 2019). Stock solution (concentration = 3 mM) of the chemical was prepared by dissolving granulated pyrene (Sigma-Aldrich, purity N 99 %) with acetone (≥99.8 %, Merck KGaA, Germany). The prepared stock solution was stored in a refrigerator in 50 ml amber bottles wrapped in aluminium foils to prevent phototoxicity. Each of the pyrene concentrations investigated in this study was prepared by simply diluting a subsample of the stock solution with acetone.

The copepods were exposed to the above stressors incubated in Duran bottles (volume = 1 L) mounted in water baths set to appropriate temperatures, as described above (Figure 3.5). Each treatment was set up in triplicates. The bottles were filled with fresh seawater filtered through an 8 µm mesh sieve. The pollutants were added where needed. The food concentration used was 280 µgC. l^{-1}, the

maximum, non-limiting food concentration suggested for *Temora stylifera* by Gréve (2014). Two males and five females of the copepods were used for each of the experiments. This sex ratio reflects personal observations of the species from field samples. The duration of each of the experiments was 24 hours. Before the experiments, the copepods were acclimatised to each experimental condition for 12 hours in line with previous studies (Punnarak, Jarayabhand, & Piumsomboon, 2017).

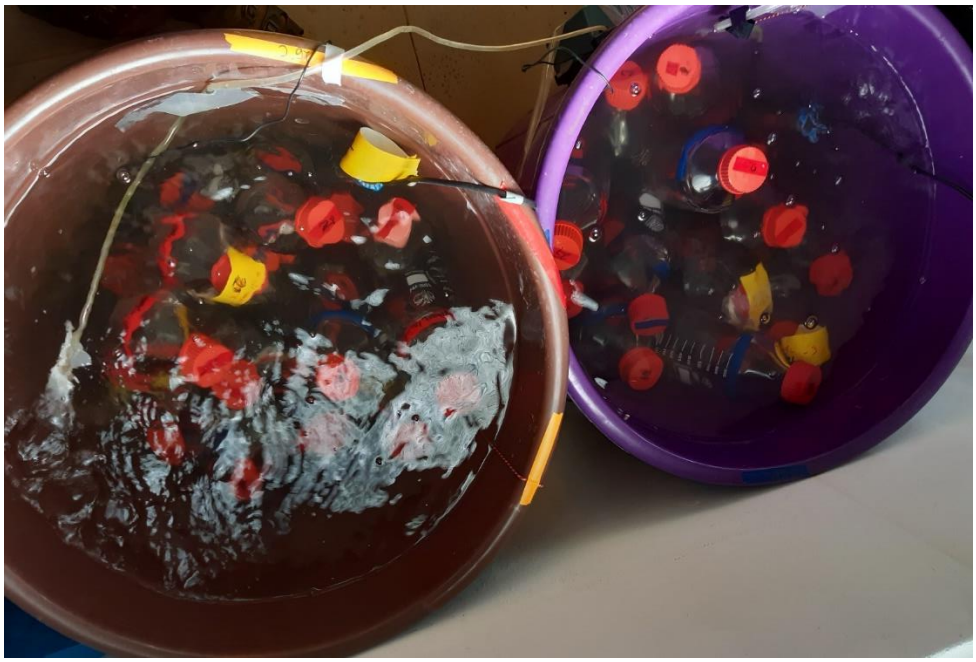

Figure 3.5: Temperature treatments established with water baths. Specific temperatures were established in different baths using thermostatic heater (EHEIM Thermo control 200).

3.3.3 Assessment of mortality rate, production of faecal pellets and eggs

The production of faecal pellets and eggs was determined following the approach in a previous study (Carlotti, Rey, Javanshir, & Nival, 1997). To do this, the experimental bottles containing the copepods were first inverted 2-3 times and then slowly filtered through a 200 µm sieve. This was to remove the copepods from the water. Copepods trap on the mesh were washed into a Petri dish. The mortality rate was determined by observing the number of copepods alive or dead under a microscope. Dead copepods appeared pale white. The filtrate was further filtered through a 20 µm mesh sieve in order to trap both faecal pellets and eggs produced by the copepods. These were then washed into a Petri dish, stained with Lugol's solution and counted under a binocular microscope (Leica M 50) with a magnification of x4.

3.4 Data Analysis

3.4.1. Evaluating the changes in the concentration of organic nutrients and primary production in coastal marine waters of Ghana

Changes in the ratio of inorganic nutrient (nitrate: phosphate), and primary production with respect to the different water depths, sampling stations and months, and transects (see materials and method section) were evaluated using a Multi-way ANOVA at an alpha level of 0.05. Tukey pairwise comparison (post hoc test) was used to determine the means responsible for the statistical difference (if any) with the aid of Minitab (version 19.1). Regarding the nutrient ratios, nitrate and phosphate were first converted into moles by dividing their weight-based

concentration (mg. l^{-1}) by 62 and 95 (g/mole), respectively. Their ratios were then computed from the calculated molar values.

3.4.2. Determination of the abundance and composition of copepods in Ghana's coastal marine waters.

Copepod abundance within the different water depths, sampling stations and months, and transects were computed by dividing the total number of copepods by litres of water filtered. The percentage composition (%) of each taxonomic group was also calculated using by the equation below:

$$(\%) = \frac{No.of\ copepods\ in\ each\ taxonomic\ group}{Total\ No.of\ \ copepods\ in\ all\ taxonomic\ groups} X100 \qquad 1$$

3.4.3. Quantification of individual effects of pollutants and sea surface warming on selected vital rates of *Temora stylifera*

Effects of the individual stressors on the egg production rate (EPR), faecal pellet production (FPR) and mortality rate (MR) of the copepod were examined using regression analysis. The Equations for calculating EPR, FPR and MR are given below:

$$FPR = \frac{Number\ of\ faecal\ pellets\ produced\ per\ day}{Individual\ number\ of\ Copepods} \qquad 2$$

$$EPR = \frac{Number\ of\ eggs\ produced\ per\ day}{Individual\ number\ of\ Copepods} \qquad 3$$

$$MR = Number\ of\ dead\ copepods\ per\ day \qquad 4$$

A linear regression analysis was used to determine the relationship between these selected vital rates and the different levels of the stressors. The significance of the relationship was determined using the coefficient of determination (R^2) and the P-value of the regression. Almost all the relationships were linear; however, the relationship between the rate of faecal pellet production and the different pyrene concentrations was non-linear. Hence the relationship was described by a Gaussian equation given below:

$$F = F_{max} * e^{-0.5\left\{\left(\frac{P-P_1}{b}\right)^2\right\}} \quad 5$$

where F_{max} is the maximum obtainable rate of faecal pellet production, *P* represents the concentration of the pollutants, P_l is the pollutant level beyond which faecal pellet production began to fall whereas *b* describes the width of the response curve.

3.4.4 Quantification of individual effects of pollutants and sea surface warming on selected vital rates of *Temora sp.*

A multiway ANOVA was used to determine the combined effects of the stressors on the copepods. Tukey pairwise comparison (post hoc test) was used to determine the specific difference among means. Warming effect on the toxicity of both cadmium and pyrene was examined using probit analysis (Finney, 1971). Using this analysis, the concentration of the pollutant at which 50 % of the experimental copepods were killed was determined. The outcome of this analysis is termed the LC_{50}. The proportion of copepods killed in each of the experimental treatments was arcsine-transformed prior to the probit analysis. The proportion was determined as a simple ratio between the number of copepods at the beginning of each experiment and those found dead at the end of the experiment. A sigmoid dose-response model

exemplified in Figure 3.6 was then used to determine the LC_{50} values for the various treatments. This analysis was done for each of the pollutants administered at different temperatures. After this, a regression analysis was used to determine the relationship between the LC_{50} values and temperature.

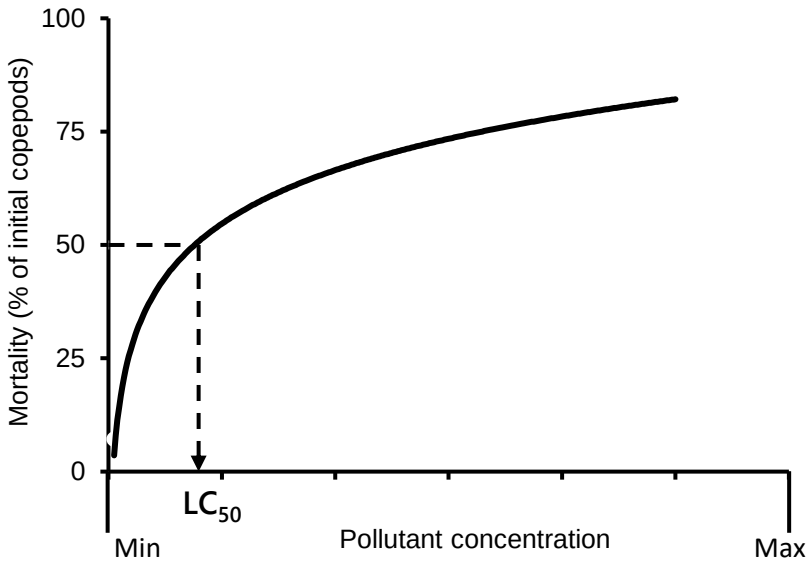

Figure 3. 6: A sigmoid dose-response model for LC_{50}

Statistical analysis of the individual, as well as combined effects of the warming and the pollutants on the selected vital rates of the copepod, were carried out using multivariate ANOVA and Tukey's comparison test (post hoc test).

3.5 Chapter summary

The materials and methods used to execute the research, as well as the study locations, have been described in detail in this chapter. Statistical analytical tools and software are employed to make inferences have also been indicated.

CHAPTER FOUR

RESULTS

This chapter presents the results obtained from field sampling and laboratory experiments, respectively. The findings are presented using relevant Tables, graphs, charts, diagrams and photographs. Standard errors (± SE) around average estimates have also been indicated in Tables and charts.

4.1 Results from Field Investigations

Concentration of inorganic nutrients (nitrate and phosphate), phytoplankton and mesozooplankton abundance were monitored based on the method used in this study. The results are presented in the following sections:

4.1.1 Dissolved inorganic nitrate and phosphate

Based on their average molar masses, highest nitrate: phosphate ratio recorded was approximately 141:1. This was obtained at the mid-water column (10 m depth) of station 3 along the Western Coast Transect in May 2019. The least nitrate: phosphate ratio was ≈ 8:1 and was recorded at the surface (top 5 m depth) of station 3 on the Central Coast Transect. This observation was made in April 2019. Figures 4.1-4.3 show the nitrate to phosphate ratios recorded at three different stations and water depths in each of the coastal zones.

Regarding data on individual transects, the highest nitrate to phosphate ratio recorded on the Western Coast Transect was approximately 141:1 whiles the least was approximately 15:1. These data were obtained within the mid-water column and the top 5 m of the water at station 3 located further away from the shoreline in May and April 2019, respectively (Figure 4.1). Multivariate ANOVA results

showed that there was a significant difference (p = 0.043) between the N:P ratios within the different water depths of this coast (see Table 2 for significant differences). Largely, the outcome from Tukey's comparison test showed that N:P ratios at the top 5m of the water were significantly lower than ratios observed at the mid and deep-water column

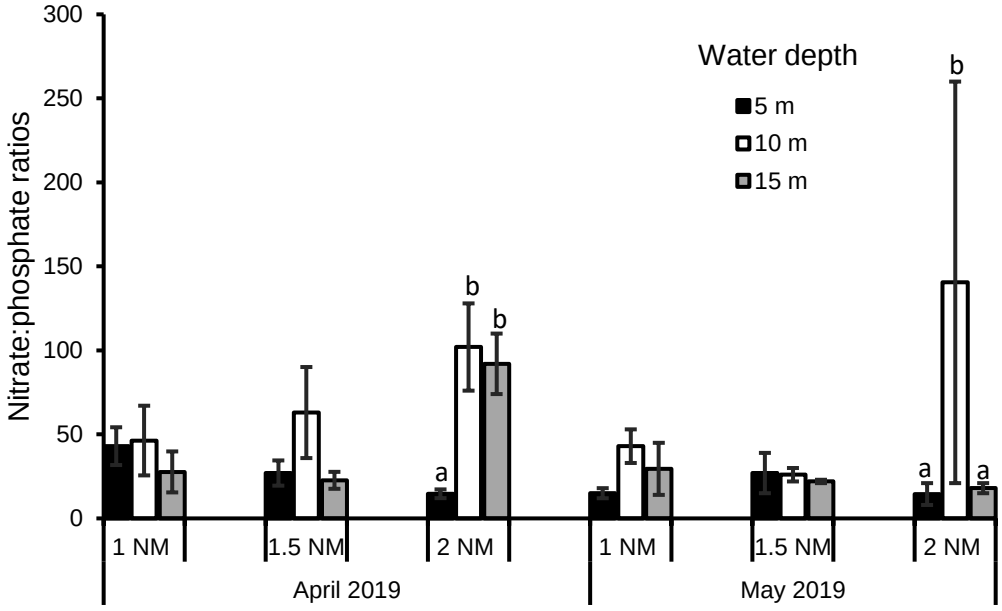

Figure 4.1: Nitrate: phosphate ratios (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Western Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets: lower case alphabets compare monthly observations at different depths within the stations.

For example, in April 2019, at station 3, N:P ratio was approximately 42 % higher at the mid-water column compared with the top 5 m of the water (Tukey post hoc test; Figure 4.1). In contrast with changes witnessed at the different water depths, N:P ratios obtained from the different sampling stations as well as at the different sampling periods did not differ significantly from each other (see Table 2 for significant differences).

Table 2: *ANOVA comparison of nitrate to phosphate ratios recorded in April and May 2019 on the Western Coast Transect of Ghana*

Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling months	1	0.161	0.161	1.8	0.195
Water depths	2	0.662	0.331	3.7	**0.043**
Sampling Stations	2	0.470	0.235	2.62	0.097
Sampling months* Water depths	2	0.204	0.102	1.14	0.340
Water depths*Sampling Stations	4	1.071	0.268	2.99	**0.044**
Error	20	1.791	0.090		
Total	31	3.568			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

On the Central Coast Transect, least average nutrient ratio (≈ 8:1) was recorded within top 5m of the water on the station at further away from the shoreline. The highest nutrient ratio (≈ 109:1) was recorded within the top-water column at the station closest to the shoreline. Both findings were made in April (Figure 4.2). In contrast with findings made on the Western Coast Transects, N:P ratios within this coast were not significantly different ($p = 0.538$) in terms of the different water depths. Consequently, there were no significant differences between findings made on the different stations along with the different periods of sampling (Table 3).

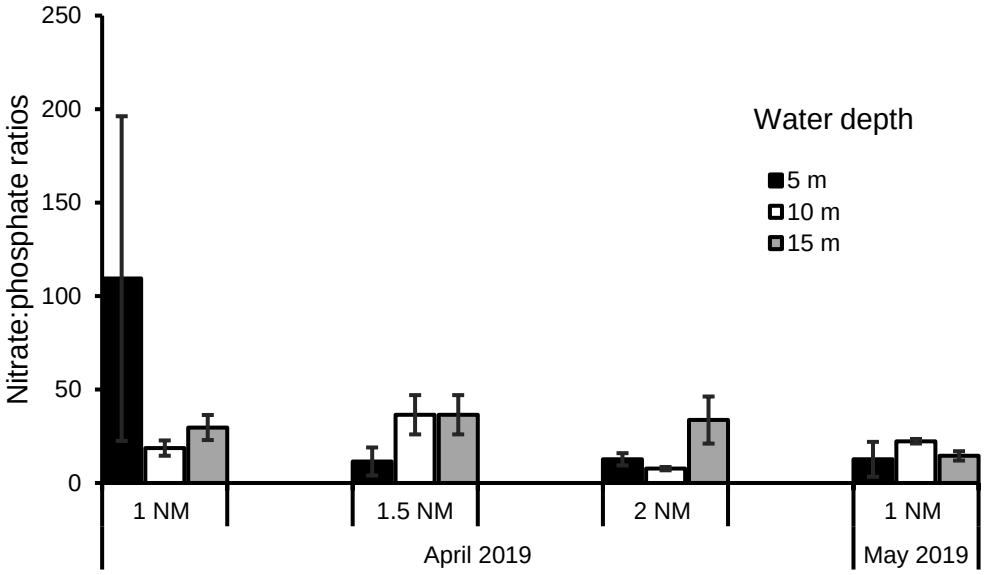

Figure 4.2: Nitrate: phosphate ratios (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Central Coast Transect of Ghana.

Table 3: *ANOVA comparison of nitrate to phosphate ratios recorded in April and May 2019 on the Central Coast Transect*

Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling month	1	0.02746	0.02746	0.54	0.259
Water depth	2	0.55407	0.27704	5.45	0.538
Sampling Stations	2	0.18106	0.09053	1.78	0.205
Sampling month*Water depth	2	0.09953	0.04976	0.98	0.393
Water depth*Sampling Stations	4	0.55952	0.13988	2.75	0.057
Error	20	1.01659	0.05083		
Total	31	2.23458			

On average, the highest ratio of the nutrients (≈ 98:1) on the Eastern Coast Transect was recorded within the mid-water column of station 1 located closest to the shoreline. The least ratio (≈ 17:1) was obtained at the top 5 m water column on station 2. These findings were made in both sampling months (Figure 4.3). Similar

to the Western Coast Transect, nutrient ratios obtained within this coast changed significantly ($p = 0.009$; Table 4) at the different water depths as well as the different sampling stations. Primarily, N:P ratios recorded at the mid-water column was approximately 13 % higher than the ratios recorded at the surface and in deep-waters (Tukey's post hoc test; $p < 0.05$). Also, along this coast, N:P ratios recorded at station 1, which is closest to the shoreline, was ≈ 13 % higher compared with those recorded at the stations located further away from the shoreline. Temporal nutrient ratios were also significantly different ($p = 0.001$; Table 4). The outcome of the Tukey's post hoc test showed that the nutrient ratios observed were higher by ≈ 14 % in April than in May (Figure 4.3).

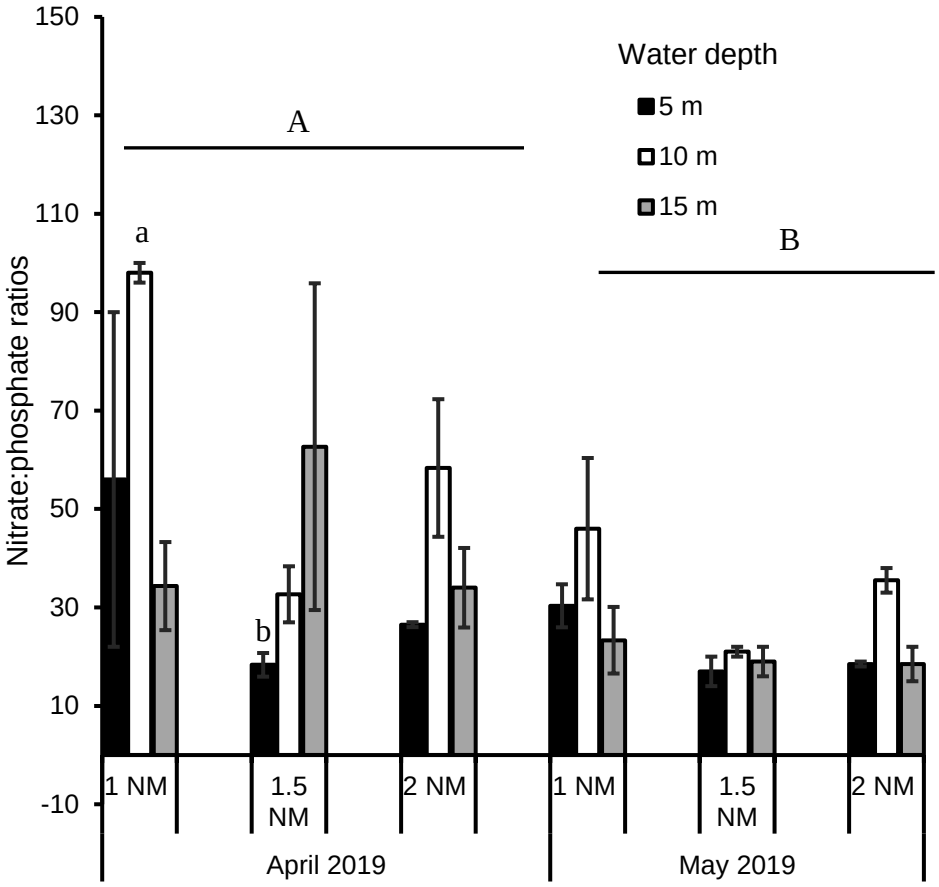

Figure 4.3: Nitrate: phosphate ratios (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Eastern Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets: lower case alphabets compare monthly observations at different depths within the stations; upper case alphabets compare observations made on different months within a station.

Table 4: *ANOVA Comparison of the nitrate to phosphate ratios recorded in April and May 2019 on the Eastern Coast Transect of Ghana*

Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling months	1	0.516	0.516	12.67	**0.001**
Water depths	2	0.455	0.227	5.59	**0.009**
Sampling Stations	2	0.347	0.173	4.26	**0.025**
Sampling months* Water depths	2	0.051	0.026	0.63	0.54
Sampling months*Sampling Stations	2	0.005	0.003	0.06	0.94
Water depths*Sampling Stations	4	0.327	0.082	2.01	0.121
Sampling months*Water depths*Sampling Stations	4	0.076	0.019	0.47	0.758
Error	27	1.099	0.041		
Total	44				

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

On average, the N:P ratio along the entire coast of Ghana was 36:1. A comparative analysis of the nutrient ratios across all the coastal zones, sampling stations, months and water depths showed significant differences in the data collected at different coastal zones ($p = 0.002$), sampling months ($p = 0.003$) and stations ($p = 0.000$) and water depths ($p = 0.003$; Table 5). Post hoc comparison of the results for the different coastal zones showed that the nutrient ratios on the Western and Central Coast Transects were significantly higher (≈ 19-21 %) than the ratios obtained on the Eastern Coast Transect (see post hoc test results in Table 5). The ratios of the nutrients significantly decreased by ≈ 9 % from April to May 2019 (Figures 4.1-4.3). In terms of sampling depths, nutrient ratios recorded within the mid-water column was significantly higher (≈ 18 %) compared with the findings

at the surface and the bottom parts of the water, and independent of the coastal area, sampling months and stations (Table 5 for significant difference). The ratios on the station located 1 NM away from the shoreline was also significantly higher (≈ 19 %) than those recorded at stations located at 1.5 NM and 2 NM, which were similar (Figures 4.1-4.3).

Table 5: *ANOVA comparison of nitrate to phosphate ratios recorded in April to May 2019 from the three coastal zones of Ghana*

ANOVA Comparison						
Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value	Significant source of variations
Coastal zones	2	0.894	0.447	6.65	**0.002**	Eastern Coast Transect
Sampling months	1	0.643	0.642	9.56	**0.003**	April & May
Water depths	2	1.455	0.727	10.82	**0.000**	Mid-water column
Sampling Stations	2	0.439	0.31	3.27	**0.043**	station sited at 1 NM
Coastal zones*Sampling months	2	0.018	0.009	0.13	0.876	
Coastal zones*Water depths	4	0.184	0.046	0.68	0.605	
Coastal zones*Sampling Stations	4	0.487	0.122	1.81	0.135	
Sampling months*Water depths	2	0.136	0.068	1.01	0.368	
Sampling months*Sampling Stations	2	0.021	0.01	0.15	0.858	
Water depths*Sampling Stations	4	0.754	0.189	2.81	0.031	
Coastal zones*Sampling months*Water depths	4	0.140	0.035	0.52	0.721	
Coastal zones*Water depths*Sampling Stations	8	0.952	0.119	1.77	0.095	
Sampling months*Water depths*Sampling Stations	4	0.268	0.067	1	0.414	
Error	79	5.310	0.067			
Lack-of-Fit	6	0.380	0.063	0.94	0.473	
Pure Error	73	4.929	0.068			
Total	120	12.172				

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

4.1.2 Concentration of phytoplankton on the field

Phytoplankton concentrations were measured in Relative Fluorescence Units (RFU). The results are shown in Figures 4.4-4.6. On average, highest phytoplankton concentration (126292.4 ± 17267.97 RFU. l^{-1}) occurred within the top 5 m of the water. This was recorded in April at station 1 on the Eastern Coast Transect. Lowest concentration (11174.8 ± 507.2442 RFU. l^{-1}) was recorded at a similar station in May along the Western Coast Transect but within the top 5 m of the water.

In relation to data on individual transects, phytoplankton concentration differed significantly at the different water depths as well as the sampling periods (see Table 6 for significant difference). At the different water depths, phytoplankton concentrations recorded at the top 5 m water column on the station closer to the shoreline was ≈ 36 % higher than the concentration recorded at the mid and lower water columns. In contrast with this observation, phytoplankton concentrations recorded on the mid-station were higher by ≈ 49 % at the mid-water column in comparison with findings made at the top 5 m water column. Both findings were made in April (Figure 4.4). In general, phytoplankton concentrations recorded on the Western Coast Transect declined by ≈ 19 % from the mid-water and deep-water columns to the top 5 m of the water (Figure 4.4; Tukey's comparison test). Also, the recorded concentrations declined by ≈ 75 % from April to May 2019 of the sampling period (Figure 4.4; Tukey's comparison test).

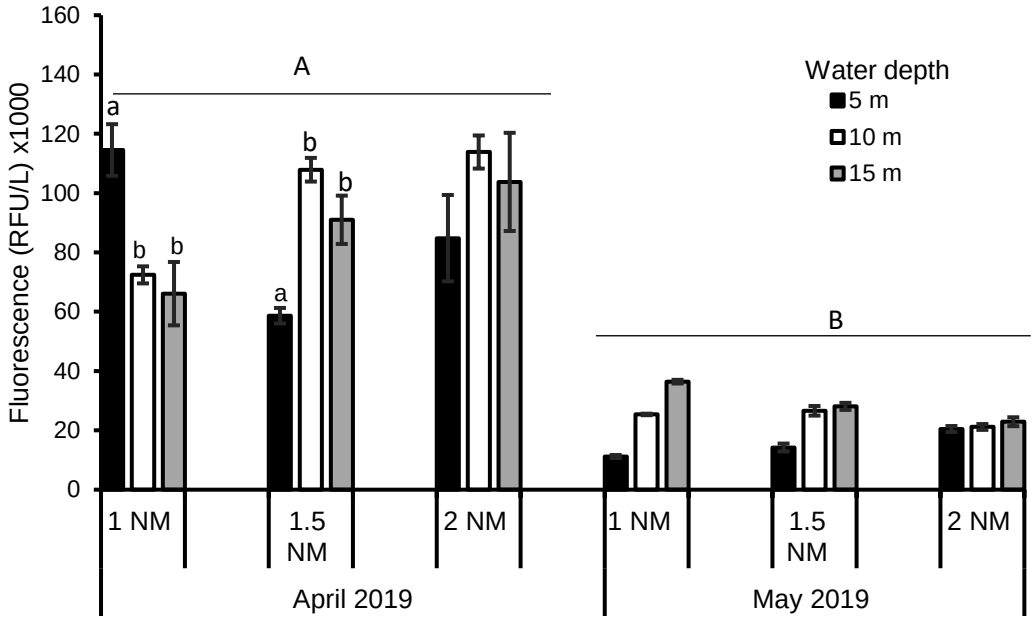

Figure 4.4: Phytoplankton fluorescence (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Western Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets: lower case alphabets compare monthly findings at different depths within the stations; upper case alphabets compare observations made on different months within a station.

Table 6: *ANOVA comparison for phytoplankton fluorescence recorded from April to May 2019 on the Western Coast Transect of Ghana*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling months	1	5E+10	5E+10	360	**0.000**
Water depths	2	1E+09	5E+08	3.63	**0.038**
Sampling Stations	2	4E+08	2E+08	1.47	0.245
Sampling months*Water depths	2	7E+08	3E+08	2.27	0.120
Sampling months*Sampling Stations	2	8E+08	4E+08	2.71	0.082
Water depths*Sampling Stations	4	3E+09	8E+08	5.13	**0.003**
Sampling months*Water depths* Sampling Stations	4	5E+09	1E+09	8.29	**0.000**
Error	32	5E+09	1E+08		
Total	49	7E+10			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

Similar to the Western Coast Transect, phytoplankton concentrations on the Central Coast Transect changed significantly at the different water depths ($p = 0.018$; Table 7). In April 2019, phytoplankton concentrations recorded were significantly higher by ≈ 9 % at the top-water column compared to the lower water column along this transect (Figure 4.5). Also, the mid-water at station 3, which is further away from the shoreline, phytoplankton concentrations recorded were higher ≈ 8 % than the concentration occurring at the deep-water column (Figure 4.5). Phytoplankton concentration on the Eastern Coast Transect did not change significantly at the different sampling stations along with the periods of sampling (see Table 7 for significant difference).

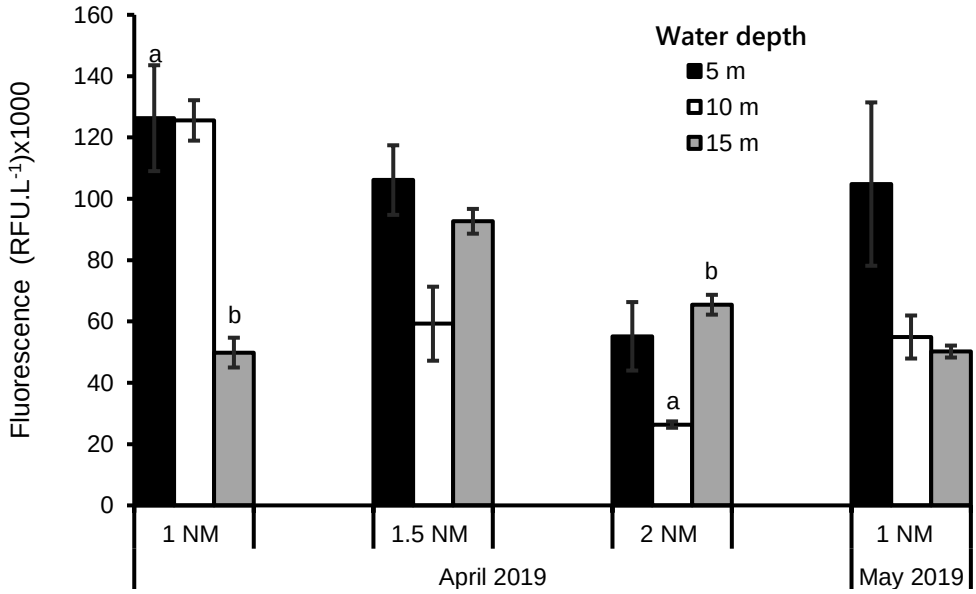

Figure 4.5: Phytoplankton fluorescence (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Central Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets: lower case alphabets compare the monthly observations at the different depths within the stations.

Table 7*: ANOVA comparison for phytoplankton fluorescence recorded in April and May 2019 on the Central Coast Transect of Ghana*

Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value
sampling months	1	$3.3*10^9$	$3.3\ 10^9$	2.15	0.163
Water depths	2	$6.4*10^9$	$3.2*10^9$	4.74	**0.018**
Sampling stations	2	$8.9*10^9$	$4.5*10^9$	6.62	**0.005**
sampling months*Water depths	2	$4.3*10^9$	$2.1*10^9$	3.9	**0.034**
Water depths*Sampling stations	4	$9.9*10^9$	$2.5*10^9$	3.7	**0.016**
Error	26	$1.7*10^9$	$6.7*10^8$		
Lack-of-Fit	3	$8.2*10^9$	$2.7*10^9$	6.73	**0.002**
Pure Error	23	$9.3*10^9$	$4.1*10^9$		
Total	34	$4.5*10^{10}$			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

Phytoplankton concentrations on the Eastern Coast Transect differed significantly at the different water depths ($p = 0.000$; Table 8). The outcome of the

Tukey comparison test showed that largely, phytoplankton concentrations recorded in deep-water columns were higher than the concentration in mid and surface water columns. For example, in April 2019, phytoplankton concentrations recorded at all sampling stations were 9-10 % higher in the deep-water columns compared with the mid and surface water columns (Figure 4.6). Phytoplankton concentrations at station 3 was higher (≈ 7 %) in April than its concentration in May 2019 (Figure 4.6; see Table 8 for significant difference).

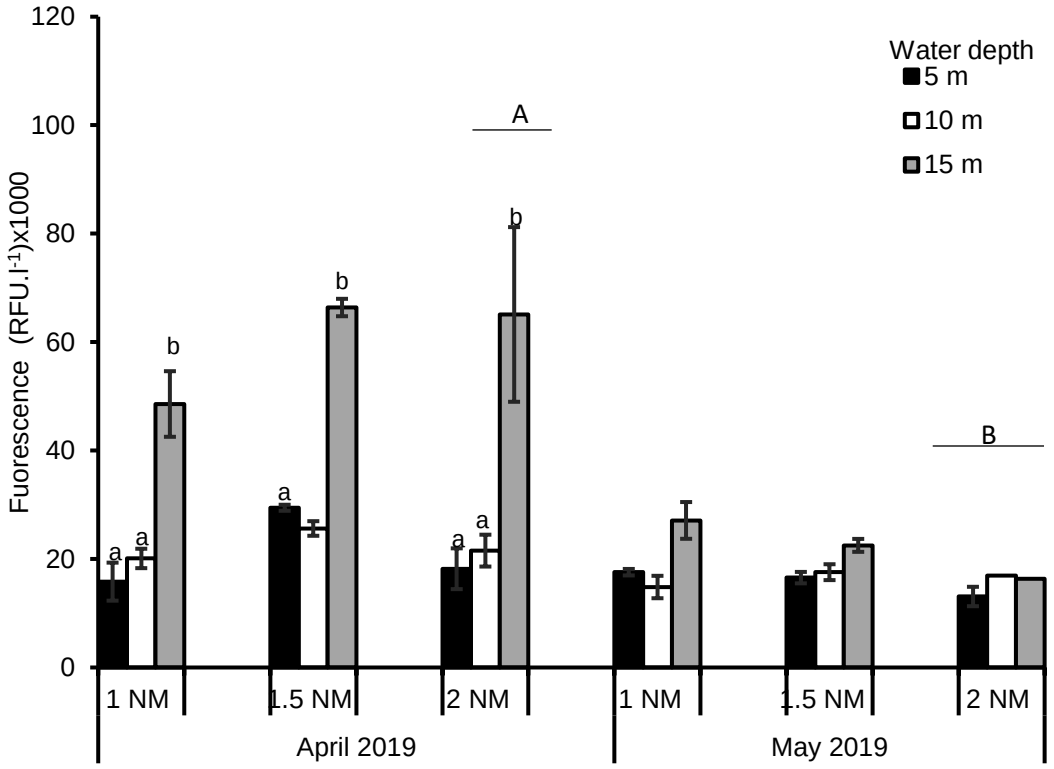

Figure 4.6: Phytoplankton fluorescence (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Eastern Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets; lower case alphabets compare monthly findings at different depths within the stations; upper case alphabets compare findings made on different months within a station

Table 8: *ANOVA comparison for phytoplankton concentration recorded from April to May 2019 on the Eastern Coast Transect of Ghana*

Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling months	1	3E+08	3E+08	31.05	**0.000**
Water depths	2	8E+08	4E+08	45.2	**0.000**
Sampling Stations	2	1E+08	5E+07	5.71	**0.008**
Sampling months*Water depths	2	2E+08	8E+07	9.67	**0.001**
Sampling months*Sampling Stations	2	8E+06	4E+06	0.49	0.615
Water depths*Sampling Stations	4	3E+08	8E+07	9.12	**0.000**
Sampling months*Water depths*Sampling Stations	4	2E+08	4E+07	4.77	**0.004**
Error	29	2E+08	8E+06		
Total	46	2E+09			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold text

Considering both spatial and temporal variations along all three transects, the ANOVA results showed that there was a significant difference between the fluorescence recorded within the three coastal zones, water depths, sampling months and stations (Table 9). The outcome from the Tukey's comparison test showed that phytoplankton concentration on the Eastern Coast Transect was significantly lower (≈ 60 %) than those on the Western and Central Coast Transects. Also, there were significant differences in the fluorescence recorded on the different sampling months; fluorescence increased (by 43 %) from April to May. In terms of water depths, fluorescence was significantly lower (by ≈ 35 %) within the mid-water column compared with the deep and surface water columns. The fluorescence at the station located at 2 NM from the shoreline was significantly lower (by ≈ 28 %) than those on the stations located at 1.5 and 1 NM from the shoreline. There was no significant interaction between the values within the different coastal zones and sampling months; also, there were no significant

interactions between the values obtained at the different sampling stations and months (Table 9). As a consequence, intersections between the coastal zones, water depth and sampling stations had no significant relationship to the level of fluorescence observed in this study (Table 9).

Table 9: *ANOVA comparison of phytoplankton concentrations recorded in April to May 2019 from the three coastal zones of Ghana*

ANOVA Comparison						
Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value	Significant source of variation
Coastal zones	2	2.6E+10	1.3E+10	56.12	**0.000**	Eastern Coast Transect
Sampling months	1	1.3E+10	1.3E+10	55.59	**0.000**	April & May
Sampling depths	2	6E+09	3E+09	12.91	**0.000**	Mid-water column
Sampling Stations	2	4.2E+09	2.1E+09	9.16	**0.000**	Station sited at 2 NM
Coastal zones*Sampling months	2	3.8E+10	1.9E+10	83.4	**0.000**	
Coastal zones*Sampling depths	4	1.2E+10	3.1E+09	13.23	**0.000**	
Coastal zones*Sampling Stations	4	1.1E+10	2.8E+09	12.09	**0.000**	
Sampling months*Sampling depths	2	5.5E+09	2.7E+09	11.86	**0.000**	
Sampling months*Sampling Stations	2	3E+08	1.5E+08	0.65	0.525	
Sampling depths*Sampling Stations	4	6E+09	1.5E+09	6.53	**0.000**	
Coastal zones*Sampling months*Sampling depths	4	3.7E+09	9.3E+08	4.03	**0.005**	
Coastal zones*Sampling depths*Sampling Stations	8	1.2E+10	1.5E+09	6.38	**0.000**	
Coastal zones*Sampling months*Sampling Stations	4	7E+09	1.8E+09	1.25	0.293	
Sampling months*Sampling depths*Sampling Stations	4	2.7E+09	6.7E+08	2.89	**0.027**	
Coastal zones*Sampling months*Water depths*Sampling Stations	8	1E+10	1.2E+09	0.89	0.529	
Error	86	2E+10	2.3E+08			
Lack-of-Fit	6	3.7E+09	6.1E+08	3.03	**0.010**	
Pure Error	80	1.6E+10	2E+08			
Total	127	1.78E+11				

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

4.1.3 Relationship between nitrate to phosphate ratio and phytoplankton concentration

The relationship between nitrate to phosphate ratios and phytoplankton concentration on each of the three sampling transects along the coast of Ghana is shown in figures 4.7-4.9. Considering the Western Coast Transect, phytoplankton concentration increased with increasing nitrate to phosphate ratio (Figure 4.7). Approximately 9 % increase in the phytoplankton concentration could be attributed to the variations in the N:P ratio. However, the changes were not significantly different ($p > 0.05$).

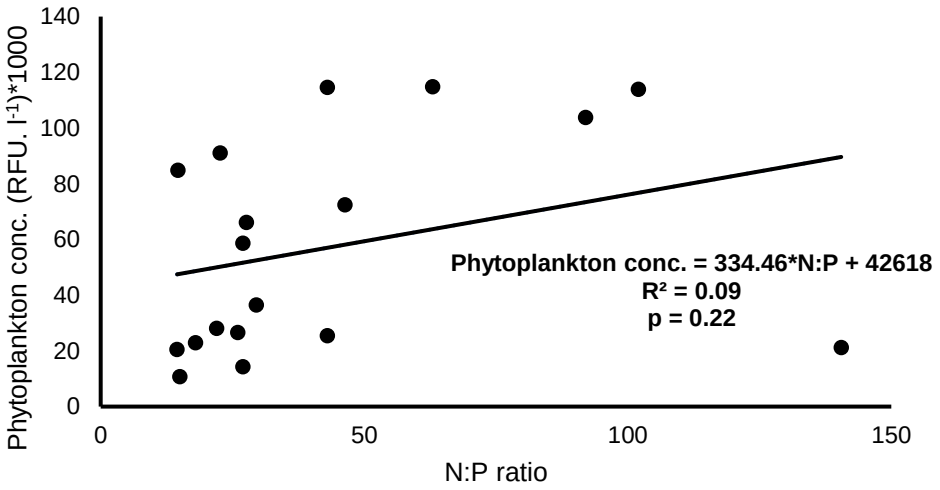

Figure 4.7: Relationship between phytoplankton concentration and N:P ratio on the Western Coast Transect of Ghana

Phytoplankton concentrations on the Central Coast Transect also increased with increasing N:P ratios. This is similar to the relationship observed on the Western Coast Transect. Approximately 11 % of the variations in phytoplankton

concentration could be attributed to changes in N:P ratio. Similar to the Western Coast, the variations on the Central Coast transects were not statistically significant.

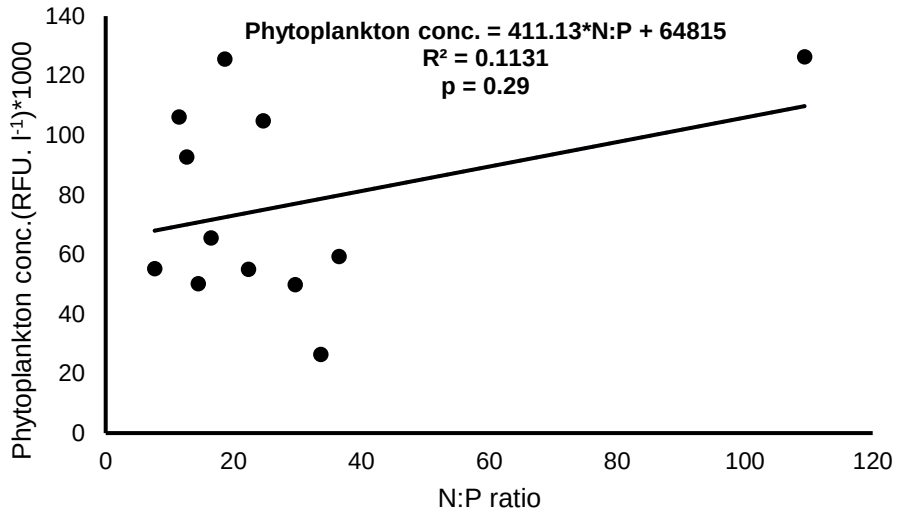

Figure 4.8: Relationship between phytoplankton concentration and N:P ratios on the Central Coast Transect of Ghana

In contrast with observations on the Western and Central Coast Transects, phytoplankton concentrations on the Eastern Coast Transect decreased with increasing N:P ratios (Figure 4.9). However, only 2 % of the variations in the phytoplankton concentration could be associated with changes in phytoplankton concentration. However, the variations were not statistically significant.

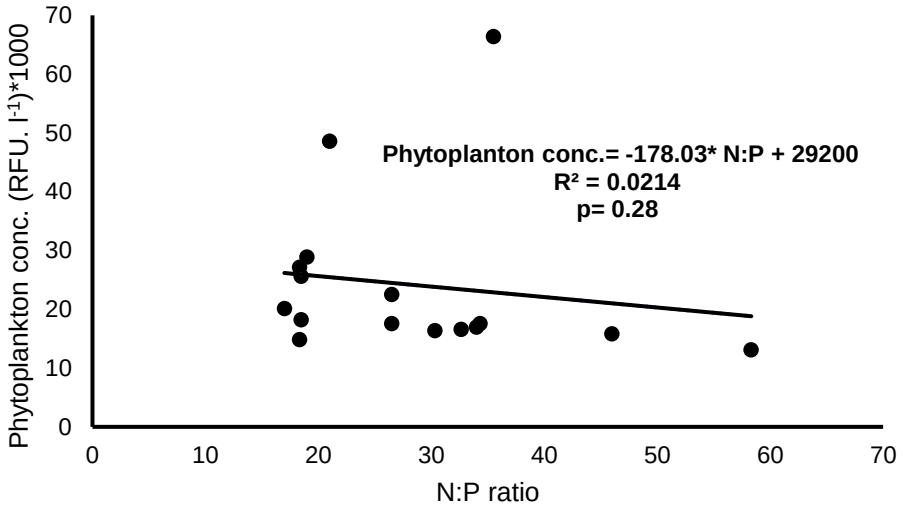

Figure 4.9: Relation between phytoplankton concentration and N:P ratio on the Eastern Coast Transect of Ghana

4.1.4 Abundance of Copepods

On average, and over the entire study period, highest abundance (1276 ind. l^{-1}) of copepods was recorded along the Central Coast Transect in April 2019 at station 3 within the top 5 m of the water. Minimum abundance recorded was 4 ind. l^{-1}. This was recorded in both months of the study at different stations within all the three coastal zones. Figures 4.10-4.12 shows the abundance (ind. l^{-1}) of copepods obtained along the coast of Ghana.

Regarding findings on the transect sited on the Western Coast Transect, copepod abundance changed significantly only with sampling months ($p = 0.003$; Table 10). The outcome of the post hoc comparison showed that abundance decreased (≈ 17 %) significantly from April to May 2019 (Figure 4.10; Table 10). Copepod abundance did not differ at the different sampling depths as well as sampling stations.

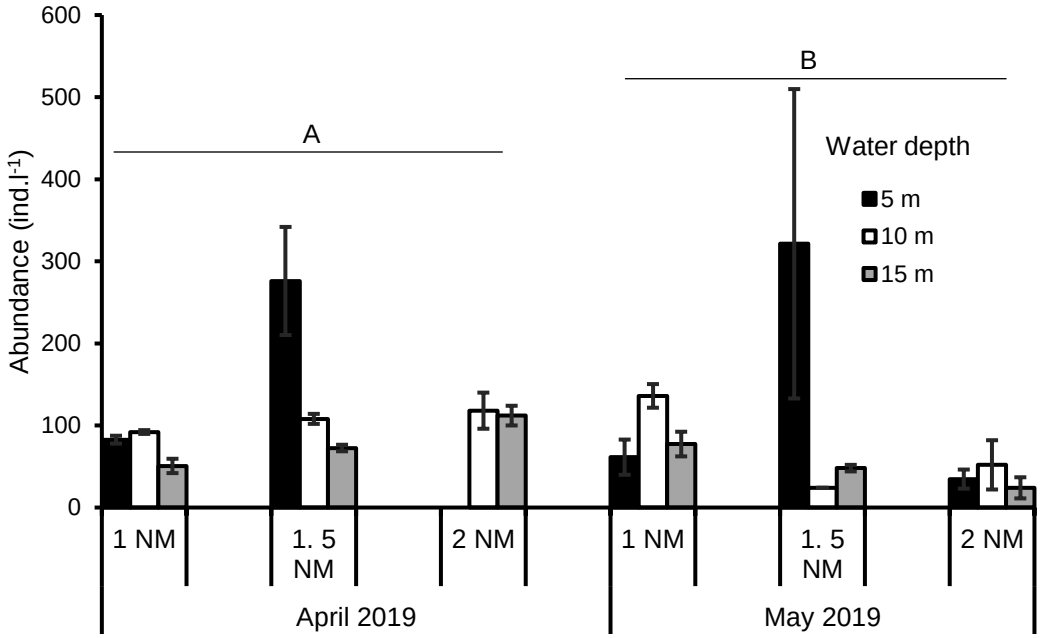

Figure 4.10: Copepod abundance (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Western Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabetsupper case alphabets compare findings made on different months within a station

Table 10: *ANOVA comparison of copepod abundance (ind. l^{-1}) recorded in April to May 2019 on the Western Coast Transect of Ghana*

Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling months	1	0.919	0.919162	10	**0.003**
Sampling stations	2	0.017	0.008395	0.09	0.913
Water depths	2	0.134	0.067038	0.73	0.49
Sampling months*Sampling stations	2	0.812	0.406087	4.42	**0.020**
Sampling months*Water depths	2	0.382	0.191204	2.08	0.141
Sampling stations*Water depths	4	0.490	0.12238	1.23	0.316
Sampling months*Sampling stations*Water depths	4	0.705	0.17617	1.77	0.157
Error	33	3.032	0.091876		
Total	42	4.723			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

Similar to the Western Coast Transect, copepod abundance on the Central Coast Transect changed significantly between the different sampling months ($p = 0.001$; Table 11). The outcome of the Tukey's post hoc test suggests that abundance

increased significantly by approximately 33 % from April to May 2019 (Figure 4.11). The abundance within this coast did differ at the different sampling stations as well as the different water depths.

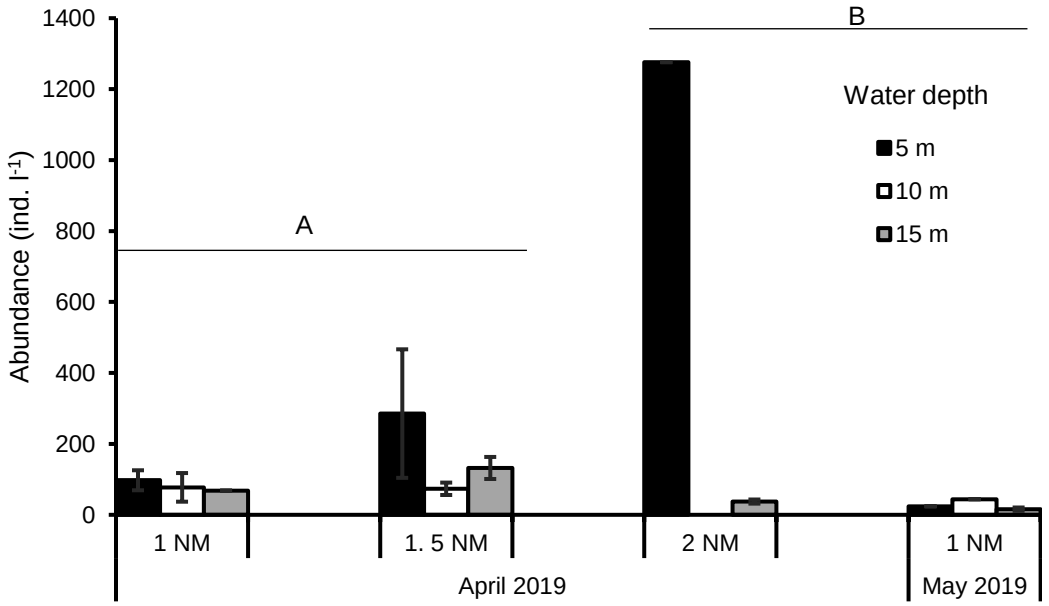

Figure 4.11: Copepod abundance (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Central Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets; upper case alphabets compare findings made on different months within a station

Table 11: *ANOVA comparison of copepod abundance (ind. l^{-1}) recorded from April to May, 2019 on the Central Coast Transect of Ghana*

Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling months	1	1.1868	1.187	22.04	**0.001**
Sampling stations	2	0.6941	0.347	1.93	0.175
Sampling depths	2	0.9236	0.462	2.57	0.106
Sampling months*Sampling depths	2	0.216	0.108	0.6	0.559
Error	17	3.0493	0.179		
Lack-of-Fit	4	1.9631	0.491	5.87	**0.006**
Pure Error	13	1.0861	0.084		
Total	23	5.3179			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

On the Eastern Coast Transect, abundances of the copepods differed significantly between the different periods of sampling (Figure 4.12; Table 12). Abundance increased by ≈ 50 % from April to May 2019. In contrast, with the Western and Central Coast Transects, copepod abundance on Eastern Coast changed at the different water depth as well as the different sampling stations (see Table 12 for significant difference). In terms of water depth, the abundance recorded was generally lower at the mid-water and surface water column compared with the deep-water column (Tukey post hoc test). At station 2 in May, the abundance at the deep-water column was ≈ 64 % higher than the abundance observed at the mid and surface water column (Figure 4.12). Also, copepod abundance decreased by ≈ 66 % from the deep-water column to the mid-water column (Figure 4.12). This observation was made in May at station 3 located further away from the shoreline. Concerning copepod abundance at the different sampling stations, the observed abundance decreased by ≈ 40 % from the station closest to

the shoreline to the station further away from the shoreline in April. Also, the abundance at the station closest to the shoreline was higher by ≈ 31 % in April compared to the abundance at the same station in May (Figure 4.12).

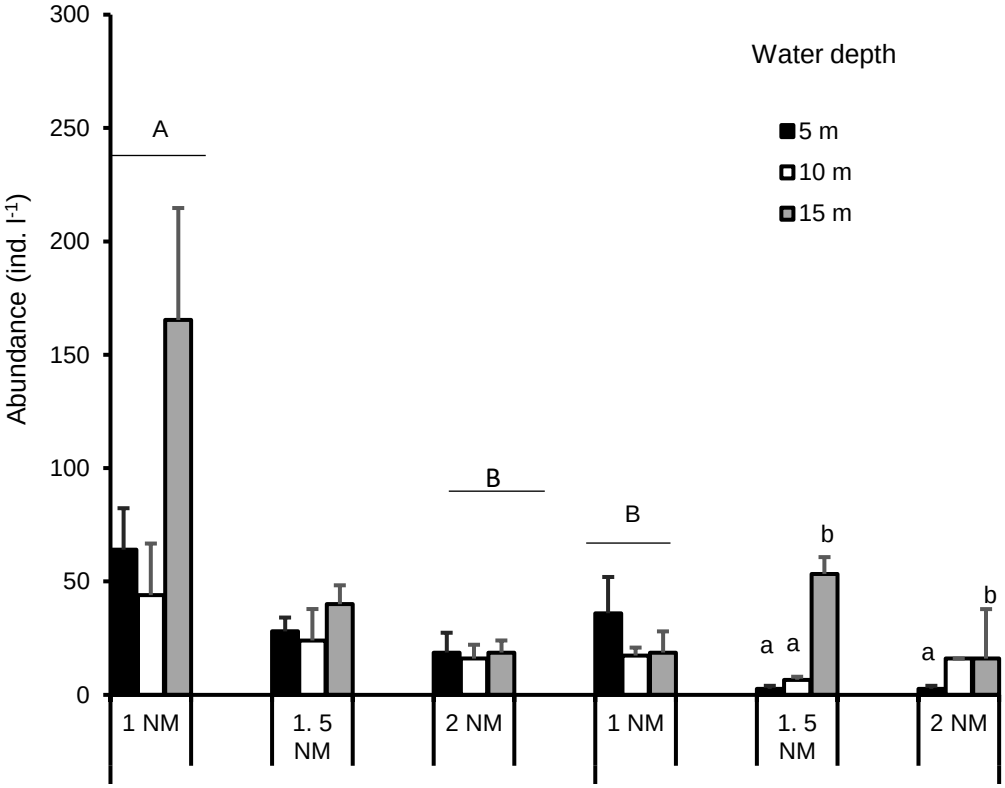

Figure 4.12: Copepod abundance (average ± standard error) recorded at different stations (1, 1.5 and 2 NM) on the Eastern Coast Transect of Ghana. Findings that were significantly different (Tukey post hoc test; $p < 0.05$) are shown using different alphabets (within stations) and numerals (between stations)

Table 12: *ANOVA comparison of copepod abundance (ind. l^{-1}) recorded in April to May 2019 on the Eastern Coast Transect of Ghana*

Source variations	DF	Adj SS	Adj MS	F-Value	P-Value
Sampling months	1	1.2152	1.215	16.48	**0.000**
Sampling stations	2	1.5963	0.798	10.82	**0.000**
Water depths	2	2.1104	1.055	14.31	**0.000**
Sampling months*Sampling stations	2	0.7171	0.359	4.86	**0.015**
Sampling months*Water depths	2	0.7223	0.361	4.9	**0.015**
Sampling stations*Water depths	4	0.2705	0.068	0.92	0.468
Sampling months*Sampling stations*Water depths	4	0.9227	0.231	3.13	**0.030**
Error	28	2.0649	0.074		
Total	45	9.6947			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

Considering all the three transects with respect to spatial and temporal variations, the ANOVA results showed that there was a significant difference ($p = 0.000$; Table 13) between the copepods abundance recorded within the three coastal zones. Similar results were recorded when the sampling stations and water depths were compared (Table 13). Tukey's post hoc test showed that copepod abundance on the Eastern Coast Transect was significantly lower (≈ 82 %) than the abundance on the Western and Central Coast Transects (Figure 4.10-4.12). The abundance recorded on the Western and Central Coast Transects were not significantly different. In terms of the sampling stations, abundance was significantly higher (≈ 69 %) at the station located 2 NM from the shoreline compared with the stations closer to the shoreline. Abundance in the deep-water column was significantly lower (≈ 59 %) compared to the ones recorded in the mid and upper water column, which was similar to each other.

Table 13: *ANOVA comparison of copepod abundance (ind. l^{-1}) recorded in April to May 2019 from the three coastal zones in Ghana*

ANOVA Comparison						
Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value	Significant source of Variation
Coastal zones	2	5.17	2.59	23.88	**0.000**	Eastern Coast Transect
Sampling months	1	1.22	1.22	11.23	**0.001**	April & May
Sampling stations	2	0.12	0.06	0.56	0.576	Station sited at 2 NM
Water depths	2	0.06	0.03	0.30	0.743	Deep water Column
Coastal zones*Sampling months	2	0.23	0.11	1.05	0.354	
Coastal zones*Sampling stations	4	1.38	0.35	3.19	**0.018**	
Coastal zones*Water depths	4	1.63	0.41	3.76	**0.008**	
Sampling months*Sampling stations	2	0.26	0.13	1.20	0.307	
Sampling months*Water depths	2	0.74	0.37	3.41	**0.038**	
Sampling station*Water depths	4	0.53	0.13	1.22	0.308	
Coastal zones*Sampling months*Water depths	4	0.35	0.09	0.81	0.522	
Coastal zones*Sampling months*Sampling stations	4	1.90	0.48	1.95	0.109	
Coastal zones*Sampling stations*sampling depths	8	0.90	0.11	0.46	0.882	
Sampling months*Sampling stations*sampling depths	4	0.63	0.16	1.46	0.224	
Coastal zones*Sampling months*Sampling stations*sampling depths	8	1.50	0.19	0.77	0.630	
Error	79	8.56	0.11			
Lack-of-Fit	8	2.53	0.32	3.72	0.001	
Pure Error	71	6.03	0.08			
Total	112	26.92				

. Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

4.1.5 Relation between copepod abundance (ind. l^{-1}) and phytoplankton concentration (RFU. l^{-1})

Copepod abundance observed within all the three coastal zones increased with increasing phytoplankton concentration. Respectively, the 29 and 31 % variations in copepod abundance on the Western and Central Coast Transects could be attributed to changes in phytoplankton concentration (Figures 4.13 A&B). On the Eastern Coast Transect, only 0.2 % of the variations could be attributed to changes in phytoplankton concentration (Figure 4.13 C). The variations recorded on the Western and Central Coast transects were statistically significant ($p < 0.05$); however, the variations recorded on the Eastern Coast Transects were not statistically significant ($p > 0.05$).

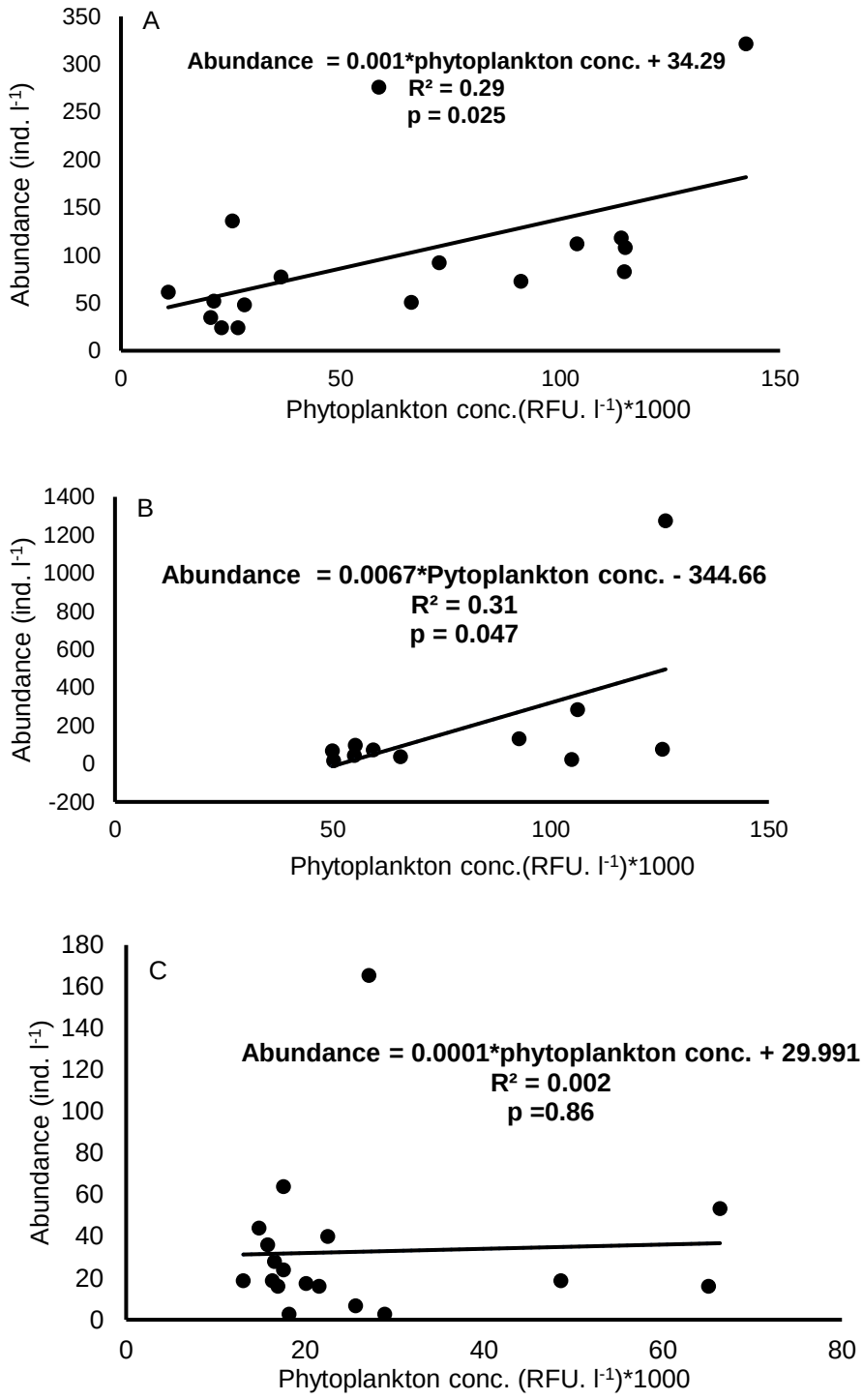

Figure 4.13: Relationship between copepod abundance and phytoplankton concentration on the Western (A) Central (B) and Eastern (C) Coasts of Ghana.

4. 1. 6 Taxonomic Composition of Copepod.

The focus of the present study was to understand the effect of multiple environmental stressors on marine copepods as they constitute the bulk (approximately 70-97 %) of the zooplankton biomass in the ocean (Wilson, 1987; Verity & Smetacek, 1996; Turner, 2004; Uttieri, 2018). As a result, this section of the report is focused on determining the taxonomic composition of copepods in marine waters of Ghana. The Copepods were identified to the lowest taxonomic level.

Four main Orders of copepods were identified based on this approach. These were Order Calanoida, Cyclopoida, Poeciliostomatoida, and Harpacticoida. Figure 4.14 presents the contribution of these Orders to the total number of copepods observed during the study. The composition was dominated by copepods belonging to the Order Calanoida (52 %) followed by Cyclopoida (26 %), Poecilostomatoida (13 %), and Harpacticoida (9 %).

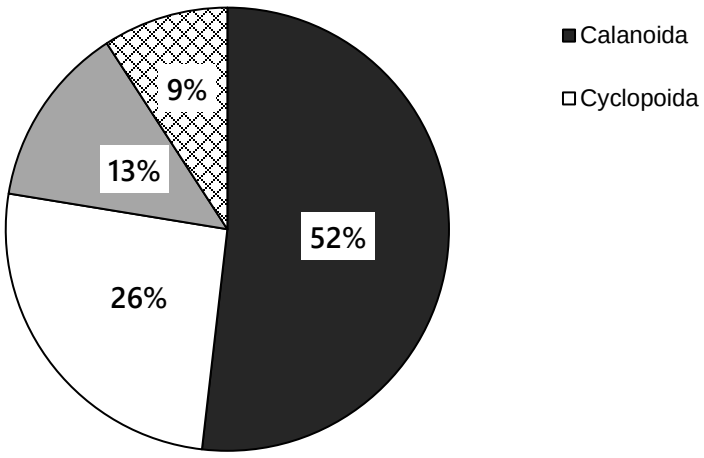

Figure 4.14: Composition of Copepods found at different depths and stations along all transects combined

Figures 4.15-4.17 present the composition of copepods recorded in each of the three Coastal zones considered in this study. On the Western Coast Transect, results showed that there were significant differences in the compositions of copepod Orders recorded (Table 14). Largely, the highest composition (51.3 %) of copepods recorded on the Western Coast Transect belonged to the Order Calanoida. This was followed by copepods belonging to the order Cyclopoida (30.2 %), Poecilostomatioda (9.5 %) and Harpacticoida (9 %), respectively (Figure 4.15; see Table 14 for significant difference). In terms of sampling months, there was a significant difference in copepod composition collected along the Western Coast Transect (Table 14). Outcome from the Tukey's post hoc test showed that the contribution of the Order Calanoida to the total number of copepods collected in April was not significantly different from that of Order Cyclopoida in the same month. However, the composition of copepods belonging to the Order Cyclopoida was significantly lower in April than May among the copepods ($p = 0.000$; Table 14). The composition of copepods was not different at different water depths as well as sampling stations (Figure 4.15; Table 14).

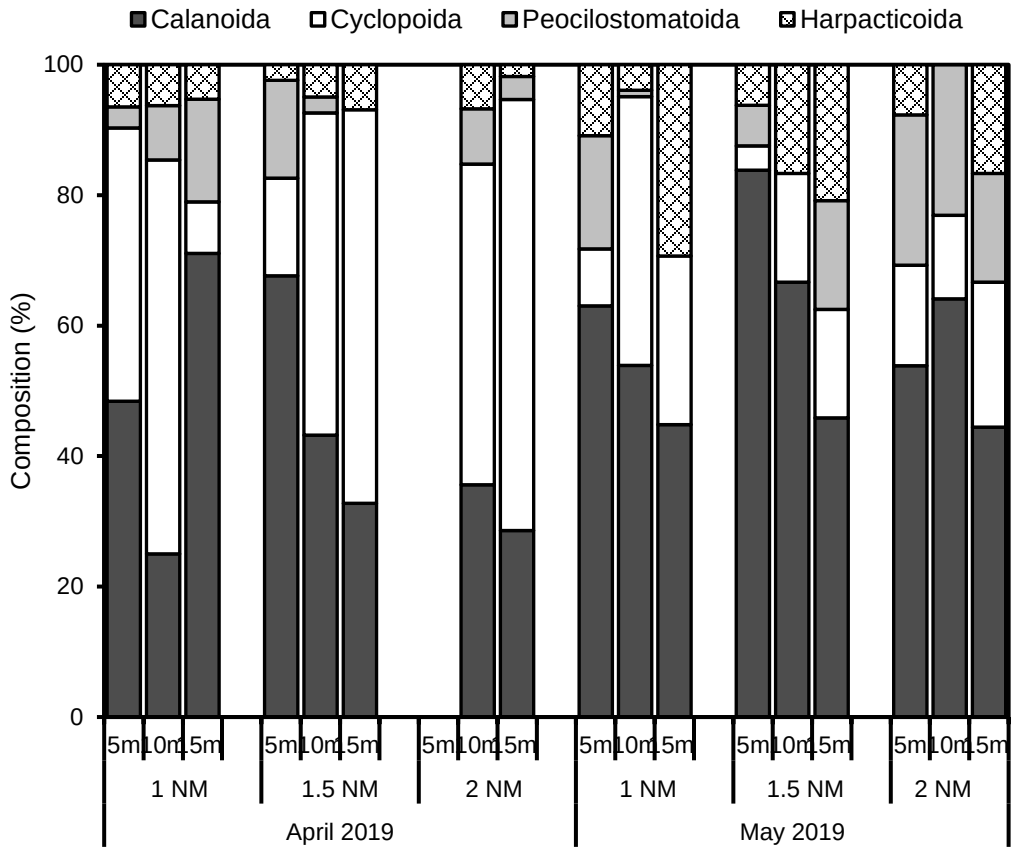

Figure 4.15: Contribution of the different taxonomic groups to the total number of copepods observed at different stations (1, 1.5 and 2 NM) on the Western Coast Transect of Ghana.

Table 14: *ANOVA comparison of the composition of different taxonomic groups to the total number of copepods recorded in April to May 2019 on the Western Coast Transect of Ghana*

Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value
Copepod Orders	3	1.95	0.65	78.22	**0.000**
Sampling months* Copepod orders	3	0.47	0.16	18.86	**0.000**
Sampling stations*Copepod orders	6	0.12	0.02	2.34	0.063
Water depths*Copepod orders	6	0.12	0.02	2.44	**0.054**
Sampling months*Sampling stations*Copepod orders	6	0.12	0.02	2.37	0.060
Sampling months* Water depths*Copepod orders	6	0.11	0.02	2.23	0.074
Sampling stations*Sampling depths*Copepod orders	12	0.40	0.03	4.05	**0.002**
Sampling months*Sampling stations* Water depths*Copepod Orders	12	0.26	0.02	0.31	0.985
Error	25	0.21	0.01		
Total	67	4.10			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

On the Central Coast Transect, the different taxonomic groups of Copepod contributed differently to the total number of copepods ($p = 0.000$; Table 15). Similar to the Western Coast Transect, copepods belonging to the Order Calanoida constituted the highest copepod composition (51.7 %) on the Central Coast Transect. Unlike the findings on the Western Coast Transect, Order Calanoida was followed by Order Peocilostomatoida (22 %) on the Central Coast. Copepods belonging to the order Cylopoida and Harpacticoida contributed the least to the copepod composition on the Central Coast Transect. The composition of the copepods also was significantly different at the different depths of the water at the Central Coast Transect (see Table 15 for significant difference). Peocilostomatoida composition of the copepods on average decreased significantly, by 80 %, from the surface to the lower depths for some of the stations (Figure 4.16). In contrast, Harparticoida compositions of the copepods increased by nearly 84 % from the top 5 m to the lower 15 m of the water for some of the stations (Figure 4.16).

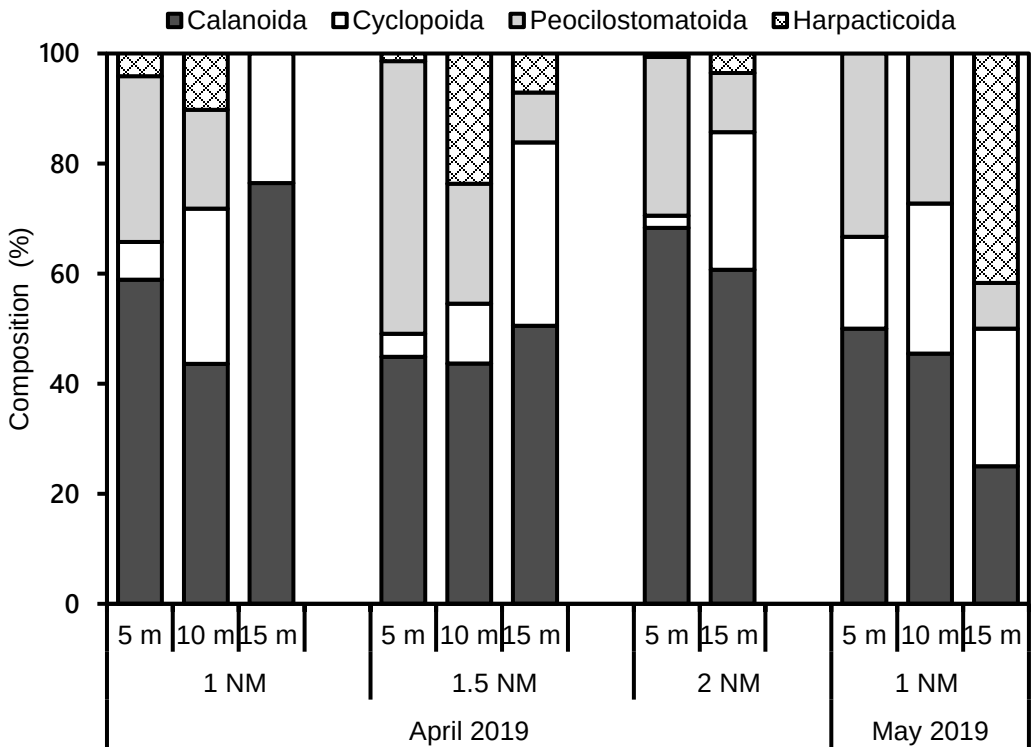

Figure 4.16: Contribution of the different copepod taxonomic groups observed at different stations (1, 1.5 and 2 NM) on the Central Coast Transect of Ghana.

Table 15: *ANOVA comparison of the composition of different taxonomic groups to the total number of copepods recorded in April to May 2019 on the Central Coast Transect of Ghana*

Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value
Copepod Orders	3	0.36507	0.12169	23.88	**0.000**
Sampling months*Copepod Orders	3	0.10503	0.03501	6.87	**0.003**
Sampling stations*Copepod Orders	6	0.07999	0.01333	2.62	0.051
Water depths*Copepod Orders	6	0.25634	0.04272	8.38	**0.000**
Sampling months* Water depths*Copepod Orders	6	0.20603	0.03434	6.74	**0.001**
Sampling months*Sampling Stations*Copepod Orders	6	0.01565	0.00261	0.03	1.000
Sampling stations* Water depths*Copepod Orders	12	0.20743	0.01729	0.23	0.995
Sampling months*Sampling stations*Water depths*Copepod orders	12	0.2646	0.02205	0.36	0.967
Error	31	1.8746	0.06047		
Total	43	2.1392			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold text

The Order Calanoida was the most dominant ((53 %) taxonomic group occurring on the Eastern Coast Transect, just like on the Western and Central Coast Transect. This was followed by copepods belonging to the Orders Cyclopoida (≈ 28 %), Peocilostomatoida (≈ 9 %) and Harpacticoida (≈ 10 %) (Figure 4.17). Copepod composition changed at different water depths (Table 16). Largely, the outcome of the post hoc test showed that the composition of copepods belonging to the Order Calanoida was 32-48 % higher at the surface water column compared to the mid and deep-water columns. However, the composition of copepods belonging to the Order Cyclopoida decreased largely by 76 % from the deep water and mid-water column to the surface-water column on the Eastern Coast Transect. In terms of the sampling period, the composition of Order Calanoida on this coast was highest by ≈ 44 % in May than its composition observed in April (Tukey's post hoc test; Figure 4.17). Respectively, copepods belonging to the Orders Hapacticoida and Poecilostomatoida declined by ≈ 75 and 89 % respectively from April to May of the sampling period.

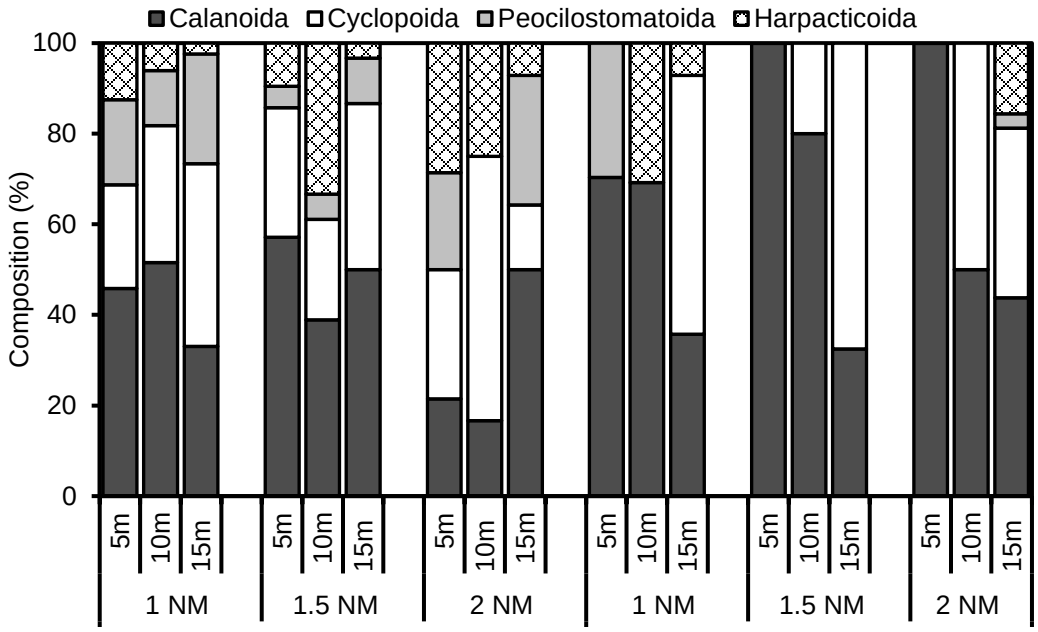

Figure 4.17: Contribution of the different copepod Orders observed at different stations (1, 1.5 and 2 NM) on the Eastern Coast Transect of Ghana.

Table 16: *ANOVA comparison of the composition of different taxonomic groups to the total number of copepods recorded in April to May 2019 on the Eastern Coast Transect of Ghana*

Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value
Copepod Orders	3	3.26	1.09	146.37	**0.000**
Copepod Orders*Sampling months	3	0.74	0.25	33.33	**0.000**
Copepod Orders* Sampling stations	6	0.16	0.03	3.67	**0.016**
Copepod Orders*Water depths	6	0.96	0.16	21.61	**0.000**
Copepod Orders* Sampling months* Sampling stations	6	0.16	0.03	3.16	**0.017**
Copepod Orders* Sampling months* Water depths	6	0.94	0.16	21.13	**0.000**
Copepod Orders*Sampling stations*Water depths	12	0.58	0.05	6.48	**0.000**
Copepod Orders*Sampling months*Sampling stations*Sampling depths	12	0.32	0.03	3.16	**0.008**
Error	17	0.13	0.01		
Total	71	7.26			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

Regarding all three transects, the ANOVA results showed that there was a significant difference between the composition of copepod Orders occurring within the three coastal zones as well as the sampling months and water depths (Table 17). The outcome of the Tukey post hoc test showed that the composition of copepods belonging to the Order Calanoida on the Central Coast Transect was higher by ≈ 44 % than its composition on the Western Coast Transect. Conversely, the composition of copepods belonging to the Order Cyclopoida was higher by ≈ 79 % instead on the Western Coast Transect compared to its composition on the Central Coast Transect. Considering the sampling periods, the outcome of the post hoc test showed that in April, the composition of copepods belonging to the Order Calanoida was ≈ 36 % lower compared with its composition observed in May. However, the composition of copepods belonging to the Order Cyclopoida was higher (≈ 55 %) in April than its composition observed in May. In terms of water depth, the composition of copepods belonging to the Order Calanoida occurred much (≈ 32 %) more on the surface-water column than its composition observed on the deeper-water column. In contrast, the composition of copepods belonging to the Order Cyclopoida was higher by 75-81 % within the middle and lower water depth than its composition at the Surface-water depth (Tukey's post hoc test; Table 17).

Table 17: *ANOVA comparison of copepod compositions observed in April to May 2019 within the three coastal zones of Ghana*

ANOVA Comparison						
Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value	Significant source of variation
Copepod Orders	3	5.61	1.87	84.3	**0.000**	Calanoida & Cyclopoida
Coastal zones*Copeod Orders	6	0.46	0.08	3.43	**0.004**	Calanoida & Cyclopoida
Sampling months*Copepod Orders	3	0.98	0.33	14.71	**0.000**	Calanoida & Cyclopoida
Sampling stations* Copepod Orders	6	0.03	0.01	0.24	0.961	Calanoida & Cyclopoida
Water depths*Copepod Orders	6	1.03	0.17	7.74	**0.000**	
Coastal zones*Sampling stations*Copepod Orders	12	0.28	0.02	1.07	0.399	
Coastal zones*Water depths*Copepod Orders	12	0.38	0.03	1.41	0.177	
Sampling months*Sampling stations*Copepod Orders	6	0.2	0.03	1.52	0.181	
Sampling months*Water depths*Copepod Orders	6	0.75	0.12	5.6	**0.000**	
Sampling stations*Water depths*Copepod Orders	12	0.34	0.03	1.29	0.237	
Sampling months*Sampling stations*Water depths* Copepod Orders	12	0.24	0.02	0.9	0.547	
Error	86	1.91	0.02			
Total	170	13.13				

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

4.2 Results from Laboratory Investigations

Faecal pellet, egg production and mortality rates of *Temora Stylifera*–a calanoid copepod- were measured at different levels of warming and concentrations of heavy metal and petroleum pollutants. Warming scenarios used were + 1, 2 and 3 °C above 28 °C, which is the temperature of Ghanaian coastal waters during the stable hydrographic period. The heavy metal pollutant used was cadmium. It was presented as cadmium chloride ($CdCl_2$) at concentrations of 0.1, 1.0, 10.0 and 100.0 µg /L. The petroleum pollutant used was pyrene. Its effects were investigated at concentrations of 0, 1.0, 10.0 and 100.0 nM. The outcome of these laboratory experiments are presented in the following sections.

4.2.1 Effect of the individual stressors on *Temora stylifera*

Warming

Figure 4.18 (A, B and C) presents the effects of warming on faecal pellet, egg production and mortality rates of *Temora stylifera*. Faecal pellet and egg production rates of the copepod decreased significantly with increasing temperature (Figures 4.18 A & B; Table 18). Faecal pellet production rate was significantly higher ($p = 0.000$) at the ambient temperature, T of 28 °C and T + 1 °C. It decreased by nearly 30 and 49 % at T + 2 and 3 °C (Figure 4.18 A). Egg production rate was also significantly higher ($p = 0.004$) at the ambient temperature. It decreased significantly by approximately 88 % at T +1 and 2 °C and 100 % at T + 3 °C. The mortality rate also increased with temperature. However, the ANOVA test showed that the changes in the mortality rate were not significantly different ($p = 0.059$) at the different temperatures (see Table 18 for significant difference).

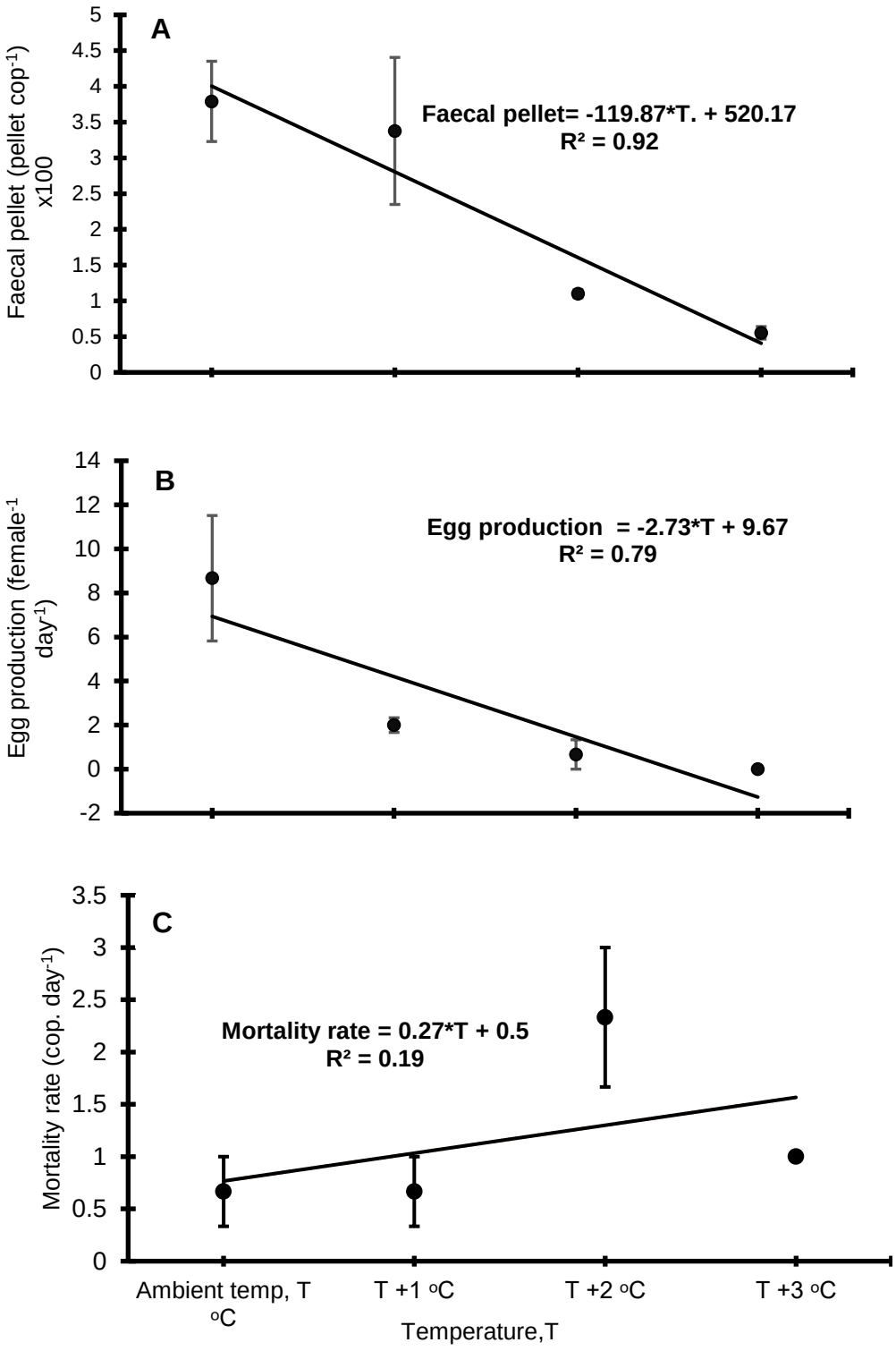

Figure 4.18: Effect of warming (mean ± SE) on faecal pellet (A), egg production (B) and mortality (C) of *Temora stylifera*

Table 18: *ANOVA testing for the effect of warming on selected vital rates of Temora stylifera.*

Selected vital rates	Source of variations	DF	Adj SS	Adj MS	F-Value	P-Value
Faecal pellet production	Temperature	3	1.391	0.464	20.83	**0.000**
	Error	8	0.178	0.022		
	Total	11	1.569			
Egg production	Temperature	3	0.330	0.120	10.39	**0.004**
	Error	8	0.085	0.011		
	Total	11	0.414			
Mortality rate	Temperature	3	5.667	1.889	3.78	0.059
	Error	8	4.000	0.500		
	Total	11	9.667			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

Cadmium

The effect of cadmium on the experimental copepod is shown in Figure 4.19 (A, B and C). The production of faecal pellets by the copepods generally decreased with increasing concentrations of cadmium pollutants (Figure 4.19 A). However, the decrease was not significantly different ($p = 0.443$) at the different cadmium concentrations (Table 19). In contrast, egg production rate increased markedly, and linearly with increasing concentrations of the cadmium (Figure 4.19 B). Judging from the slope of the relationship, it increased by ≈ 44 % per each 0.62 µg. l^{-1} unit increase in the concentration of the pollutant.

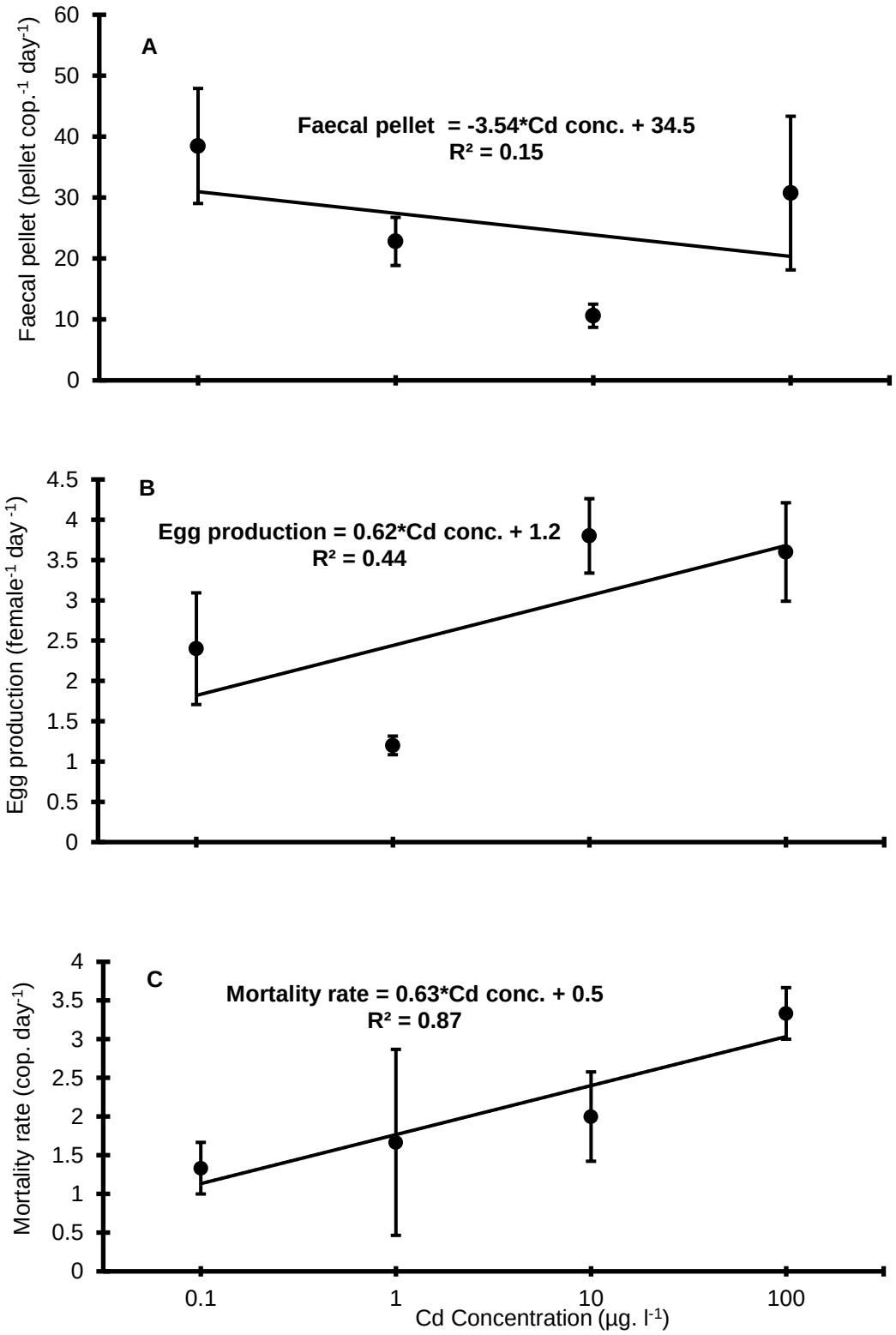

Figure 4.19: Effect of different concentrations of Cd on the rates (mean ± SE) of faecal pellet (A), egg production (B) and mortality (C) of *Temora stylifera* at 28 °C

The mortality of the copepod, on the other hand, increased linearly with the concentration of Cd; based on the gradient of the relationship, with an increase of approximately 63 % per every µg increase in the level of pollutants per litre of water (Figure 4.19 C).

Table 19: *ANOVA Test for the effect of cadmium pollution on selected vital rates of Temora Stylifera*

Selected vital rates	Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Faecal pellet production	Cd	3	0.125	0.042	0.92	0.443
	Error	8	3033	379.1		
	Total	11	7379			
Egg production rate	Cd	3	0.407	0.136	2.7	0.062
	Error	8	11938	1492		
	Total	11	26564			
Mortality rate	Cd	3	48.25	16.083	7.15	**0.012**
	Error	8	18	2.25		
	Total	11	66.25			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold text

Figure 4.20 (A, B and C) shows the effect of pyrene on faecal pellet, egg production and mortality rates of *Temora stylifera.* Faecal pellet production of the copepod increased with increasing concentration of pyrene only to a level after which it began to fall when the concentration of the pollutant was increased further (Figure 4.20 A). This effect (denoted as F) can be described using the Gaussian relationship below:

$$F = F_{max} * e^{-0.5\left\{\left(\frac{P-P_l}{b}\right)^2\right\}} \quad 5$$

Where F_{max} is the maximum obtainable rate of faecal pellet production, P represents the concentration of the pollutants, P_l is the pollutant level beyond

which faecal pellet production began to fall whereas *b* describes the width of the response curve. Values for the parameters of the equation are provided in the Table below:

Table 20: *Parameters estimated for Guassian relationship (equation 5) describing the effect of pyrene on the rate of faecal pellet production by Temora stylifera*

Parameter	Mean ± Standard error	R^2
F_{max} (pellets per copepod per day)	131.87 ± 229.79	0.67
P_l (nM of pyrene per litre of water)	5.41 ± 0.61	
B	1.94 ± 0.19	

The rate of faecal pellet production was 48 % higher at a pyrene concentration of 1 nM compared with the rates observed at pyrene concentration of 100 nM (Figure 4.20 A).

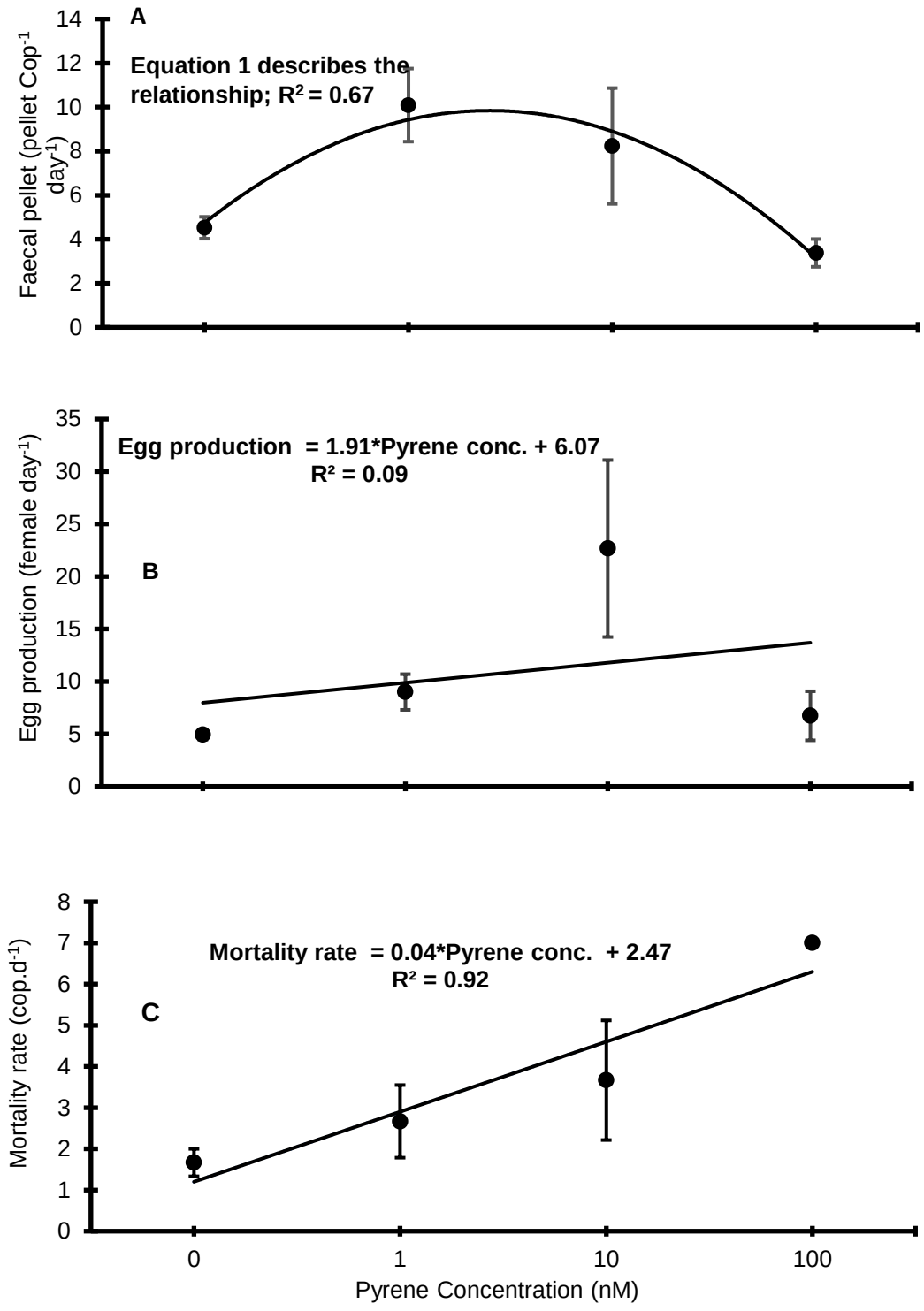

Figure 4.20: Effect of different pyrene (PY) concentrations on the rates (mean ± SE) of faecal pellet production (A), egg production (B) and mortality (C) of *Temora stylifera* at 28 °C.

Egg production rate, on the other hand, increased with increasing concentration of pyrene (Figure 4.20 B). Nonetheless, the changes were not statistically significant ($p = 0.022$; Table 21). Mortality rate of the copepod (Figure 4.20 C) also increased with increasing concentration of the pollutant; it was ≈ 75 % higher at the highest pyrene concentration (= 100 nM) compared with observations made at the lowest concentration of the pollutant (= 1 nM; Figure 4.20 C).

Table 21: *ANOVA Test for the effect of pyrene pollution on selected vital rates of Temora stylifera*

Selected vital rates	Source of variation	DF	Adj SS	Adj MS	F-value	P-Value
Faecal pellet production	Pyrene conc.	3	0.417	0.139	5.69	**0.022**
	Error	8	0.195	0.024		
	Total	11	0.613			
Egg production	Pyrene conc.	3	0.584	0.195	3.6	0.065
	Error	8	0.433	0.0541		
	Total	11	1.017			
Mortality rate	Pyrene conc.	3	48.25	16.083	7.15	**0.012**
	Error	8	18	2.25		
	Total	11	66.25			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

4.2.2 Combined Effect of Multiple Stressors on *Temora stylifera*

Figure 4.21 illustrates the interactive effect of warming and cadmium pollution on selected vital rates of *Temora stylifera*. The results showed that generally, at each concentration of cadmium pollutants, the rates of faecal pellet production decreased significantly ($p = 0.005$) with increasing temperature. At

the lowest level of cadmium (concentration = 0.1 µg. l^{-1}), the highest rate of faecal pellets production (27.5 ± 6.7 cop^{-1} day^{-1}) was obtained when the experimental temperature was 28 °C. Faecal pellets decreased by 50-60 % when the effect of cadmium was evaluated at all the warming scenarios (28 °C + 1, 2 and 3 °C). Similar declining trends occured at cadmium concentrations of 10 and 100 µg. l^{-1} (Figure 4.21 A). Similar to the above report on faecal pellet, egg production rate of the copepods decreased significantly ($p = 0.002$) with increasing temperature (Figure 4.21 B; Table 22). However, Tukey pairwise comparison of the result showed that the decline was significant only at cadmium concentrations of 10 µg. l^{-1} ($p < 0.05$). At this concentration, the highest rate of eggs production (12.01 ± 4.0 $females^{-1}$ day^{-1}) was observed when the temperature was increased by 1 °C. The rate of egg production decreased by ≈ 64 and 77 % when temperature was increased by +2 and 3 °C, respectively.

The mortality of the copepod increased with increasing temperature (Figure 4.21 C). ANOVA showed no significant changes in the mortality of the copepod based on the combined, interactive effect of temperature and cadmium pollution (Table 22). However, the concentration of cadmium at which 50 % mortality of the copepods occurred (LC_{50}) generally decreased with increasing temperature (Figure 4.22); it was highest (368.2 µg. l^{-1}) at a temperature of 28 °C, and decreased progressively when the temperature was raised by 1-3 °C.

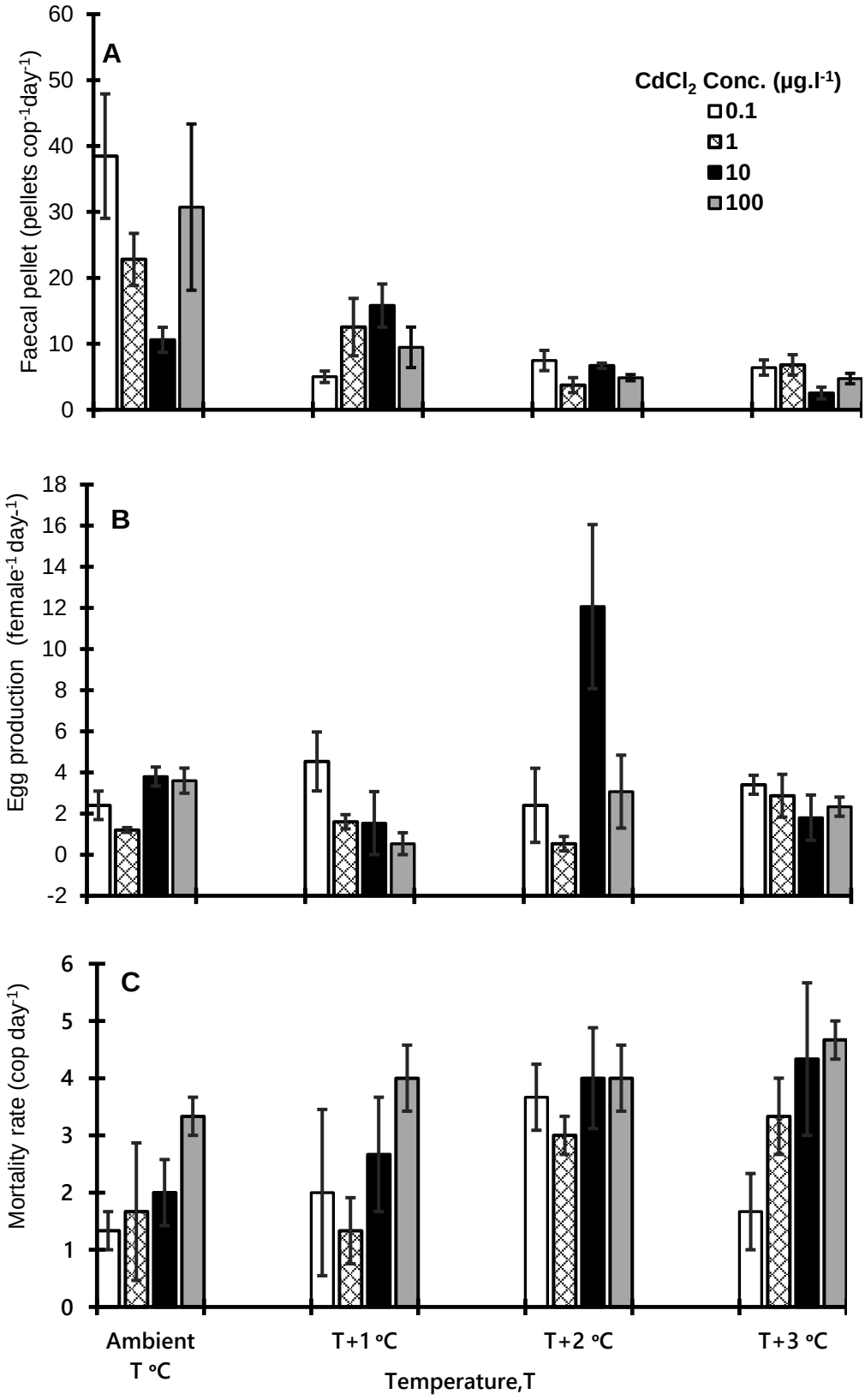

Figure 4.21: Combined effect of warming and Cd pollutant on the rates (mean ± SE) of faecal pellet production (A), egg production (B) and mortality (C) by *Temora stylifera.*

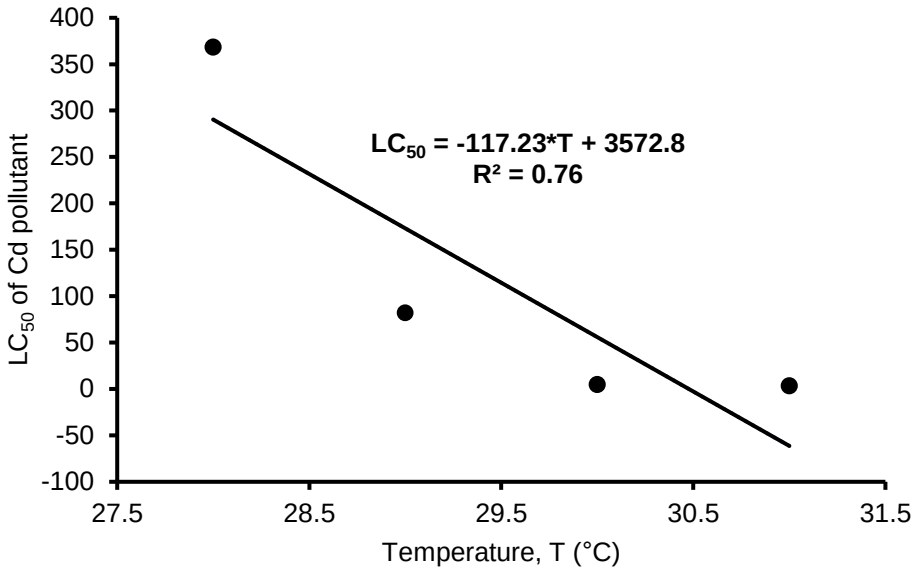

Figure 4.22: The effect of temperature on the lethal concentration of cadmium

Table 22: *Two-way ANOVAs Testing for the individual and combined effect of temperature and pyrene pollution on selected vital rates of Temora stylifera*

Selected vital rates	Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Faecal pellet production	Temperature	3	3.34	1.11	24.48	**0.000**
	$CdCl_2$	3	0.13	0.04	0.92	0.443
	Temperature*$CdCl_2$	9	1.39	0.15	3.4	**0.005**
	Error	32	1.45	0.05		
	Total	47	6.31			
Egg production rate	Temperature	3	0.24	0.079	1.56	0.217
	$CdCl_2$	3	0.41	0.14	2.7	0.062
	Temperature*$CdCl_2$	9	1.79	0.20	3.97	**0.002**
	Error	32	1.61	0.05		
	Total	47	4.04			
Mortality rate	Temperature	3	0.43	0.14	3.73	**0.021**
	$CdCl_2$	3	0.54	0.18	4.61	**0.009**
	Temperature*$CdCl_2$	9	0.22	0.02	0.63	0.766
	Error	32	1.24	0.04		
	Total	47	2.42			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts.

The combined effect of pyrene pollution and warming on the *Temora Stylifera* is shown in Figure 4.13. The combination of the two stressors had a significant effect on the rates of faecal pellets as well as egg production (see Table 23 for significant difference). At a pyrene concentration of 1 nM, the highest rate of faecal pellet production (26.57 ± 5.7 cop^{-1} day^{-1}) was observed when the experimental temperature was increased by 2 °C (Figure 4.23 A). This was approximately 60-87 % higher than the rates observed at all the other temperatures. The rate of egg production of the copepod covaried with changes in both temperature and the concentration of the Cadmium and pyrene pollutants. Generally, it was highest when both temperature and the concentration of the pollutants were low and *vice versa* (Figure 4.23 B). However, the effect of temperature was significant only at pyrene concentration of 10 nM (Tukey's post hoc test, $p < 0.05$). At this concentration, the highest rate of egg production was observed at the control temperature of 28 °C. The rate declined by ≈ 81 % when the experimental temperature was increased by 3 °C (Figure 4.23 B). The mortality rate of the copepods increased with increasing temperature (Figure 4.23 C). ANOVA examination of the results showed no significant changes ($p = 0.335$) in the total mortality of the copepod based on the combined, interactive effect of temperature and pyrene pollution (Table 23).

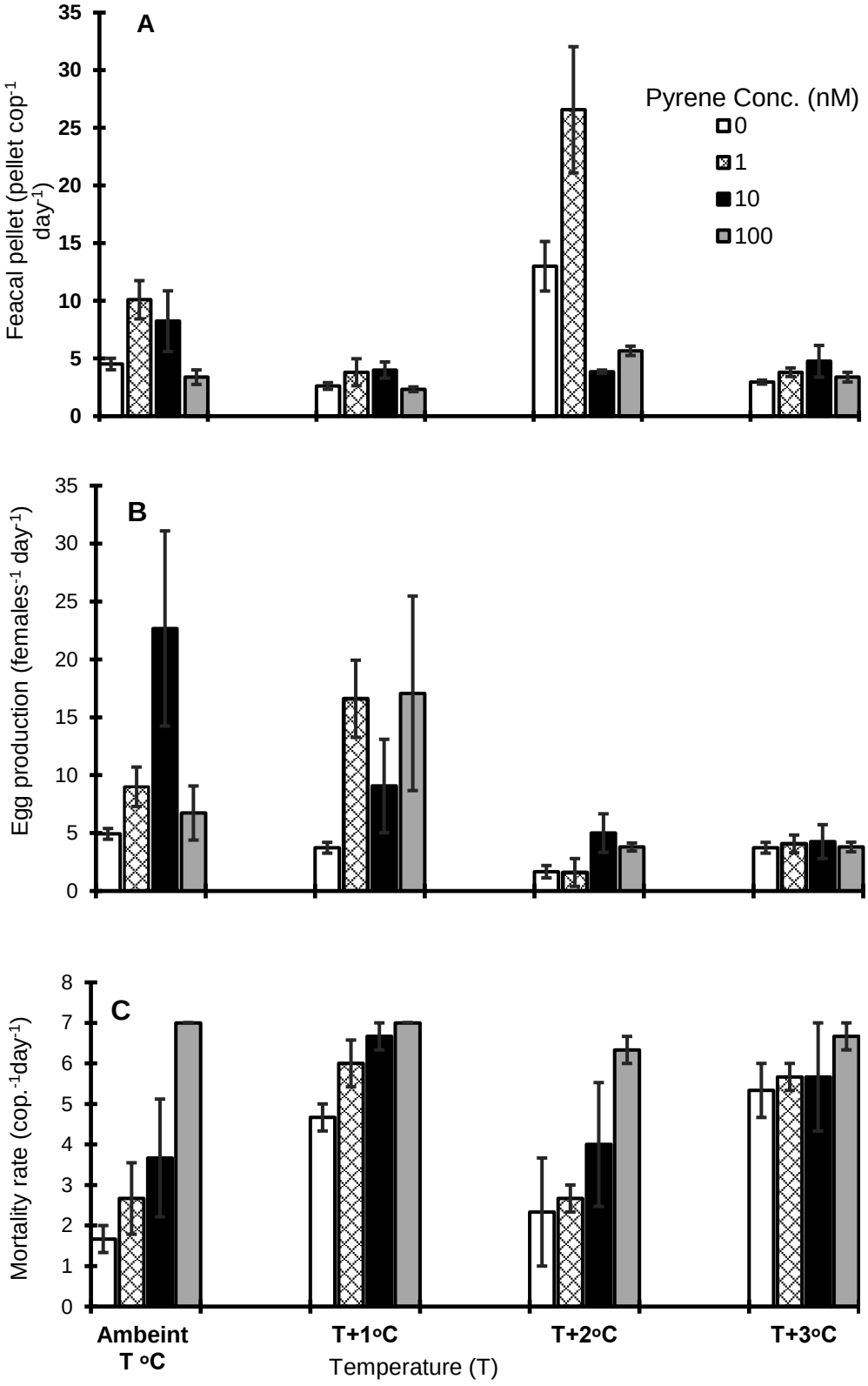

Figure 4.23: Combined effect of warming and pyrene pollutant on the rates (mean ± SE) of faecal pellet production (A), egg production (B) and mortality (C) *of Temora stylifera*

The concentration of cadmium at which 50 % mortality of the copepods by pyrene was observed (LC_{50}) increased linearly and significantly ($R^2 = 0.71$) with increasing temperature (Figure 4.24). Judging from the slope of the relationship, the increase can be said to have occurred at a rate of approximately 6 nM per every degree increase in temperature.

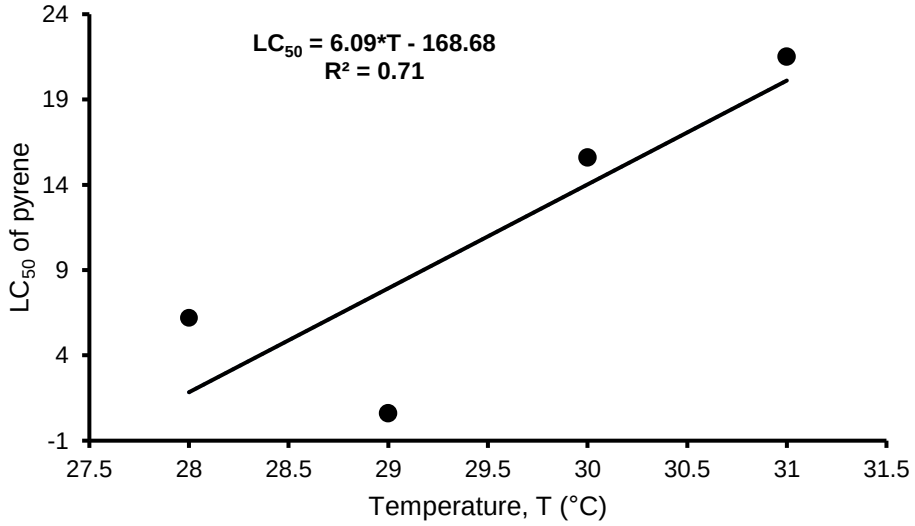

Figure 4.24: The effect of temperature on the lethal concentration of pyrene

Table 23: *Two-way ANOVAs Testing for the individual and combined effect of temperature and pyrene pollution on selected vital rates of Temora stylifera*

Selected vital rates	Source of variation	DF	Adj SS	Adj MS	F	P-Value
Faecal pellet production	Temperature	3	1.71	0.57	30.23	**0.000**
	Pyrene (nM)	3	0.72	0.24	12.67	**0.000**
	Temperature*Pyrene (nM)	9	1.05	0.12	6.18	**0.000**
	Error	32	0.60	0.019	6.18	
	Total	47	4.08		6.18	
Egg production rate	Temperature	3	18169	6056.2	6.97	**0.001**
	Pyrene(nM)	3	7072	2357.2	2.71	0.061
	Temperature*Pyrene (nM)	9	17435	1937.2	2.23	**0.046**
	Error	32	27807	869		
	Total	47	70482			
Mortality rate	Temperature	3	56.75	18.917	9.76	**0.000**
	Pyrene(nM)	3	69.75	23.25	12	**0.000**
	Temperature*Pyrene (nM)	9	20.75	2.306	1.19	0.335
	Error	32	62	1.938		
	Total	47	209.25			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

Figure 4.25 shows combined effect of cadmium and pyrene pollution on *Temora stylifera.* This effect was examined at an experimental control temperature of 28 °C. In comparison with effects observed when the pollutants were presented alone, the rate of faecal pellet production was reduced by 78 % when the copepod was exposed to cadmium and pyrene combined (Figure 4.25 A). Individually, the effects of the single pollutants were not significantly different when they were presented alone (Table 24). Egg production rate was also significantly higher by 75 % when the copepod was treated with pyrene alone compared with the observations made on cadmium and pyrene combined (Figure 4.25 B). In comparison with the single treatments, mortality rate of the copepods increased by ≈ 73 % when the animal was exposed to both pollutants (Figure 4. 25 C).

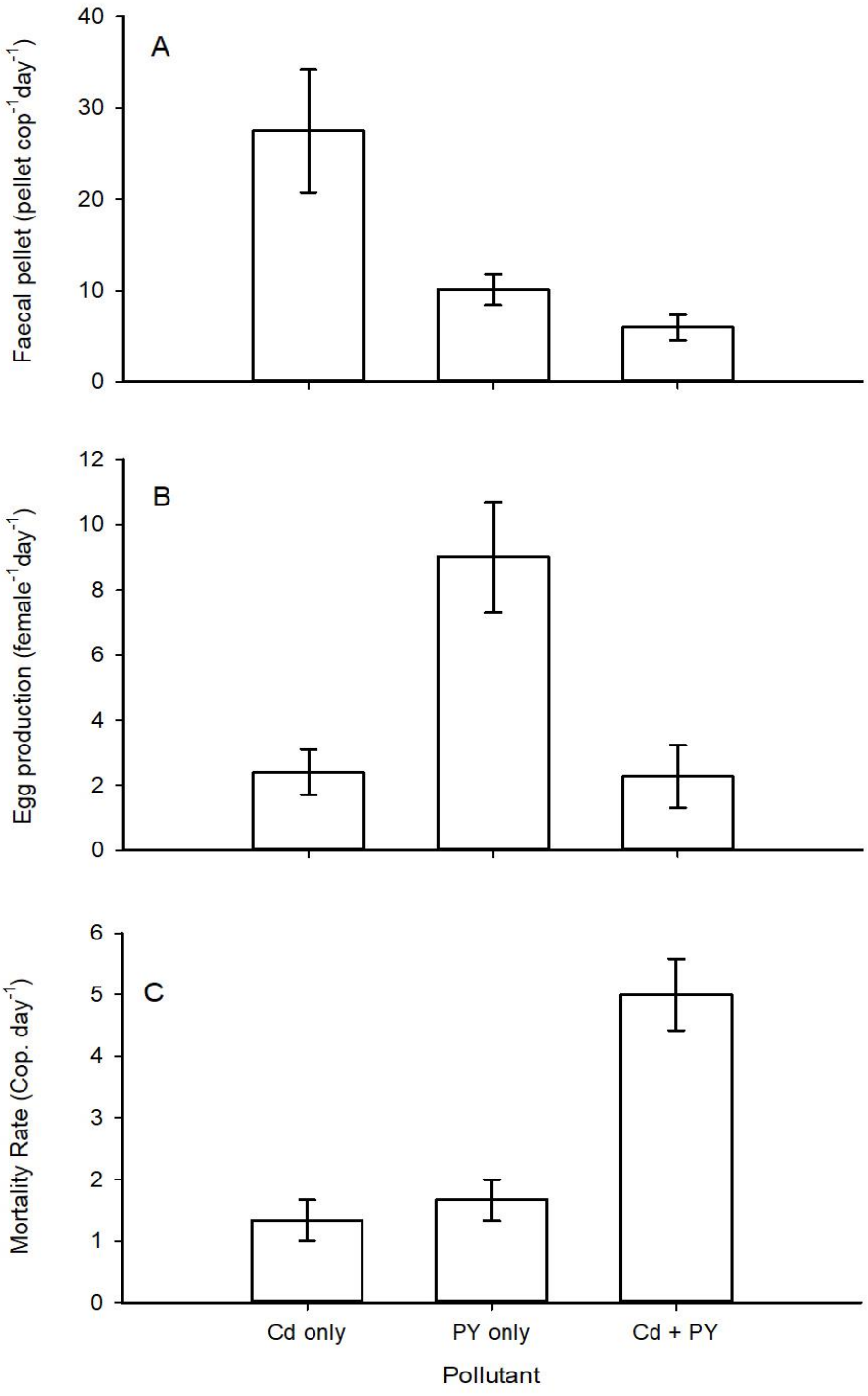

Figure 4.25: Combined effect of Cd and PY pollutant on the rates (mean ± SE) of faecal pellet production (A), egg production (B) and mortality (C) of *Temora stylifera* at 28 ° C.

Table 24: *ANOVA testing for the combined effect of Cd and PY on selected vital rates of Temora stylifera*

Selected vital rates	Source of variation	DF	Adj SS	Adj MS	F-Value	P-Value
Faecal pellet production	Pollutants	2	782.5	391.27	7.81	**0.021**
	Error	6	300.7	50.11		
	Total	8	1083.2			
Egg production rate	Pollutants	2	88.92	44.458	10.34	**0.011**
	Error	6	25.79	4.298		
	Total	8	114.7			
Mortality rate	Pollutants	2	20.667	10.333	8.45	**0.018**
	Error	6	7.333	1.222		
	Total	8	28			

Significant source of variation determined based on the post hoc test ($p < 0.05$) are shown in bold texts

CHAPTER FIVE

DISCUSSION

The focus of this study was to assess the dynamics of the plankton community within Ghana's coastal waters in relation to nutrient input, pollution (heavy metal and petroleum-based chemicals) and warming-induced by global climate change. This may be the first work to focus on the combined effect of the above global change factors on plankton in Ghana's coastal waters.

The planktonic community was characterized using phytoplankton production and mesozooplankton composition and abundance. Primary production was measured using relative fluorescence unit (RFU) in line with previous studies (Zeng, Zeng, Fischer, & Xu, 2017), and in relation to the concentration of nitrate and phosphate as these are essential for marine phytoplankton growth (Eker-Develi, Kideys, & Tugrul, 2006). The results are discussed in the following sections.

4.3 Field Observation on Nutrient and Plankton

Generally, in the ocean, the growth of phytoplankton is, in part, driven by the balance between nitrogen and phosphorus (Redfield, 1934). This is because nitrogen is a significant component of chlorophyll (essential for photosynthesis) and amino acids which are the building block of proteins. Phosphorus is essential because it helps convert other nutrients into usable building blocks for phytoplankton growth. In this study, the highest N:P ratio obtained was ≈ 141:1. This occurred on the Western Coast Transect in the mid-water column at station 3 (Figure 4.1). This is higher than the 92:1 observed by Nubi, Ajao, Oyewo, & Unyimadu, (2008) in the Nigerian coast of the Gulf of Guinea. Others working on the Ivorian coast (Arfi, Bouvy, & Menard, 2002)

have also reported relatively lower N:P ratios (50:1) than what was observed in this study. The higher ratios recorded in this study could be due to increasing human populations within the coastal areas of Ghana (Ukwe & Ibe, 2010). This increase could have led to increased organic matter pollution within the coastal marine waters of Ghana over the years. Spatial and temporal results recorded in the three coastal zones with respect to water depth, sampling stations, and duration of this study showed that the average N:P ratio was 36:1. This ratio is higher than the proposed ratio (16:1) needed for marine phytoplankton growth (Redfield, 1934), and could likely be attributed to the large inflows of nutrients from land sources (Gordon & Ibe, 2006). Over the years, the rate of organic pollution in coastal seas of Ghana has increased steadily due to over-population and industrial activities within the coastal areas (Ukwe & Ibe, 2010). Most of these organic pollutants end up in the coastal seas due to a lack of proper sewage systems (Entsua-Mensah, 2002). This may be the contributing factor to the large nutrient load observed within Ghana's coast during the present study.

In terms of the individual coastal zones, N:P ratios obtained were 9-21 % higher on the Western and Central Coast Transects than N:P ratio recorded on the Eastern Coast Transect (Figure 4.1 to 4.3). These spatial variations could be attributed to the nutrient inflows by major riverine estuaries along these coasts, including the Pra and Ankobra estuaries (Vowotor et al., 2014). This is because estuaries have long been recognized as the primary conduit for transfer of nutrients from land-based sources to coastal seas (Dame, 2008). Most riverine estuaries in Ghana are dotted along the Western and Central Coast of the country. This, in effect, increases the inflow of land-based nutrients into the coastal seas in those areas. In addition to the high number of riverine estuaries

along the coast, most industries are located on the Western and Central Coasts of Ghana and as a result, it is expected that coastal seas in these regions would be exposed to more pollutants than the coastal sea on the Eastern Coast (Personal observation). Both factors may contribute to the spatial variation in nutrients ratios obtained in the three coastal zones.

The foregoing difference in the N:P ratios were reflected by the phytoplankton concentrations in the study area (Figure 4.4-4.6). Similar to the N:P ratios, phytoplankton concentrations were highest on the Western Coast Transect and Central Coast Transect of the country (Figure 4.4-4.5; Table 8). Nutrients, especially nitrate and phosphate, are essential for phytoplankton growth (Xu, Paerl, Qin, Zhu, & Gao, 2010). This may have accounted for the spatial variations in phytoplankton concentration witnessed in this study. Hence it is expected that an increase in the N:P ratios would result in an increase in phytoplankton concentration. Also, there was a positive correlation in the phytoplankton concentration and N:P ratios obtained on the Western and Central Coast Transects. However, the correlation on the Eastern Coast Transect was negative (Figure 4.9). In consequence, there may be other factors such as solar irradiance (Piazena & Häder, 1994) and temperature controlling the growth of phytoplankton on the Eastern Coast Transect rather than the nutrient abundance.

Similar to the observations on phytoplankton concentration, copepod abundance was higher on the Western and Central Coast Transect (Figure 4.10-4.12). Also, there was a positive correlation between copepod abundance and phytoplankton concentration in all three coastal zones (Figure 4.13 A-C). This correlation between copepods abundance and phytoplankton concentrations is

widely reported in literature (e.g., Binet, Le Reste, & Diouf, 1995; Kowalczewska-madura, 2008; Vallina et al., 2014). This is mainly because copepods are the major consumers of phytoplankton in the ocean (Jones, Flynn, & Anderson, 2002; Richardson, 2008).

The copepod composition in this study was dominated by members of the Order Calanoida. They dominated all three coastal zones (Figure 4.15-4.17). Findings from this study are in agreement with other studies (Andrady et al., 2012; Bradford-Grieve, 2002; Wiafe and Freid, 2001). The dominance exhibited by the Calanoida may be due to their ability to escape predators due to their torpedo shape and presence of sensory organs, including antennule. Calanoid copepods also have the ability to reproduce sexually, helping them to delete mutations and promote good genes (Kiørboe, 2011). The least dominant order was the Harpacticoida (Figure 4.14) in tropical and subtropical marine waters. This can be attributed to their benthic nature, which makes them less dominant in the pelagic water column (Lee & Lee, 2019). This study revealed that copepod abundance is highly dependent on phytoplankton concentrations, which is also dependent on the nitrate to phosphate ratio in the water body.

4.4 Effect of Individual Stressors Alone on *Temora stylifera*

Primarily, temperature controls physiological processes of organisms, including calanoid copepods. It drives metabolic activities of these organisms (Abram et al., 2017; Alcaraz et al., 2014; Heinle, 1969). In this study, *Temora stylifera* was exposed to different levels of warming. The results indicated that warming has a significant effect on the rates of faecal pellet and egg production of the copepod (Figure 4.18 A&B). Literature search revealed no documented laboratory studies indicating the effect of warming on copepod faecal pellet

production in tropical waters. In the present study the nearly 30-49 % decrease in faecal pellet production rate with increasing experimental temperature (Figure 4.18 A) compares with other studies from temperate regions. These observations compare with studies from temperate regions. For example, studies by Carlotti et al. (1997) showed that the rate of faecal pellet production of *Centropages typicus* was higher at 15 °C than at 20 °C. Similar trends were reported in field observations by Kjellerup et al. (2012) and Smetacek (1980). Generally, faecal pellets are produced after the digestion and assimilation of ingested food items (Wotton 1994). Hence, it can be suggested that the rate of faecal pellet production by copepods is influenced by physiological processes related to food digestion and assimilation. Previous studies have shown that food ingestion by copepods, including *Temora stylifera* increases linearly with temperature (Dam & Peterson, 1988). Food digestion and assimilation, on the other hand, increases only to an optimum level and then decline with further increases in temperature due to temperature-related constraint on the reactivity of digestive enzymes (Alcaraz et al., 2014; Boscolo-Galazzo, Crichton, Barker, & Pearson, 2018). Therefore findings of the present study indicating a decline in faecal pellet production at higher temperatures (Figure 4.18 A) may be attributed to one of two reasons; (1) the copepods may not have ingested enough food for digestion or (2) the animals may have digested and over-assimilated most of the food ingested at higher temperatures. Such an event could limit the transfer of carbon from surface waters for burial in deeper part of the ocean, a process known as biological carbon pump that helps to moderate the amount of carbon dioxide in the atmosphere (Coppock et al., 2019; Steinberg & Landry 2017; Turner 2015). This is because faecal pellets produced by copepods

contribute significantly to the amount of organic matter that gets transferred from surface waters into the deeper ocean (Liszka, Manno, Stowasser, Robinson, & Tarling, 2019; Turner, 2015). Because of this, the effect of warming on faecal pellet production by copepods can limit the carbon sequestration process within marine ecosystems, and as a consequence, weaken the ability of the ocean to moderate global climate change.

It has also been demonstrated that increasing temperature generally increases energy demand for respiration and other metabolic processes (Acheampong et al., 2014; Pörtner & Farrell, 2008). As a result, the growth of organisms also increases only when increases in ambient temperature are within tolerable limits of organisms; it declines when temperature increases above the optimum levels required by organisms (Pörtner & Farrell, 2008). Specifically for marine copepods, experiments have shown that egg production increases with increasing temperature to an optimum level and then start decreasing when temperature exceeds the optimum level (Calbet & Agustí, 1999; Holste, St. John, & Peck, 2009; Lee, Ban, Ikeda, & Matsuishi, 2003). In line with these findings, the rates of egg production by the copepod in the present study decreased with the warming of the water (Figure 4.18 B). The approximately 88% decline in egg production when the temperature was increased by +1 and +2 °C above 28 °C (the average temperature of Ghana's coastal waters during the stable hydrographic period) was therefore in agreement with other studies. In addition, the recorded no egg production when the temperature was increased by +3 °C may have likely been because, at higher temperatures, organisms invest most of the energy they assimilate into metabolic maintenance to ensure their survival (Acheampong et al., 2014). This maintenance in metabolism

includes the need to satisfy energy demand for physiological processes such as thermoregulation (Bennett & Ruben, 1979), ion transport across membranes (Milligan & McBride, 1985) and protein or biomass turnover (Mente, Coutteau, Houlihan, Davidson, & Sorgeloos, 2002). It is only after the cost of maintenance has been met before the remaining substrates could be invested into egg production (Acheampong, Nielsen, Mitra, & St. John, 2012). The effect of warming on the rate of egg production observed in this study (Figure 4.18 B) suggests that it is likely for the growth of the copepod to be negatively impacted by the increase in sea surface temperature projected under global climate change (IPCC, 2018). Such an effect would directly result in reduced production of nauplii and their subsequent recruitment into the plankton population with effect on the marine food web as copepods acts as a direct link between primary producers (phytoplankton) and higher trophic organisms (e.g fish) in the Ocean.

In contrast to the observations on faecal pellet and egg production rates, the mortality of *Temora sp* did not significantly change with the different warming scenarios (Figure 4.18 C; Table 17). This result does not agree with observations from other studies on *Temora* species. For example, Holste et al. (2009) found that the mortality of *Temora longicornis* increases with increasing temperature. In the present study, no significant effect of warming on mortality occurred probably because the investigated warming scenarios (28 + 1 to 3 °C) fall close to the average range of sea surface temperatures (24 to 30 °C) reported for the waters from which the experimental copepods were collected. So, it is likely the copepods may have already adapted to survive at the higher temperatures simulated in this study.

With regard to pollution, no significant effect of cadmium on the rates of faecal pellet and egg production by *Temora stylifera* was found in the present study (Figure 4.19 A & B; Table 18). However, mortality rate increased significantly with increasing concentration of the heavy metal in the culture media (Figure 4.19 C), in agreement with observations on copepod communities including *Centropages ponticus, Temora longicornis* and *Acartia clausi* (Ensibi et al., 2017; Kuiper, 1981). Generally, these effects can arise through cadmium-induced oxidative damage during respiration (Ensibi et al., 2017; Moraitou Apostolopoulou & Verriopoulos 1979;) and morphological damages during hatching (Gentile et al., 1982; Moraïtou-Apostolopoulou & Verriopoulos, 1982 and references therein). The level of cadmium pollution simulated in this study (= 0.1-100 µg/l) is consistent with the concentration of the heavy metal (= 2-240 µg/l) in coastal marine waters of Ghana (Acquah 1988). On the bases of the results (Figure 4.19 A-C), it can be deduced that the introduction of the heavy metal into Ghana's coastal waters is already having negative impacts on the population of copepods within the country's marine ecosystem.

Pyrene is very likely to be found in most petroleum polluted waters (Gustavon, Tairova, Wegeberg, & Mosbech, 2016). The chemical impairs swimming, feeding, mating success and egg production of most aquatic animals (Bhattacharya, 1988; Cherif, Pringault, Hannaoui, & Yahia, 2015; Almeda, et al., 2016). It can also interfere with membranes and other cellular processes and lead to long-term damage to cellular structures (Neff, 1979). The findings of this study showed that increasing concentrations of pyrene significantly decreased faecal pellet production rate of *Temora Stylifera*. The 48 % decreaseobtained at the higher pyrene concentrations (Figure 4.20 A) was

similar to observations reported on the calanoid copepods such as *Calanus hyperboreus* (Nørregaard et al., 2014; Toxværd et al., 2019) and *Centropages stylifera* (Ruiz, 2019). The reason for this may be that higher concentrations of petroleum pollutants, including the pyrene used in this study, suppresses food ingestion (Almeda et al., 2016) and hence the amount of the ingested material that can be released as faecal pellet after digestion. It is, therefore, likely that pyrene as an aquatic pollutant interferes with the metabolic fates of food ingested by copepods. Such an effect may limit the availability of energy for the growth and survival of the organism. It is therefore not surprising that the mortality rate of the copepods was ≈ 75 % higher at the higher concentrations of the pyrene (Figure 4.20 C), similar to findings by Bellas & Thor (2007).

Pyrene, however, had no significant effect on the egg production rate of *Temora stylifera* in this study (Figure 4.20 B; Table 19). The current findings are in agreement with findings by Toxværd et al. (2019). This may be attributed to the observation that petroleum pollutants, including pyrene impair feeding (Van Dinh, Olsen, Altin, Vismann, & Nielsen, 2019 and reference therein), thus limiting the availability of food for use in growth. Furthermore, pyrene may suppress metabolic activity of the copepod as observed in other animals exposed to petroleum pollutants (Bayne et al., 1982). This limitation of pyrene on metabolic activity may explain observation in the present study indicating no significant effect of pyrene on egg production rate (Figure 4.20 B).

4.5 Combined Effect of Multiple Stressors on *Temora stylifera*

As established by many studies (e.g., Crain et al., 2008; Griffen et al., 2016), organisms are exposed to many environmental hazards at the same time. The combination of these factors has consequences that are not fully understood

especially in tropical systems (Griffen et al., 2016). The results of the present research showed that combined effect of warming and cadmium pollution leads to decreases in the rates of both faecal pellet and egg production (Figure 4.21 A & B). Even at the lowest concentration of cadmium (= 0.1 µg. l^{-1}), there was a 50-60 % decline in the rate of faecal pellet production when ambient temperature was increased by just 1 °C. The decline in the rate of egg production, on the other hand, was significant (by ≈ 83 %) at higher levels (=10 µg. l^{-1}) of the pollutant when the temperature was increased by 3 °C. This could be attributed to the findings that temperature increases the toxicity of heavy metals (Kim, Lee, & Lee, 2012; Noyes et al., 2009). This toxicity, among others, results in oxidative stress and accumulation of lipid peroxidation products (Sokolova and Lannig 2008), impairment of mitochondrial functions (Cherka sov et al., 2006; Sokolova 2004), damages to lysosomal system (Izagirre et al., 2014) and deoxyribonucleic acid (DNA) function (Kim et al., 2012), within aquatic organisms. It may, therefore, explain the reduced rates of faecal pellet and egg production by copepods exposed to both warming and cadmium pollution in the present study (Figure 4. 21 A & B).

The lethal concentration (LC_{50}) of cadmium at which 50 % mortality of the cultured copepods occurred in this study generally decreased with increasing temperature (Figure 4.22). The highest LC_{50} estimated for cadmium was 368.2 µg. l^{-1} which occurred at a temperature of 28 °C, corresponding to the average temperature reported for Ghana's marine coastal waters during the stable hydrographic period (GMA, 2016). This lethal concentration decreased linearly with increasing warming (+1, 2 and 3 °C) of the water (Figure 4.22, graph of LC 50). In light of these findings, it is likely that the warming of the

ocean as a result of global climate change (IPCC, 2018) may intensify the toxic effect of cadmium pollutants on marine copepods. This effect could cascade through the marine food web since copepods serve as a direct link between primary producers such as phytoplankton and higher trophic organisms such as fish. The results of this study (Figure 4.22) suggest that the toxicity of cadmium is likely to be more intense at higher temperatures, even when the concentration of the pollutant is low.

Considering the combined effect of warning and pyrene pollution, egg and faecal pellet production rates of the copepod were generally decreased when temperature was higher and *vice versa* (Figure 4.23). The effect was significant only at the lowest pyrene concentration (1 nM; Tukey's post hoc test, $p < 0.05$). At this concentration, the rate of faecal pellet production (26.57 ± 5.7 cop^{-1} day $^{-1}$) was 60-87 % higher than observations made at other experimental temperatures. Generally, the production of faecal pellets occurs only after the digestion and assimilation of ingested food items (Wotton, 1994). Hence, the extremely high rate of faecal pellet production by the copepods even when they were exposed to low levels of the pollutant at higher temperatures may be attributed to the amount of food that was likely ingested for digestion by the copepod. Previous studies (Hjorth & Gissel, 2011; Jensen, Nielsen, & Dahllöf, 2008) suggest that this process of food ingestion and digestion is limited by petroleum pollutants, including the pyrene used in this study. Under such a situation, it is expected that the growth of the copepod would also decrease since feeding limitation induced by petroleum pollutants is likely to also limit the amount of substrate that can be used for new biomass production. However, the growth of the copepod in the present study as measured using the rate of egg

production was relatively higher at lower levels of the pollutant irrespective of the temperature investigated (Figure 4.23 B). Indeed, there was 81 % decline in egg production rate when pyrene concentration was 10 nM. So, it is likely the copepod in the present study ingested more food for processing at lower levels of the pyrene at all the warming scenarios. These may explain why egg production rate of the copepod was higher at the lower levels of the pollutant in all the warming scenarios (Figure 4.23 B). In contrast, food ingestion for processing is likely to be limited at higher levels of the petroleum pollutant as indicated in previous studies (Hjorth & Gissel, 2011; Jensen et al., 2008). Hence, and as shown in Figure 4.23 B, the growth of the copepod may be limited when the concentration of pyrene is high, irrespective of the water temperature. Alternatively, higher energetic cost of metabolism at higher temperatures (Acheampong et al., 2014) may also worsen the limiting effect of the pollutant on the copepod. Such a limitation may force the copepod to invest more energy in maintenance rather than egg production (Acheampong, 2012). Indeed findings from previous studies suggest that copepods can decrease their retention of ingested petroleum hydrocarbons by as much as 50 % in some cases when ambient temperature is high (Connell & Miller, 1980; Harris, Berdugo, Corner, Kilvington, & O'Hara, 1977). This occurs as a result of increased metabolic activities. This could likely be the reason why the LC_{50} estimated for pyrene in the present study increased with increasing temperature (Figure 4.24), suggesting that the toxicity of the pollutant is likely to be low at higher and high at lower temperatures. Findings from the present study contradict the pattern of response reported by Ruiz (2019) on *Centropages velificatus* exposed to similar warming scenarios and pyrene pollution as used in the present study.

Regarding the combined effect of cadmium and pyrene, the rates of faecal pellet and egg production declined significantly when the two pollutants were combined in comparison with effects observed when the pollutants were presented alone (Figure 4.25 A&B). The mortality rate of the copepod also increased (Figure 4.25 C), suggesting a reduction in the survival of the copepod when exposed to cadmium and pyrene combined.

These findings are in agreement with observations made by Fleeger, Gust, Marlborough, & Tita (2007). They found that the interaction between cadmium and another polycyclic aromatic hydrocarbon (phenanthrene) was 2.8 times more toxic on copepods than when the chemicals act individually. Findings from the present study could be attributed to reasons stated by Gauthier et al. (2014). They made it known that the bioavailability of heavy metals in aquatic environments increases in the presence of pyrene pollutants. This is because pyrene and other hydrocarbons have the potential to alter the membrane fluidity of most aquatic animals. This effect exposes vital organs to increasing bioaccumulation of heavy metals. In addition, pyrene is capable of forming a complex with heavy metals in aquatic environments, thus increasing the uptake of metals from the water (Fleeger et al., 2007). This effect also increases bioavailability of heavy metals and, as a result, intensifies the toxicity of the metals. This could be the underlying reason for the synergistic effect of cadmium and pyrene pollution observed on the copepods in this study (Figure 4.25 A-C).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The main aim of the study was to identify the spatio-temporal dynamics of copepod communities and their supporting environments in coastal seas of Ghana. Furthermore, the combined impact of sea surface warming and pollution on selected vital rates (faecal pellet production, growth via egg production, and mortality) of *Temora stylifera*, which is the most abundant calanoid copepod in Ghana's coastal waters was determined. The study provides critical information for predicting future changes in copepod community structure. It also provides vital information for the development of useful parameters for modelling the dynamics of marine food webs under global climate change. The study showed that nutrient ratio, phytoplankton concentration and copepod abundance were highest within the Western and Central Coasts of Ghana compared to the Eastern Coast of Ghana. Also, the most abundant copepod encountered belonged to the Order Calanoida and Cyclopoida. Microcosm experiments showed that combinations of stressors (sea surface warming, cadmium and pyrene pollutant) had a synergistic impact on the selected vital rates of *Temora stylifera*.

6.2 Conclusions

The following conclusions were made in the present study:

In general, nitrate:phosphate ratios of the coastal seas of Ghana was ≈ 2 times the optimum Redfield ratio (16:1) for phytoplankton growth. Therefore,

there was probably no inorganic nutrient limitation within Ghana's nearshore waters during the time of this study.

There was a positive correlation between nutrient ratios and phytoplankton concentration as well as phytoplankton concentration and copepod abundance in coastal marine waters of Ghana. Consequently, nutrient concentration, phytoplankton concentration and Copepod abundance are higher in the Western and Central Coasts than in the Eastern Coast of Ghana.

Copepod composition in coastal seas of Ghana is dominated by copepods belonging to the Order Calanoida. The least dominant copepod Order is the Harpacticoida.

The biology of *Temora stylifera* is affected by sea surface warming and heavy metal (cadmium) and petroleum(pyrene) pollutants.

Increasing sea surface temperature and cadmium pollutants combine to affect the biology of calanoid copepods synergistically.

Sea surface warming combines with pyrene pollutants to synergistically affect some aspects of the biology of *Temora stylifera*. However, its impact on mortality rate is antagonistic.

Petroleum and heavy metal pollutants become more toxic to *Temora stylifera* when they occur together in marine ecosystems.

6.3 Recommendation

Further studies are recommended to:

1. assess the chlorophyll-a concentration of the phytoplankton occurring in the coastal zones of Ghana.
2. measure physico-chemical parameters to support spatio temporal variations of copepods

3. identify copepod taxonomic groups in coastal seas of Ghana to the highest (species) level.
4. ascertain the combined effect of warming, heavy metals and petroleum pollution across the different trophic levels in the marine food web.

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