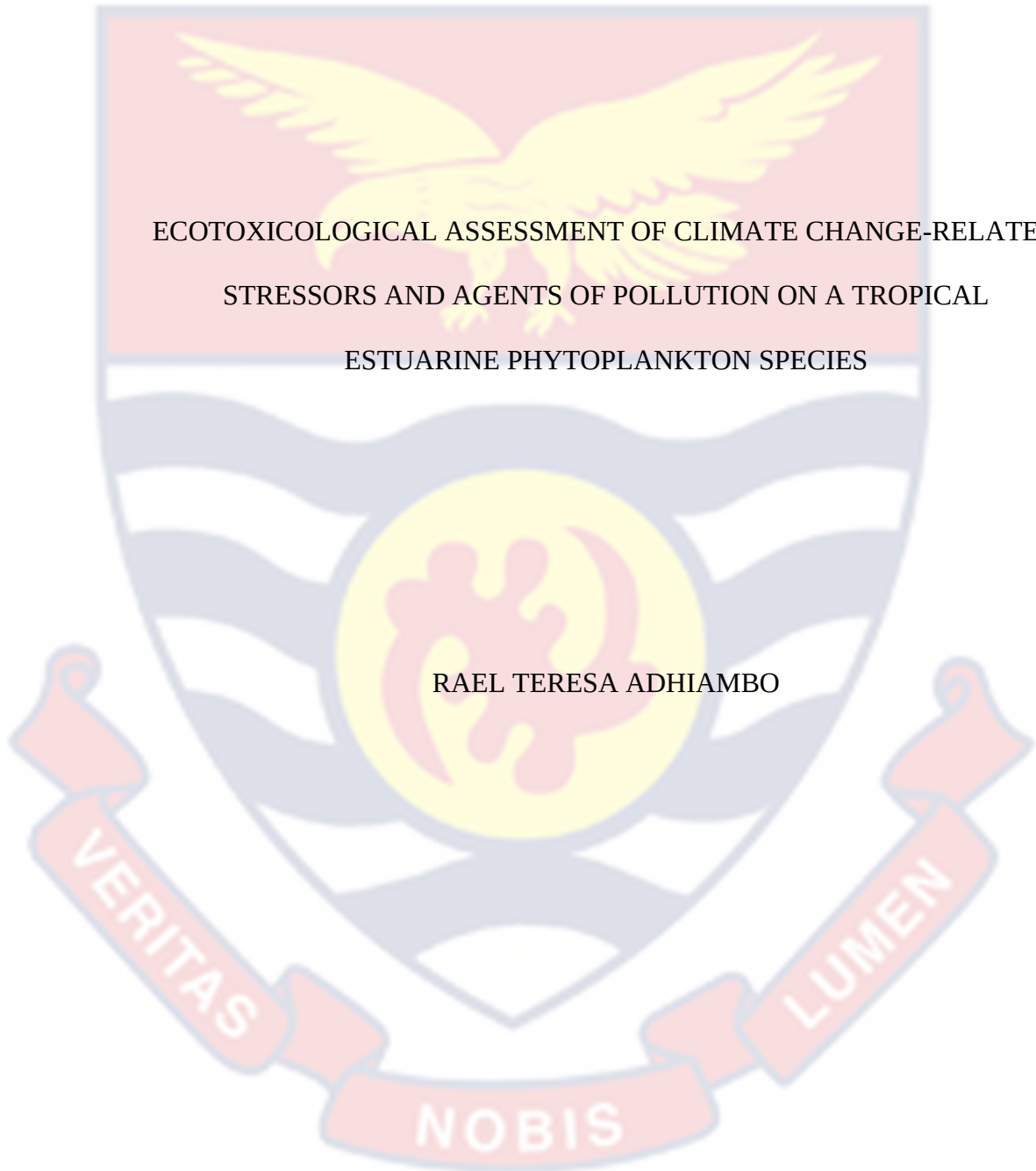


UNIVERSITY OF CAPE COAST

ECOTOXICOLOGICAL ASSESSMENT OF CLIMATE CHANGE-RELATED
STRESSORS AND AGENTS OF POLLUTION ON A TROPICAL
ESTUARINE PHYTOPLANKTON SPECIES

RAEL TERESA ADHIAMBO



2024



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The background of the page features a large, faint watermark of the University of Cape Coast crest. The crest is a shield with a red top section containing a yellow eagle with spread wings. The middle section is white with blue wavy lines. The bottom section is blue with a yellow circle containing a red stylized figure. A red banner at the bottom of the shield contains the word 'VERITAS' in white. A blue banner at the bottom of the shield contains the word 'NOBIS' in white. The text of the thesis title is centered over the red top section of the crest.

ECOTOXICOLOGICAL ASSESSMENT OF CLIMATE CHANGE-RELATED
STRESSORS AND AGENTS OF POLLUTION ON A TROPICAL
ESTUARINE PHYTOPLANKTON SPECIES

BY

RAEL TERESA ADHIAMBO

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 Doctor of Philosophy degree in Oceanography and Limnology

AUGUST 2024

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 19.8.2024

Name: Rael Teresa Adhiambo

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 19.8.2024

Name: Dr. Paul Kojo Mensah

Co-Supervisor's Signature



Date 19.8.2024

Name: Dr. Emmanuel Acheampong

ABSTRACT

This study assessed the ecotoxicological effects of climate change-related stressors (temperature, salinity, and pH) and agents of pollution (cadmium and pyrene) on selected estuarine phytoplankton adapted to the Whin Estuary in Ghana. Field investigations were first conducted to assess the abiotic environmental parameters and phytoplankton abundance, composition, and distribution within the Estuary. Diatoms, particularly from the genera *Thalassiosira*, *Navicula* and *Nitzschia*, were the most abundant phytoplankton in the selected estuary. As a result, *Thalassiosira weissflogii* cultures were isolated, acclimatised and used for ecotoxicological assessments on the impacts of change- and pollution-related stressors, and the impacts on their functional (growth rate, chlorophyll-*a* content, and biomass) and molecular attributes. The impacts on functional attribute results showed that lower salinity and pH have the greatest impact on *T. weissflogii* growth rate, chlorophyll-*a*, and biomass concentration. For instance, salinities of 1 and 5‰ resulted in more than 75% decrease in cell growth, while exposure to higher levels of combined pyrene and cadmium reduced the growth rate by more than 30%. The genotoxicity analysis revealed that exposure to high levels of pollution stressors significantly altered the molecular setup of *T. weissflogii* as portrayed by the high number of differently expressed genes (DEGs), particularly in the temperature, cadmium and pyrene treatments. This study has provided evidence on impacts of climate change-related stressors and pollution agents on *T. weissflogii* at the species and molecular genetics levels. The outcome could be critical for modelling the impacts of climate change and pollution on coastal ecosystems, particularly within the tropics.

KEY WORDS

Climate change

Differently Expressed Genes

Ecotoxicology

Gulf of Guinea

Microcosm

Thalassiosira weissflogii



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DEDICATION

To my beloved family,

Dad, Mum and Flo



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LIST OF ACRONYMS

ACECoR	Africa Centre of Excellence in Coastal Resilience
ANOVA	Analysis of Variance
Cd	Cadmium
chl _a ,	Chlorophyll- <i>a</i>
DTU-Aqua	National Institute of Aquatic Resources-Danish Technical University
EA	Estuarine Acidification
HAB	Harmful Algal Blooms
IPCC	Intergovernmental Panel on Climate Change
OA	Ocean Acidification
PAHs	Polycyclic Aromatic Hydrocarbons
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
UCC	University of Cape Coast

CHAPTER ONE

INTRODUCTION

Ghana is endowed with a vast and diverse coastline spanning approximately 560 km, and is endowed with many coastal ecosystems and resources, including 90 lagoons and 10 estuaries (Yankson & Obodai, 2007). These ecosystems play an important role in sustaining a vibrant marine fishing industry that supports the livelihoods of more than 2 million people (Ayoubi & Failler, 2012). The diverse coastal ecosystems also contribute to marine productivity by supporting the production and transportation of nutrients and organic matter to neighbouring marine ecosystems. Additionally, they serve as spawning and breeding grounds for many commercially exploited fish (Okyere, 2019). At the same time, coastal ecosystems within the Gulf of Guinea have experienced tremendous changes in their structure and functioning because of anthropogenic stressors such as pollution from organic and inorganic sources (Scheren et al., 2002), climate change impacts such as warming and erosion, flooding by sea-level rise (Sylla et al. 2016; (Appeaning Addo et al., 2018; Jonah et al., 2016), and habitat destruction (Ukwe et al., 2003). As a result, these impacts have in one way or another altered ecosystem functioning and biodiversity in Ghana's coastal areas (Okyere, 2019).

Generally, in the tropics, and particularly in Ghana, studies to understand the response of coastal biodiversity to anthropogenic stressors are mostly focused on fish and fisheries resources (Abdul et al., 2019; CRC/FoN, 2010; Lalèyè et al., 2007; Okyere 2019). On the other hand, very few studies have been undertaken on pelagic micro-organisms such as phytoplankton. This low attention to

phytoplankton research particularly within the tropics may be attributed to existing biases i.e., taxonomic biases related to the choice of study organisms, where organisms from a particular taxonomic group are disproportionately targeted for research (Culumber et al., 2019). These biases have been reported widely in ecological fields relating to research on invasive organisms (Burian, 1993), biodiversity conservation (Donaldson et al., 2017), and community assessment (Kovalenko & Reavie, 2022) and are more prevalent in areas with limited human capacities and taxonomic expertise and research funding opportunities (Carneiro et al., 2008; Santos et al., 2020; Tyree et al., 2020). Therefore, it's possible that tropical nations are often forced to focus their research efforts on fields like biomedical science, agriculture, and fisheries because these fields have a direct bearing on socioeconomic development. On the other hand, phytoplankton may not receive much attention in nations with less research opportunities due to their small size and indirect relationship to humans through intricate trophic relationships and other indicators, (Reid et al., 2019).

1.1 Background of the Study

Estuaries provide areas of intersection between freshwater and marine ecosystems. They comprise some of the most biologically productive ecosystems on earth (Sheaves et al., 2015). They also harbour several ecologically and economically viable organisms and perform several ecosystem services that support livelihoods. Estuaries also play a crucial role in the biogeochemical cycling of chemical elements by filtering out pollutants from rivers (Lancelot & Muylaert, 2011). Despite these benefits, estuarine ecosystems are facing tremendous pressure

from anthropogenic activities such as damming, over exploitation of species (particularly those that are of commercial importance), pollution from organic and inorganic contaminants as well as the introduction of alien invasive species (Miththapala, 2013). Other negative impacts associated with anthropogenic activities on estuaries include increased harmful algal blooms (HAB), oxygen depletion due to increased nutrient enrichment from rivers, degradation, habitat loss, and changes in freshwater flow into estuaries (Miththapala, 2013). The cumulative impacts of these changes on estuarine ecosystems is the alteration of their environmental parameters, habitats and community structures, and ultimately the disruption of their ability to provide ecosystem services that are critical in sustaining coastal livelihoods (Elliott & Whitfield, 2011). For instance, some of the challenges associated with HABs in estuaries include the losses of fish and shellfish production, biodiversity and trophic structure, tourism and recreation services (Adams & Greeley, 2000). Therefore, understanding anthropogenic based threats and their impacts to estuaries provides a basis for their effective management.

Climate change and pollution are two of the most important sources of stressors in many estuarine ecosystems. The various sources of pollution in coastal ecosystems include open defaecation, mining activities, industrial activities and wastes from households. As a result of these activities, different kinds of contaminants such as microplastics, nutrient pollutants, heavy metals, polycyclic aromatic hydrocarbons and pesticides, find their way into coastal water bodies and deposit themselves in the sediments or accumulate in the tissues of organisms (Faseyi, Miyittah, Yafetto, et al., 2022; Miththapala, 2013). On the other hand,

climate change represents one of the most important causes of anthropogenic-led global environmental change because of the increased emission of greenhouse gases (Sampaio & Rosa, 2020).

There are five critical global environmental changes associated with climate change i.e., increasing atmospheric and water temperatures, alterations in the hydrological cycles, sea-level rise, melting of the icepacks and declining glaciers and ocean acidification (IPCC, 2021, 2022). The Intergovernmental Panel on Climate Change (IPCC) report (2021) has shown that the world has been warming gradually over the last century as a result of increased greenhouse gases emissions. It is estimated that the rate of temperature increase has doubled within the last 50 years (Emori et al., 2016). The continued warming has led to disproportionate and catastrophic effects on the world's ecosystems, their structures and functioning, particularly those adapted to narrow ranges of climatic conditions (Gattuso et al., 2015).

Estuaries are known to be highly dynamic systems that are constantly adapting to external pressures. However, just like other ecosystems, global change factors such as pollution and climate change will impact these ecosystems beyond their abilities to respond and adapt. Global change factors for instance will alter river flow dynamics and marine environments (IPCC, 2021; Sampaio & Rosa, 2020; Vu, 2018). This will eventually affect coastal ecosystems, disrupt their structures and functions and alter their capacities to provide ecosystem services that offer critical sources of livelihoods to many communities around the world (James et al., 2013). For instance, Havens and Steinman (2015) projects that future

estuaries will become more saline due to factors such as prolonged drought, reduced inputs from freshwater inflows and sea-level rise (which will cause salty ocean waters to move into estuaries). In addition, climate change-induced temperature changes on estuaries will be mainly influenced by both stream and sea surface temperatures (SST) as well as the actions of wind (Havens & Steinman, 2015; Robins et al., 2016). Further, anthropogenic activities such as the destruction of the riparian vegetations through large-scale agriculture and deforestation will also result in stream temperature increases (Groom et al., 2011).

The combined action of climate change and pollution also impacts on estuarine pH, an important environmental parameter influencing biological activity in aquatic environments (Abdul et al. 2018). Climate change will influence pH of estuaries through the combined action of sea-level rise and increasing ocean acidification. On the other hand, pollution will facilitate acidification conditions such as the increase of carbon dioxide (CO₂) in the atmosphere (IPCC, 2021). Also, climate change will increase the accumulation and toxicity of pollutants such as metals and polycyclic aromatic hydrocarbons (PAHs) through changes in salinity, temperature, acidity, precipitation, river flow and increased extreme weather events such as storms, waves, currents, and tides in coastal areas (Fischer et al., 2014; Pontes et al., 2021). This complex relationship between climate change and pollution necessitates studies that attempt to quantify their interactions and productivity on coastal ecosystems, a gap that the current study has provided contribution to fill.

Climate change and pollution are not only global challenges, but present realities for many areas in sub-Saharan Africa (IPCC, 2021; Rose, 2015). Africa's vulnerability to global change factors is due to its overreliance on nature and ecological systems (Rose, 2015). In addition, other challenges such as poor financial, technical, and institutional capacities cripple the continent's ability to mitigate or adapt quickly to emerging global change problems such as climate change and pollution (Rose, 2015; Scheren et al., 2002). For instance, Climate change will have long term effects on precipitation patterns in sub-Saharan Africa, which will in turn affect agriculture, food production, food security and nutrition, as well as reduction in fish stocks, water security and sovereignty (Gemed & Sima, 2015).

In Ghana, pollution and climate change pose the greatest threats to coastal areas (Appeaning Addo et al., 2018; Addo and Adeyemi, 2013; Faseyi et al., 2022; Okyere, 2019). Increased coastal flooding and inundation, changes in precipitation patterns, sea-level rise and increasing temperatures comprise some of the impacts of climate change on coastal ecosystems in Ghana (Ankrah, 2018). In addition, climate change has also been associated with increased flooding and coastal inundation, saltwater intrusion into coastal ecosystems such as lagoons and estuaries and erosion. For instance, the Ministry of Environment, Science, Technology, and Innovation projected a sea-level rise of 345 mm by 2080 in the country (EPA, 2011). This sea-level rise, combined with the expected rise in temperature, will have significant effects on ecological and socio-economic systems. At the same time, estuarine ecosystems in the country such as the Volta,

Pra and Ankobra are also undergoing significant threats from over exploitation of their resources, siltation, and pollution (Okyere & Nortey, 2018). Illegal mining activities (also known as *galamsey*) and associated impacts on aquatic ecosystems have been extensively studied, especially in the Pra Estuary and Ankobra Estuary (Faseyi, Miyittah, Yafetto, et al., 2022; Okyere, 2019). The main challenges associated with *galamsey* is the release of sediments and heavy metals into aquatic ecosystems that increase the turbidity of coastal ecosystems and pose significant risks of heavy metal bioaccumulation in fish and other organisms. Yet, research on estuarine ecosystems in Ghana has focused on single aspects of environmental change. Researchers working on Ghana's coastal ecosystems have assessed the individual impacts of pollution, climate change, habitat destruction, over-fishing, or shoreline erosion (Addo and Adeyemi, 2013; 2013; Faseyi et al., 2022; Okyere and Nortey, 2018). Yet, few studies globally, within the region or in Ghana have assessed the combined impacts of environmental change on estuaries, their structure or functioning.

Phytoplankton form the basis of coastal and marine food webs and support critical biogeochemical and ecosystem services in coastal and marine ecosystems (Abdul et al. 2018; Lancelot and Muylaert 2011). It is estimated that phytoplankton alone account for nearly 50% of the global primary production. In marine ecosystems, phytoplankton primary production represent the total flux of organic carbon (energy) that these ecosystems can utilise for their metabolic processes (Falkowski and Raven 2013; Falkowski 2012). In addition, plankton also play a significant role in the biogeochemical cycling of important elements such as carbon

(C), nitrogen (N), phosphorus (P), iron (Fe) and silicon (Si) (Bonachela et al., 2016). They use these elements to carry out their metabolic processes and ultimately reintroduce them back to the environment or lock them out of circulation when their bodies or wastes sink into sediments. In addition, research has shown that environmental factors such as nutrient availability, light regimes, temperature, alkalinity, and pH greatly affect the diversity, growth, and development of plankton communities in these aquatic environments (Abdul et al. 2018; Falkowski 2012). Because of their structure and functioning, phytoplankton are often good bio-indicators of anthropogenic disturb in aquatic environments, including the impacts of climate change and pollution (Boyce, Lewis, and Worm 2010; Abdul et al. 2018; Lancelot and Muylaert 2011; Carneiro, Nabout, and Bini 2008).

1.2 Statement of the Problem

Natural systems are currently faced with multiple environmental stressors (pollution, climate change, etc) that act cumulatively to impacts on ecosystem structure, and function (Halpern, et al., 2008). Many studies have documented impacts related to individuals of these stressors (Acheampong et al., 2021; Barnes & Wurtsbaugh, 2015; Collins et al., 2014; Garcia et al., 2012), with only few studies focusing on the effects of combined stressors on natural systems (Chakraborty et al., 2011; Hernández Ruiz et al., 2021; Hindarti & Larasati, 2019). These studies show that multiple environmental stressors can act together synergistically (when the cumulative effects of combined stressors is greater than the additive sum of the effects produced by the stressors acting in isolation), and antagonistically (when the cumulative effects of combined stressors is less than the additive sum of the

effects produced by the stressors acting in isolation), even when present at the same concentration (Crain et al., 2008). This understanding is mainly derived from research on organisms adapted to high latitude (polar and temperate) areas. For instance, a study carried out Adhiambo et al., (2023) shows that approximately 80% of all the global research on phytoplankton related to climate change impacts undertaken between 2012 -2022 was carried either carried out on temperate and polar regions, or under conditions similar to these found in these regions. Oppositely, very few studies in comparison were undertaken in the tropics, and particularly African countries within the tropics. This dismal investment in phytoplankton research within the tropics is alarming, particularly when it comes to modelling the impacts of climate change on global primary production (Kwiatkowski et al., 2017). For instance, studies have shown that current standards for assessing global primary production rely heavily on extrapolations from temperate data (Flombaum et al., 2020; Kwiatkowski et al., 2017; Van De Waal & Litchman, 2020), which in turn introduce significant ($\approx 20\%$) uncertainties in model predictions (Kwiatkowski et al., 2017). To tackle this challenge, this study employed multidisciplinary approaches to evaluate the impacts of climate change and pollution on a cosmopolitan diatom that was adapted to tropical conditions.

1.3 Purpose of the Study

The present study was aimed at providing baseline data on phytoplankton communities in the Whin Estuary located in the Western Region of Ghana. This research was mainly necessitated by the lack of data on phytoplankton in the country and the region at large (Adhiambo et al., 2023; Adhiambo et al., 2024).

Research on coastal and marine areas in the country, have primarily been focused on assessing fish and fisheries resources. Oppositely, very few studies have focused on plankton, which form the basis of food chains in these ecosystems (Adhiambo et al., 2023; Adhiambo et al., 2024). The data generated from this study provides an in-depth understanding of the link between pollution and climate change and how this affects coastal ecosystems, food webs and nutrient cycling and can be used in the development of species-specific parameters for modelling the dynamics of coastal food webs under global change.

1.4 Research Objectives

The main objective of the study was to assess the individual and combined impacts of climate change and pollution on estuarine phytoplankton communities based on species adapted to Whin Estuary.

The specific objectives of the study were to:

- i. investigate the abundance, distribution and composition of phytoplankton communities in the Whin Estuary.
- ii. quantify the individual effects of climate change (temperature, salinity and pH) and pollution (cadmium and pyrene) stressors on the functional attributes of *Thalassiosira weissflogii*.
- iii. evaluate the combined effects of climate change (temperature, salinity and pH) and pollution (cadmium and pyrene) stressors on the functional attributes of *Thalassiosira weissflogii*.

- iv. Assess the genotoxicity of combined impacts of climate change (temperature, salinity and pH) and pollution (cadmium and pyrene) stressors on *Thalassiosira weissflogii*.

1.5 Research Hypotheses

The following hypotheses were derived for the research:

- i. Phytoplankton abundance and distribution in the Whin Estuary gradient was dependent on existing physiochemical parameters
- ii. Climate change and pollution stressors reduced *Thalassiosira weissflogii* functional attributes
- iii. Climate change induced stressors hasten molecular change in *Thalassiosira weissflogii*

1.6 Significance of the Study

Plankton communities play a crucial role in carbon biogeochemical cycling and maintaining food chains in coastal ecosystems. This study provides an important baseline for understanding the existing plankton community structures in the coastal ecosystems in Ghana as well as how they respond to individual and multiple stressors of climate change and pollution.

This work has provided empirical data that will support the attainment of the Global Sustainable Development Goals (SDGs) particularly, SDG 13: Climate Change Action, and SDG 14: Life below water. The data will also feed on the developmental aspirations within the region as envisioned by The African Union Agenda 2063, the World Bank's Agenda 2030 on ending poverty and promoting shared prosperity and Ghana's National Climate Change policy.

1.7 Delimitations of the Study

Whin Estuary located in the Western Region of Ghana was selected for the case study to determine the existing phytoplankton community structure in estuarine ecosystems in Ghana. The estuary was selected because it had previously been documented to be among the most undisturbed and productive coastal ecosystems in Ghana (CRC/FoN, 2010).

To assess the response of experimental organisms to stressors, three functional attributes i.e., growth rate, chlorophyll-*a* and dry biomass concentration were chosen because they served as proxies for aquatic productivity, and also the bulk of energy available for transfer to the next trophic levels.

For the molecular analysis, temperature (32 °C), salinity (35 ppt) and pH 5 in combination to varying concentrations of cadmium and pyrene were selected. These were based on the projected impacts of global change on coastal ecosystems in Ghana from literature review (i.e., increased salinity, warming and acidification).

1.8 Limitations of the Study

During phytoplankton analysis, samples from same transects were pooled together in order to cut down on the costs and time required to carry out their identification and analysis. This did not adversely affect any analyses since the data on the different transects, seasons and tidal periods per station were still maintained. For the molecular analysis, samples with the pH, cadmium and pyrene combinations could not be analysed due to tissue deterioration during transportation and handling at the genetic laboratory. These results have been omitted and only the ones for temperature and salinity reported in this thesis.

CHAPTER TWO

LITERATURE REVIEW

This section reviews the different concepts relevant to the objectives of the study. It entails literature on estuarine ecology, multiple impacts of climate change and pollution on coastal ecosystems, as well as the ecology of phytoplankton and their response to climate change and pollution stressors.

2.1 Estuarine ecology

Estuaries by their definitions are very dynamic places on Earth formed in coastal areas where oceanic waters intrude into indentations in the landform and meet freshwaters flowing off the land. One of the most popularly used definitions of the term estuary comes from Pritchard (1967): *“An estuary is a semi-enclosed coastal body of water, which has a free connection with the open sea, and within which seawater is measurably diluted with freshwater derived from land drainage”*. However, this definition is restrictive and excludes other types of estuarine features such as coastal lagoons, coastal bays, and intermittent saline lakes. For purposes of this study, the definition of estuaries will be as follows *“a semi-enclosed coastal body of water which is connected to the sea either permanently or periodically, has a salinity that is different from the adjacent open ocean due to freshwater inputs or evaporation and includes a characteristic biota*. The estuary extends upstream to the limit of influence by the sea (including tidal rise)”

As a result of their location, estuaries are subject to continuous changes in environmental parameters such as wind, water and sediment, which results in high variabilities in their biochemical compositions. Estuaries lie in the transitional areas

between freshwater and marine environments and are highly influenced by river inflows, exchanges between the river and sediments, as well as the water-atmosphere linkages (Lancelot & Muylaert, 2011). Estuaries undergo various changes in depth, physical energy (i.e., currents and turbulence), water clarity, salinity, biodiversity, chemical parameters, and oxygen concentrations in the sediments as one moves through from the outlet of the river into the sea.

The concept of estuaries being the most biologically productive areas on earth has different meanings. For instance, it can be as a result of the productive plants in the estuarine ecosystem, the existence of very productive fish in these ecosystems or the high amount of organic matter that is produced than used up (Day et al., 2012). Studies undertaken by Day et al. (2012) highlight that the high productivity in estuaries is primarily because of photosynthetic activities of the nanophytoplankton, phytoplankton, submerged vascular plants, periphyton, benthic algae, tidal marsh detritus and land run off. Estuaries are also recipient of high levels of sediments, nutrients and organic matter from land surface run-offs that result in their high productivity. In addition, the existence of physical energies such as wind, waves, riverine currents, and tides facilitate their high productivity by enabling the mixing of nutrients in both the sediments and water column (Day et al., 2012).

A typical estuarine food web is has the following distinct characteristics; a high level of interaction between the water column and sediments, a variety of primary producers, a highly complex and interconnected food web structure and generalist feeders (Day et al., 2012). Figure 1 below gives a summary of the major

structural and functional attributes of estuarine ecosystems, including the flow of energy across the different trophic levels. In contrast to the open sea, which has only phytoplankton, estuaries have different types of primary producers, ranging from phytoplankton, algae, salt marsh plants, sea grasses and mangroves (Day et al., 2012). However, the most recognisable estuarine food webs are the ones initiated by phytoplankton primary production. Other important aspects of estuarine food webs include the variety of plants (seagrasses and benthic algae) that grow in shallow waters and the intricate flow of food and inorganic nutrients in the water column, then to the bottom, and back up again in the water column to facilitate survival of benthic organisms such as oysters, clams and mussels. This flow of energy from phytoplankton and detritus converges in the topmost carnivores that are categorised as generalist feeders, which comprise a wide range of organisms, including trout, striped bass, flounder, birds, as well as mammals such as seals and dolphins.

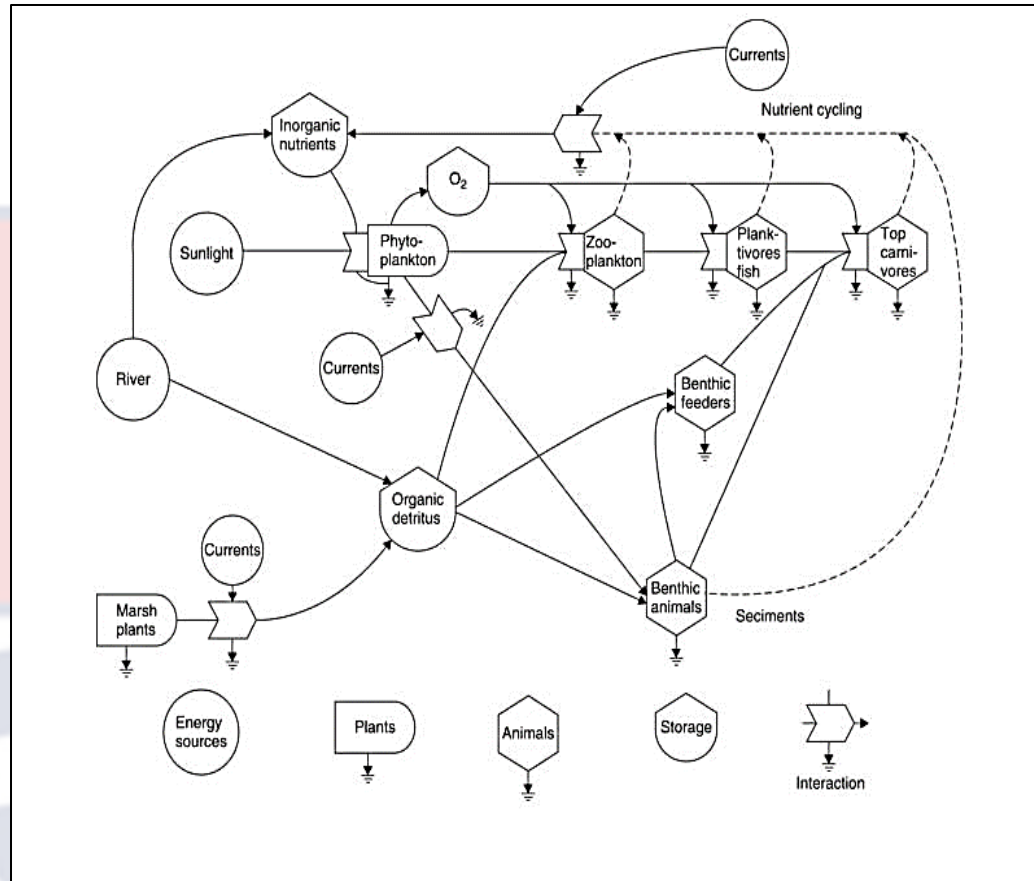


Figure 1: Energy flow in estuarine ecosystems (adopted from Day et al., 2012)

2.1.1 The role of estuaries in the provision of ecosystem services

Estuaries are critical for the survival of many organisms. They provide areas of habitat, food, favourable conditions, maintain biogeochemical balance, and filter out pollutants from rivers, thus sustaining high numbers of ecologically and economically important organisms (Nagelkerken et al., 2015). Ecologically, they provide several services that underpin various human use and values. Firstly, estuaries provide important sources of food such as fish, birds, and shellfish. The high productivity in estuaries attracts a high number of fish, birds, and mammals. In addition, estuaries are often referred to as the “nurseries of the sea” as they provide important spawning areas for fish and shellfishes. They also offer several

provisioning services including raw materials such as medicines, fish products, timber, and vegetation products.

Estuaries provide key regulation and maintenance services through a series of biological, physical and chemical processes. These services are diverse and range from regulation of waste, nutrient cycling and storage, climate regulation and carbon sequestration, sediment formation and stability, and shoreline protection. These regulation and maintenance services play an important role in maintaining ecosystem integrity and mitigating human impacts on estuarine and marine environments (Thrush et al., 2013). In addition, they also provide key community services through the provision of habitats and a wide pool of genetic resources. They also safeguard coastal ecosystems from extreme. Estuaries also contribute to human wellbeing through the provision of several social services such as cultural and spiritual heritage, recreation and tourism, aesthetics, cognitive benefits such as education and scientific research, non-use benefits as well as speculative “option” values.

2.1.2 Challenges facing estuaries

Although estuaries provide several critical ecosystem services that sustain millions of people, they are currently facing several anthropogenic challenges that impact their physical, chemical, and biological structures. Currently, estuaries rank among the most heavily impacted aquatic ecosystems on earth because of the combined effects of anthropogenic activities from inland sources and within their waters or surrounding areas (Kennish & Paerl, 2010). Currently, the main drivers of change for global estuarine ecosystems include urbanisation unplanned and

uncontrolled settlements particularly in watershed areas and increased population and demand for resources (Kennish, 2002). Many impacts of these drivers may be subtle and gradually build up over time, ultimately resulting in catastrophic disasters for humans and the ecosystems. For instance, it is estimated that only 12% of the world's 177 longest rivers are unobstructed and run freely from their sources to the sea/ocean (Deinet et al., 2010). This means that the remaining rivers receive a form of obstruction from dams or dikes, thereby depriving downstream ecosystems the minerals and nutrients that make them productive.

Estuarine ecosystems depend on flows from inland sources, which are important in creating vital coastal features. Anthropogenic activities have altered the natural sedimentation process in estuaries. Agricultural, mining, deforestation and industrial activities that remove vegetation result in increased sedimentation by increasing sediment loads that are washed downstream (Miththapala, 2013). Further, development activities such as road construction reduce the permeability of inland surfaces, which enables the flow of water and sediments to coastal areas. Increased sedimentation results in the increase of turbidity, which affects productivity of estuarine ecosystems (Kennish & Paerl, 2010). Human activities such as dredging that remove sediment from estuarine ecosystems can also negatively affect the estuarine ecology, particularly benthic organisms, while activities such as development of dams and diversion of water upstream significantly impairs quality and quantity of water reaching estuarine ecosystems (Miththapala, 2013). The reduction of freshwater inputs downstream results in reduced flushing and consequently increased estuarine salinity, which can spread

upwards towards the river or infiltrate into groundwater aquifers, resulting in saltwater intrusion. An increase in salinity result in changes in biological composition by favouring the increase of euryhaline species. Certain human actions such as paving in urban areas could also lead to increase of surface run offs into estuaries and lagoons, thereby reducing salinity levels in estuaries.

Coastal ecosystems all over the world are facing considerable threats from habitat destruction and resource overexploitation (Duarte et al., 2008). Land use changes upstream such as deforestation and conversion of land for agricultural purposes have a profound impact on estuarine water resources as much as the destruction that occurs within them. The resultant impacts of these activities is reduced stream flows and inflows into estuaries, increased sedimentation and changes in biota (Waycott et al., 2009). The over-exploitation of estuarine resources may inadvertently coincide with habitat destruction. For instance, the over-exploitation of fisheries resources is a particularly common threat facing estuaries and this is enhanced by the sheltered and safe environments provided by these water bodies as opposed to marine fishing whereby the waters are exposed to harsher elements such as wave action. Additionally, activities such as recreation and irresponsible tourism may further damage estuaries and endanger organisms (Waycott et al., 2009).

Estuaries, just like many other ecosystems, have also been significantly affected by the introduction of alien invasive species. Increased human activities such as aquaculture and tourism have resulted in the introduction of these invasive species that compete with native species to become established in the ecosystem.

When invasive species displace native flora and fauna, it results in the disruption of ecosystem services and even contributes to severe economic damages (Miththapala, 2013). For example, aquatic invasive plants such as the water hyacinth have caused disruptions in estuarine ecosystems. Their physical characteristics such as filamentous roots and broad leaves clog waterways, block underwater channels, and prevent sunlight from reaching submerged primary producers, thereby affecting the rate of production and biodiversity.

2.1.3 Estuaries in Ghana

Ghana's coastal zone spans approximately 560 km (Lukhabi et al., 2024) and is endowed with different types of ecosystems and resources of socio-economic importance and is divided into three regions (the east coast, central coast and west coast). The east coast of Ghana spans from Aflao in the East to Prampram, the central coast spans approximately 321 km long and are comprised mainly of rocky beaches interspaced with short sections of sandy beaches, and the west coast stretches from the Ankobra river to the country's border with Ivory Coast (Amlalo & Oppong-Boadi, 2015). Majority of Ghana's major river i.e., Ankobra, Volta and Pra flow into the Atlantic Ocean, thereby contributing the sustenance of downstream coastal ecosystems.

Estuarine ecosystems in Ghana are important as they provide significant ecosystem services that support the livelihoods of riparian dwellers (Ankrah, 2018). All the estuarine ecosystems in the country are exploited for their resources and contribute immensely to Ghana's vibrant fishing industry. These estuarine ecosystems have also undergone significant changes in their biological, physical

and chemical characteristics because of the pressure from anthropogenic activities from inland and coastal sources (Faseyi et al. 2022; Okyere and Nortey 2018; Jonah et al. 2016). The country has a total of 10 estuaries, including Volta, Kakum, Pra, Butre, Akwiada, Nyan, Owuku, Ankobra Amanzule and Whin, as well as more than 90 coastal lagoons and associated wetlands that spread along the entire stretch of the coastline (Yankson & Obodai, 2007).

The Whin Estuary is found on the western coast of Ghana in Whin River (CRC/FoN, 2010). The mouth of the estuary is located on the western coast of Ghana within the Takoradi-Sekondi Metropolis. The banks of the estuary are a mixture of sandy shores to the western side and rocky beaches on the eastern side and are characterised by thick vegetation with thickets of mangrove stands. The estuary supports fishing activities which provide the major source of livelihood for adjacent communities. Whin has been reported as being one of the most productive and least perturbed estuaries by anthropogenic activities in Ghana (Atindana et al., 2019; CRC/FoN, 2010) which makes it the ideal aquatic environment to assess the composition and distribution of organisms.

2.2 Climate change and pollution impacts on estuarine ecosystems

Globally, climate change and pollution represent two important major environmental challenges facing coastal ecosystems. The main aim of this study was to understand impacts of pollution and climate change on estuarine phytoplankton communities. This section provides an in-depth account of climate change, pollution and their impacts on estuarine ecosystems.

2.2.1 Climate change and its impact on estuarine ecosystems

Climate change refers to the systematic change in the long-term state of the atmosphere over multiple decades or longer (Luber & Lemery, 2015). It is estimated that anthropogenic activities alone have contributed to approximately increase global temperatures by 1.0 °C, above pre-industrial levels (Masson-Delmotte et al., 2022). The Intergovernmental Panel for Climate Change (IPCC) has shown that Global temperatures are likely to rise by 1.5 °C by 2052 if emissions continue at the current rate (IPCC, 2021). The main concern globally is, therefore, to reduce anthropogenic greenhouse gas emissions and to protect existing and future ecosystems and livelihoods. It is this same concern that necessitated world leaders to come together and commit themselves to keeping global temperatures rise below 2 °C above pre-industrial levels during the historic 2016 Paris Agreement. Carbon dioxide (CO₂) is the main greenhouse gas that contributes to global warming, and it is released mainly through the combustion of fossil fuel. In the United States of America for instance, CO₂ accounted for nearly 80% of human induced greenhouse gas emission, followed by methane (CH₄) and nitrous oxide (N₂O) at 12% and 6% respectively (US EPA, 2015). Other important greenhouse gases include black carbon (soot and particulate matter) and various fluorinated gases i.e., hydrofluorocarbons (HFCs).

The impacts of climate change on ecosystems will be disproportionate and vary for different ecosystems. A study carried out by Harrison, et al. (2015) for instance, outlines that sectors that are linked to agriculture, forest, biodiversity, coastal areas, energy, transportation and water resources will be the most affected.

Coastal ecosystems play a crucial role in climate regulation and nutrient cycling. However, climate change induced stressors are having profound consequences on these ecosystems (Herr, 2009). For instance, studies have highlighted that global warming is already resulting in increased ocean stratification, sea-level rise, reduced sea-ice cover, disruptions in oceanic circulation, precipitation, and rainfall input (Doney et al., 2012). Combined with the already existing anthropogenic-led challenges, climate change could alter biodiversity, species composition and productivity of the oceans.

From the foregoing, it is apparent that coastal ecosystems, including estuaries, are facing anthropogenic-driven climate change-related threats despite their numerous important ecological and economic roles. These threats could have serious impacts on estuaries and their ability to provide vital ecosystem services (Robins et al., 2016). Thus, under this section, some impacts of climate change on coastal ecosystems, especially tropical estuaries are reviewed. These impacts, which also are climate change indicators include earth and ocean surface temperature rise, increased acidification, sea-level rise, increased sea salinity, increased precipitation and freshwater runoff, and increased coastal storms.

Sea surface temperature

Studies have shown that sea surface temperature (SST) has increased since the 1970's at a rate of 0.11 °C per decade (IPCC, 2022). In addition, results from various climate change simulations suggest that global temperatures will be 1.1 to 5.4 °C warmer in 2100 than it is today (Solomon, 2007). Temperature is an important parameter in estimating estuarine water quality and increased water

temperatures can increase the extent of hypoxia, pathogen levels as well as the frequency of harmful algae blooms (Brown et al., 2016). Solar radiation, temperatures of river, air, the ocean, and changes in river discharge are among the primary drivers that influence estuarine water temperature (Tanaka et al., 2021). However, the effect that climate change will have on estuarine water temperature will vary with different estuaries depending on their configuration, type, and location within the estuary. For instance, the upper levels of estuaries will be influenced by stream temperature, while the lower portions will be dependent on temperature changes in the ocean, rate of sea-level rise and wind action (Robins et al., 2016). A study by Groom et al., (2011) has also shown that land use changes upstream such as destruction of riparian vegetation, agriculture, and deforestation result in increased stream temperatures, which ultimately affect estuarine water temperatures. Many organisms, particularly in tropical ecosystems are adapted to specific temperature ranges and, therefore, even small temperature changes can result in their mortality or changes in their distribution (Halpern et al., 2015). Ghana's climate has become drier and unpredictable in recent times. For instance, temperatures are projected to change by 2.0 °C and 3.9 °C for 2050 and 2080, respectively in the coastal areas (Ankrah 2018). These changes in temperatures are having profound impacts on coastal ecosystems in Ghana, particularly on the fisheries industry which supports the livelihoods of about 10% of the country's population (Ankrah, 2018; Effah et al., 2023).

Increased acidification

Although oceans absorb a significant amount of carbon dioxide (CO₂) released into the atmosphere to buffer the earth from the effects of increased carbon emissions, this action ultimately contributes towards ocean acidification by lowering pH of the oceans. It is estimated that CO₂ emissions have increased from approximately 280 ppm in preindustrial times to nearly 384 ppm by 2007 (Doney et al., 2012). Fossil fuel combustion, land use changes and deforestation form the main anthropogenic activities responsible for the increased CO₂ in the atmosphere. It is projected that future CO₂ levels will reach 1,071 ppm by 2100 given the current rate of emissions (Gattuso et al., 2015). Similarly, increased CO₂ concentrations have resulted in decreased global water pH (about 0.1 globally), making the current sea water about 1.25 more acidic than it was in the 1750s (Gattuso et al., 2015). This decrease in pH is expected to continue by an additional 0.4 by the end of the century if CO₂ emissions continue at the current rate. Ultimately, the decrease in pH will have extreme impacts on aquatic ecosystems, particularly, in the development of shells in many marine animals, which will further affect communities that depend on the shellfish industry for their livelihoods (Brown et al., 2016).

In comparison, estuaries are defined primarily by the magnitude and seasonality of freshwater inputs, the existing physical processes and chemical gradient. Estuarine acidification is therefore a different concept from ocean acidification in terms of the acidification mechanism, amplitude and timeframes associated with pH change (Marshall et al., 2016). While ocean acidification is

defined by the variation in atmospheric CO₂ over a long period of time, estuarine acidification is a more dynamic multifaceted process usually involving not only atmospheric CO₂ but also biological CO₂ generation and mineral acidification (Marshall et al., 2016). Naturally, estuarine acidification taking place by either the biological or mineral acidification are likely to vary proportionally at different timescales. For instance, mineral acidification is expected to change seasonally due to the high acidic content in freshwater inflow, while biogenic acidification is influenced by tide and mixing of sea water with poorly buffered but nutrient-rich estuarine water (Marshall et al., 2016). Generally, acidification caused by exchange of CO₂ from the atmosphere is not a concern in many estuaries because estuarine surface water residence time is short, which does not allow for significant dissolved inorganic (DIC) accumulation and the fact that most shallow estuaries are also super saturated with CO₂. In large estuaries, however, longer residence times, low pCO₂ values resulting from high biological CO₂ uptake and more ocean influence on water masses play an important role in their acidification (Cai et al., 2021).

Climate change will intensify estuarine acidification in two main ways: (1) through increased oceanic acidity that will be driven by other climate change induced impacts such as sea-level rise that will facilitate more seawater intrusion into estuaries; (2) climate change driven changes on river run-off coupled with increased anthropogenic activities will facilitate conditions that support acidification and changes to pH due to additional nutrients (Glamore et al., 2016). In addition, increased flooding events in estuarine ecosystems may flush rainwater into estuaries causing episodes of acidification. Currently, limited studies have been

undertaken in Ghana on estuarine and ocean acidification. However, efforts are currently being made by the International Atomic Energy Agency and the Centre Scientifique De Monaco to understand the effects of acidification on the West African coastal waters in a co-ordinated research project (CRP) (Nunoo et al., 2016).

Sea-level rise

It is estimated that global sea-levels have risen to about 8 inches in the last 100 years, which is driven by increasing temperatures directly through expansion of water molecules as oceans become warmer and indirectly by the addition of freshwater into oceans due to the melting of ice caps and glaciers (Rahmstorf, 2007). The Intergovernmental Panel on Climate Change (2021) further projects sea-level rise between 0.38 and 0.77 m by 2100, which will have significant shoreline changes globally. Additionally, sea-level rise (SLR) will affect estuarine hydrodynamics, which can potentially influence the size and distribution of inter-tidal wetlands, and ultimately affecting biodiversity in these ecosystems (Robins et al., 2016). In Ghana, sea levels are projected to rise every decade by an average of 0.3 cm, from 3.6 cm in 2010 to about 34.5 cm by 2080 (EPA, 2011). This anticipated rise in sea-level will affect estuarine ecosystems by increasing their water levels, thus potentially leading to inundation of adjacent low-lying coastal lands, recession of the shoreline towards the land, shore erosion and destruction of coastal and stormwater infrastructure (Hanslow et al., 2018). Sea-level rise combined with increased temperature is expected to affect socio-economic activities such as fishing, and tourism associated with estuarine ecosystems.

Therefore, understanding the complex impacts of SLR on estuarine ecosystems can promote their sustainable management.

Increased sea salinity

Ocean salinity refers to the concentration of salts (mainly sodium chloride) in sea water. The salinity of the ocean comes primarily from land-based sources (i.e., weathering of rocks) through surface run-off from rivers for thousands of years and provides a good indicator of the status of global water cycle and atmospheric measurements (Talley, 2002). Salinity levels in coastal ecosystems affect other features of the ocean such as density, waves, pressure, currents, freezing point, temperature as well as the type of organism that will be found in a particular ecosystem (Talley, 2002). Again, salinity levels in coastal and marine ecosystems are highly influenced by evaporation, precipitation, river-runoff, wind action and melting of the ice caps (Curry et al., 2003). Climate change driven salinity changes in aquatic ecosystems are disproportionate across the world. For instance, studies undertaken in the Atlantic Ocean between 15°S and 42°N indicated a salinity increase in the upper 500 m, whereas the North Atlantic Region between 42°N to 72°N region showed a reduction in salinity due to increased freshwater inputs (Bindoff et al., 2007).

Estuaries form important places where oligohaline waters from river run-offs meet the euhaline ocean waters. Therefore, salinity in this area is because of the tidal effects and changing freshwater inflow from rivers. Species composition along the gradient of an estuary is highly influenced by salinity. For instance, freshwater organisms are limited to the upper reaches of the estuary, while marine

organisms inhabit the estuarine mouth. There are some species that are adapted to intermediate levels of salinity and, therefore, acclimate to any salinity levels. Salinity levels influence various other aspect of estuarine ecology including turbidity resulting from flocculation and outbreak of salinity specific diseases (Tallam & White Jr, 2023). In addition, changes in salinity levels such as those associated with droughts and storms can greatly affect estuarine species composition. Climate change will have an impact on estuary salinity by either altering river flow or through the actions of continued sea-level rise (Stocker, 2014). For instance, a study by Havens & Steinman (2015) projects that future estuaries will be saltier due to prolonged incidences of droughts, reduced inputs from freshwater inflows and sea-level rise, which will cause salty ocean waters to move into estuaries. Therefore, understanding how estuarine organisms will respond to these projected higher salinity changes could give an insight on future estuarine community structures and food webs. In the Gulf of Guinea, a study undertaken by Dovlo (2016) on the seasonal variability of temperature and salinity showed that the average sea salinity was estimated to be 35.24‰, with the highest observations being made in the upwelling seasons at 35.56‰.

Changes in precipitation and freshwater runoff

Climate change is expected to change global precipitation patterns whereby arid areas are expected to become dryer, while wet areas, particularly in the mid and high latitudes, are expected to receive greater amounts of precipitation (Emori et al., 2016). The Intergovernmental Panel on Climate Change, (2021) has shown that the frequency of heavy precipitation events has increased over majority of land

regions with projections further showing that precipitation increases will occur near the equator, particularly in the Pacific, Arctic and Antarctic areas. On the other hand, precipitation is expected to reduce by up to 20% in the Mediterranean and Southern Africa regions, while areas such as Chile, Western Australia and Central America are expected to become 10% drier. Current climate information shows heavy precipitation events, and the trend is expected to continue (IPCC, 2021). The highest increases in heavy precipitation events are expected to occur in areas such as Central Africa and Southern Asia (Fischer et al., 2014). These changes in precipitations will consequently affect the amount of freshwater runoff on land and ultimately into coastal ecosystems such as estuaries.

Ghana is already experiencing changes in precipitation because of both climate variability and climate change (Ankrah, 2018). For instance, studies have shown that the amount of rainfall in Ghana has been consistently decreasing and becoming more erratic (Asante & Amuakwa-Mensah, 2014). These changing precipitation and surface run-off patterns are expected to significantly influence coastal ecosystems by altering water quality parameters, sedimentation, nutrient loads, flushing and residence times, particularly for intermittent open estuaries and estuarine ecology (Glamore et al., 2016).

Increase coastal storms

Climate change is expected to increase the frequency and intensity of coastal storms, and there are reported increases in storm intensity and wave height across the Pacific Ocean in recent times (IPCC, 2021). It is expected that these events associated with climate change and climate variability will consequently

influence sea-level rise, shoreline erosion and estuarine bio-geodynamics. Coastal storms, heavy waves and sea-level rise can result in significant changes in estuarine ecosystems such as coastal inundation. Large storm events may also cause increased water that may carry pollutants, sediments and nutrients into estuaries over a short period of time and exacerbate the occurrence of stressors such as acidification.

2.2.2 Pollution and its impact on estuarine ecosystems

Estuaries are very productive ecosystems because of their long water residence times and their ability to cycle nutrients numerous times before they are flushed into the ocean. These same characteristics that make them productive also increase their vulnerabilities to nutrient over-enrichment and its associated effects (Kennish & Paerl, 2010). Pollution is perhaps the most important threat to estuaries. Agricultural, urban, and industrial runoff are among the largest sources of pollution to estuarine ecosystems; fertilisers and domestic wastes increase the levels of nitrogen and phosphorous in estuaries leading to eutrophication (Miththapala, 2013).

Some aquaculture activities may be carried out in estuaries, whereby farms discharge nutrient rich effluent and sediments into the environment, thereby polluting the waters. In Sri Lanka for instance, shrimp farming is an important socio-economic activity. However, majority of shrimp farms lack appropriate infrastructure and effluent treatment plants, thereby discharging their untreated effluents into the surrounding waterways, which affects the water quality for human and aquatic lives (Jones et al., 2018).

Pollution is a major challenge in Ghana and most of the water resources in the country have been severely polluted (ISSER, 2020). The Institute of Statistical, Social, and Economic Research outlined that the key drivers of water pollution are rapid population growth, urbanization, unsustainable agricultural and industrial activities, and negative attitudes toward the environment (ISSER, 2020). These drivers of water pollution contribute to the discharge of sewage from domestic sources, release of inorganic contaminants from industrial sources, and leaching of fertilizers and pesticides from agricultural areas. In addition, illegal mining “galamsey” has impacted estuaries because they are on the “receiving end” of anthropogenic activities both terrestrial and marine-based sources.

Eutrophication and its subsequent impacts on ecosystems represent one of the major threats to estuarine ecosystems in Ghana (Mahu et al., 2023). As stated earlier, estuaries are highly productive areas with high levels of organic matter. However, human activities such as the indiscriminate disposal of nutrients from agricultural and domestic sources can contribute to their eutrophication status. Some of the negative impacts associated with eutrophication in estuaries include reduction in water quality due to hypoxia, increased primary productivity, frequent algal blooms, and eventual mortality of biota due to low levels anoxia (Damar et al., 2021).

“Galamsey” or illegal mining activities and their associated impacts on estuarine water quality have been extensively studied in Ghana, particularly on the Pra and Ankobra river systems (Faseyi et al., 2022; Okyere and Nortey, 2018; Okyere 2019). The process involves the excavation, removal, grinding, and

washing of soil and earth materials to extract gold from its ore. Usually, this extraction also involves the use of heavy metals such as mercury, lead and arsenic (Hayford et al., 2009), which ends up in the water column, sediment and biota. In low concentrations, these metals are usually not a cause of concern and can be utilized by aquatic organisms for their metabolic and physiological processes such as respiration, photosynthesis and absorption of major nutrients (Gavhane et al., 2021). However, increased concentrations of these metals in the water column, sediments and organisms pose ecotoxicological risks to ecosystems. Other important sources of heavy metals in Ghanaian estuaries include agricultural activities (Alloway, 2013), industries (Darko et al., 2017), and domestic effluents (Fianko et al., 2007).

Another important threat associated with illegal mining activities in Ghana is extremely high levels of turbidity. Recent studies reported high levels of turbidity of up to 1000 ppm in the Pra Estuary, which is directly attributed to illegal mining activities along the river gradient (Okyere, 2019). This high turbidity influences other important aspects of estuarine structure and function such as the levels of dissolved oxygen, temperature, nutrient concentration and photosynthetic activity (Okyere 2019).

Naturally, estuaries act as biogeochemical barriers that trap petroleum products because of their strong salinity gradients (Chizhova et al., 2020). As a result, estuarine ecosystems may contain higher levels of polycyclic aromatic hydrocarbons (PAHs) than the surrounding coastal and riverine ecosystems. Polycyclic aromatic hydrocarbons form the most important constituents of crude

oil, and anthropogenic activities that contribute to their presence in estuarine ecosystems include oil spills during extraction, refining, transportation and consumption, as well as of oil discharge of effluents. It is estimated that estuarine sediments could contain PAH concentrations of more than 100 ng/L, which is above the established lowest pollution levels for PAH compounds on aquatic environments. Nearly 2.7 million barrels of petroleum is produced within the Gulf of Guinea coastline in a single day (Hernández Ruiz et al., 2021). This makes the area particularly prone to oil spill accidents with release of PAHs into the environment.

Polycyclic aromatic hydrocarbons are not readily soluble in water but sink to the sediment of estuarine ecosystems by attaching to other organic and inorganic particulate materials (Tolosa et al., 2004). Reported toxic effects of PAHs on aquatic organisms include immunotoxicity, embryonic abnormalities, and cardiotoxicity. For example, Honda & Suzuki (2020) and Almeda et al., (2013) similarly reported that PAHs disrupt membrane fluidity of aquatic organisms, which altered feeding, embryonic development and reproduction in animals. Further, PAHs have also been suggested to affect the development of humans immune system (Armstrong et al., 2004).

Pyrene is one of the most distributed PAHs (Essumang, 2010), and most studies in Ghana's aquatic ecosystems have primarily focused on fish (Bandowe et al., 2014; Nyarko & Klubi, 2011; Palm et al., 2011), while other studies have also been undertaken on zooplankton (Asiedu, 2020; Hernández Ruiz et al., 2021). This study investigates impacts of pyrene on tropical phytoplankton.

In fact, the impacts of climate change and pollution stressors, whether individually or in combination have the potential to disrupt estuarine ecosystem functions. These impacts happen concurrently in nature to cause adverse effects on coastal ecosystems all over the world. This study, therefore, investigated the impact of individual as well as combined stressors of climate change and pollution on phytoplankton functional and molecular attributes.

2.3 Phytoplankton Roles in Coastal Ecosystems

Phytoplankton comprise one of the most taxonomically diverse group of organisms on earth, with over 20,000 known species that are distributed across various aquatic environments around the world (Falkowski et al., 2003). They have played a central role in promoting human existence as they provided first sources of oxygen for the planet, which led to the development of modern photosynthetic-driven food webs. These organisms have continued to play important ecological and biogeochemical roles in aquatic ecosystems, which are often overlooked (Falkowski et al. 2003). Ecologically, phytoplankton account for nearly half of the global primary production. In turn, the organic matter is made available to heterotrophs after the subtraction of what they use for their own metabolic activities (i.e., net primary productivity (NPP)). The NPP is important, particularly in marine ecosystems, because it represents the only source of energy that these ecosystems can utilise for their metabolic processes (Falkowski et al., 2003).

In addition to primary production, phytoplankton also play a significant role in the cycling of important biogeochemical elements i.e., carbon (C) and nitrogen (N) by utilising these elements and releasing them back into the environment as

either particulate or dissolved organic matter. Phytoplankton also mediate the biological carbon pump which is a major component of the global carbon cycle where atmospheric carbon (iv) oxide is transferred from the atmosphere through primary production by mostly phytoplankton into the underlying ocean sediment (Hülse et al., 2017).

Socio-economically, many of the products that sustain current human populations such as petroleum and natural gas trace their origins from phytoplankton (Falkowski et al., 2003). Majority of the calcareous remains of plankton deposited over millions of years, are converted into building materials. These products represent metabolic processes that occurred over millions of years that involve the transport of phytoplankton to the ocean floor once they died (Falkowski et al., 2003).

Despite their numerous importance, some species of phytoplankton have been known to form toxic algal blooms which have direct impacts on humans and other biodiversity in aquatic ecosystems (Assmy & Smetacek, 2009). Phytoplankton blooms in aquatic ecosystems are usually caused by increased levels of limiting nutrients i.e., phosphorous and nitrogen, which increase the assemblages of phytoplankton beyond their normal mortality rates. In addition, once the limiting nutrients are depleted, the phytoplankton groups usually die out and accumulate in the water column and use up oxygen as their bodies decompose, leading to anoxic conditions. It is estimated that only 5% of the thousands of phytoplankton species can form toxic algal blooms (Assmy & Smetacek, 2009). In recent times, there has been an increase in the occurrence of HABs primarily attributed to anthropogenic

activities such as the spread of fertilisers from agricultural and domestic sources. The main challenges associated with HABs include decreased sunlight to estuarine ecosystems, low dissolved oxygen and production of toxins that could bioaccumulate in shellfish, fish and other organisms, ultimately having significant impacts on human health (Tsikoti & Genitsaris, 2021).

2.3.1 Functional attributes as tools for assessing phytoplankton response to stressors

Functional traits represents the characteristics of organisms that mediate biological processes such as growth, reproduction and survival and are vital in assessing the response of organisms to their environments (Violle et al., 2007). This duality of functional traits being at both the crossroads between ecosystems and the environment provide an important tool for researchers to use in understanding environmental change (Díaz et al., 2013). Some of the traits that can be used for phytoplankton studies include cell sizes, phosphate affinity, light affinity, maximum growth rate, motility, mixotrophy, buoyancy, reproduction (egg production) and chlorophyll-*a* production, among others (Wentzky et al., 2020). To assess the impact of climate change and pollution stressors in this study, functional attributes that describe estuarine ecosystem productivity, including growth rate, chlorophyll-*a* and biomass were assessed.

Chlorophyll-a

Chlorophyll represents the colour pigment found in plants, algae and phytoplankton that enables them to photosynthesise. As a photoreceptor, chlorophyll absorbs light energy, which is used by the plants to produce organic

compounds (Falkowski and Raven 2013). Thus, phytoplankton biomass represents one of the most important parameters in aquatic sciences. Environmental stressors such as increased acidification, water temperatures and contaminants can influence the production of chlorophyll-*a* in phytoplankton, ultimately affecting biomass production and food chains in higher trophic levels.

The effects of temperature on phytoplankton productivity has been widely researched (Acheampong et al., 2021; Gittings et al., 2018; Thomas et al., 2012). Other studies have also been undertaken to understand stressors such as acidification (Hancock et al., 2020), salinity (Azhikodan & Yokoyama, 2016), heavy metal (Hindarti & Larasati, 2019) and crude oil (Huang et al., 2011) stressors on phytoplankton productivity as well. Studies have also found that the chlorophyll-*a* concentrations in the world's oceans have been reducing. Studies by Boyce et al., (2010) estimated global-averaged declines of 1% chlorophyll-*a* concentration per year. They attributed this decline to physical drivers such as sea surface temperatures which consequently decreased the availability of nutrients in the aphotic zones of the ocean, limiting the growth of phytoplankton. Other studies have supported Boyce et al., (2010) by reporting on the relationship between sea-surface warming and decrease of global chlorophyll-*a*, phytoplankton biomass and primary production (Boyce et al., 2014; Mackas, 2011). Increased stressors such as acidification, increased salinity and CO₂ have also been found to reduce chlorophyll-*a* in phytoplankton (Barnes & Wurtsbaugh, 2015; Hyun et al., 2020).

Growth and development

Growth rate refers to the change in the number of cells or organisms in an experiment and is usually expressed as a function of time. Thus, growth rate may be calculated by dividing the change in number of organisms to change in time. The determination of growth rate is important in understanding how environmental parameters regulate growth over space and time. For phytoplankton, the growth rate is a direct indicator of fitness within an environmental niche and serves as a basis for estimating primary production in aquatic ecosystems (Behrenfeld et al., 2005; Boyd et al., 2013; Fanesi et al., 2017). As stated previously, phytoplankton growth rate depends mainly three things i.e., the availability of carbon dioxide, sunlight and nutrients. In natural environments, however, factors such as the amount of grazing from primary consumers further influence the growth rate of phytoplankton (Chen et al. 2021). Growth rates of phytoplankton may be determined directly by cell counts and biomass estimations as well as indirectly through the measurement of chlorophyll-*a* concentrations (Laws, 2013).

Climate change induced stressors have been reported to alter the growth rate of phytoplankton and zooplankton in many microcosm experiments. For Instance, studies by Acheampong et al., (2021) found that sudden increase in surface water temperatures decreased diatom and dinoflagellate growth rates by about 75% and 78-86%, respectively. They noted that responses to these sudden events were mainly influenced by the time it took for the warming effect to manifest and degree of stress suffered. Other studies by Garzke et al., (2015) showed that surface water warming decreased copepod body size and abundance. In a separate study, Berge

et al., (2010) showed that low pH and the corresponding increase in CO₂ had no significant effect on phytoplankton growth rate, which they attributed to the organism's ability to adapt quickly to the stressors. Other study by Othman et al., (2018) revealed that increased PAH concentrations resulted in a significant decreased phytoplankton growth.

Biomass

Biomass of phytoplankton also represents the amount of “food” is passed higher trophic levels and the amount of pollution within an environment (biotic and detritus components of particulate organic carbon (POC) (Legendre & Michaud, 1999). Furthermore, majority of the research that evaluates the impacts of nutrients, turbidity, toxicants, and hydrological modifications require measurements of phytoplankton biomass (Agawin et al., 2000).

Generally, studies about the impacts of stressors on phytoplankton biomass show that high exposure to environmental stressors have adverse impacts (Bergström & Karlsson, 2019; Christiansen et al., 2019). Chakraborty et al., (2011) and Barnes & Wurtsbaugh, (2015) showed that high salinity levels decreased the total biomass in freshwater phytoplankton due to osmoregulatory stress.

2.3.2 Phytoplankton response to stressors at molecular level

Functional traits such as chlorophyll-*a*, growth rate, particulate organic carbon content and bulk protein enhance understanding of environmental change effects in phytoplankton communities (Díaz et al., 2013). However, the use of these functional traits assessments alone provides a limited understanding of how phytoplankton communities adapt to stressors induced by climate change,

particularly given the rapid adaptation rates of these organisms response to environmental stressors (Litchman et al., 2012). These provides some level of uncertainties, particularly for coastal ecosystems, which are currently under enormous stress from anthropogenic activities (Miththapala, 2013).

Research has shown that phytoplankton species are expected to respond to environmental stressors through processes that enhance phenotypic plasticity, species sorting and replacement and through molecular change (genetic adaptations), through selection of new genotypes (Polechová & Storch, 2008; Urban et al., 2012). Therefore, there is a need to move beyond assessment of functional attributes alone when evaluating plankton response to environmental stressors to include genotoxicity assessment.

Genetic variation within a species highly influences the level of adaptations to changes within their environment. The degree of genetic variation is later expressed at phenotypic levels to ensure the survival and reproduction of individuals within a population (Litchman et al., 2012). Current phytoplankton studies are now attempting to understand these adaptations at the molecular level using modern biotechnological tools such as Polymerase chain reaction (PCR) and analysis of rDNA/rRNA-based technologies (Alemzadeh et al., 2014). For example, studies by Bailey et al., (2017) on the effects of acidification (low pH) found significant transcriptomic level changes in *Calanus glacialis* despite there being no observable changes on functional traits. Their study showed that increased acidification facilitated an altered gene expression in activities such as ion exchange, DNA transcription, molecular chaperones and processes involved in

locomotor activity, which may have allowed the copepod to buffer itself against the changes in low pH.

In a separate study, Schaum et al., (2018) utilised molecular sequencing techniques to investigate the impact of thermal stress on marine diatoms at molecular level in the marine diatom *Thalassiosira pseudonana*. After about 300 generations of selection, their study identified candidate single-nucleotide variants (SNVs) in the evolved populations compared to the ancestral clone. They concluded that this evolution could have contributed to some of the observed phenotypic adaptations to the different warming treatments. Other study by Lohbeck, Riebesell, and Reusch (2012) to determine the response of the coccolithophore *Emiliana huxleyi* to acidification found revealed that increased CO₂ levels resulted in lower growth and calcification rates in *E. huxleyi*. However, their study could not agree on whether the existing genotypic diversity was responsible for the phenotypic observations due to influence of many versus single genotypes in the experiments.

2.3.3 Phytoplankton community structures along the coast of Ghana

The existence of actual estuarine phytoplankton assemblages has been a great subject of debate among scientists. Studies have shown that there is no evidence of true estuarine phytoplankton assemblages but rather the distribution of these species occurs along salinity gradients (Muylaert et al., 2009). However, studies show that some organisms have been evolved to adapt to the intermediary salinity in brackish environments (Lancelot & Muylaert, 2011). Further Lancelot & Muylaert, (2011) argue that phytoplankton species dominance and development

in estuarine environments are driven by complex interplays between activities occurring in the watersheds and processes from the regional ocean basins. Phytoplankton communities in Ghana's inshore and offshore ecosystems comprise diatoms, dinoflagellates, and coccolithophores (deGraft-Johnson et al., 2010). Majority of studies on phytoplankton in Ghana have primarily been undertaken in freshwater ecosystems such as the Bui Dam and Lake Bosomtwe (Alhassan, 2015; Awortwi et al., 2022; Awortwi et al., 2015). In marine ecosystems, the main species of diatoms in Ghana belong to the genera *Skeletonema*, *Nitzschia*, *Chaetoceros*, *Rhizosolenia* and *Thalassiosira*, and occur particularly during the upwelling season (Anang, 1979). Within brackish ecosystems, studies carried out by CRC/FoN, (2010) on the Whin Estuary similarly found a total of 25 genera of phytoplankton, which comprised mainly of green and blue algae, as well as diatoms. The literature on phytoplankton laboratory experimental research in Ghana is sparse with Acheampong et al., (2021) microcosm experiments to determine the effects of marine heatwaves on *Thalassiosira weissflogii* (a diatom) and *Gymnodinium* sp (a dinoflagellate) being probably the only encountered study.

2.3.4 Biology and distribution of *Thalassiosira weissflogii*

Thalassiosira weissflogii (Grunow) (Fryxell & Hasle, 1979) is a cylindrical diatom that occurs singly or in groups, sometimes in a gelatinous matrix (Figure 3). Each single cell can measure anywhere from 4–32 µm in diameter. The shape of the diatom varies in different aquatic environments and is highly influenced by salinity. *Thalassiosira weissflogii* was originally isolated from Blankenberge, Belgium, is considered a cosmopolitan euryhaline species (Hasle et al., 1996). *T.*

weissflogii is a cosmopolitan euryhaline species, frequently recorded in polluted fresh and brackish rivers and streams (Heudre et al., 2021).

Even though the diatom is non-toxic, it has been often associated with algae that form blooms in red tides (Bauder et al., 2001). The diatom grows well in waters with high pH (around 8–9.4) and salinities (>5) (Heudre et al., 2021). Depending on its environment, various nutrients i.e., include nickel, zinc, iron, nitrogen, and silicon may be growth limiting or have a controlling effect on cell processes (Heudre et al., 2021).

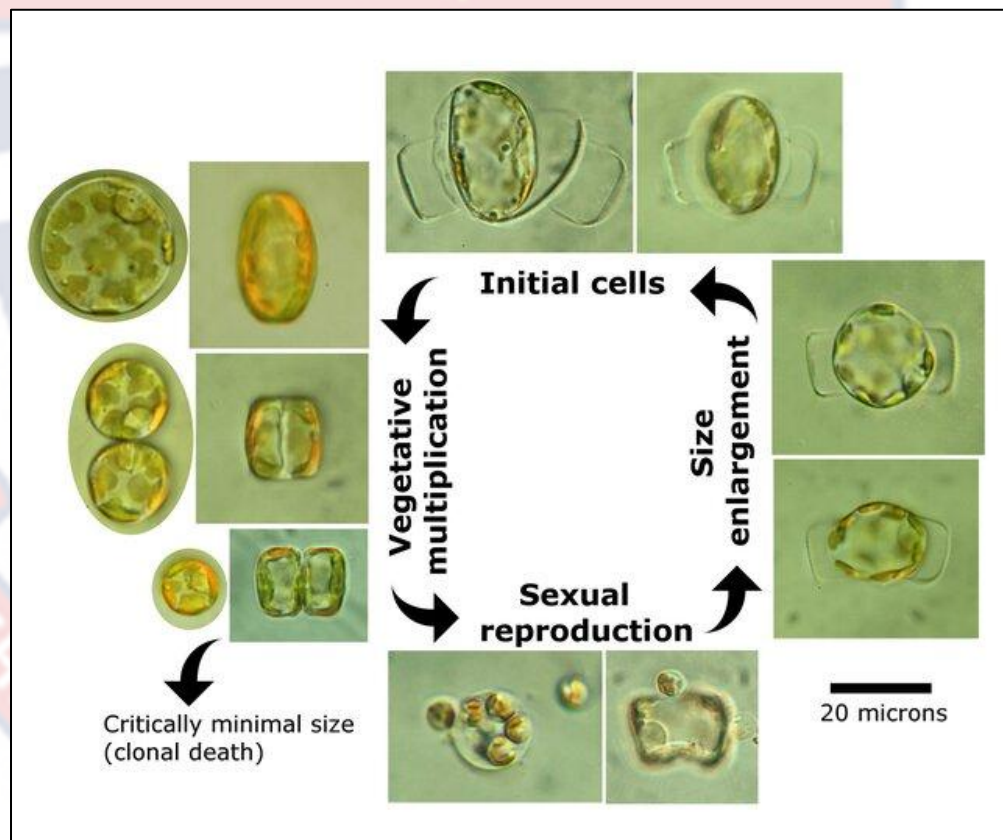
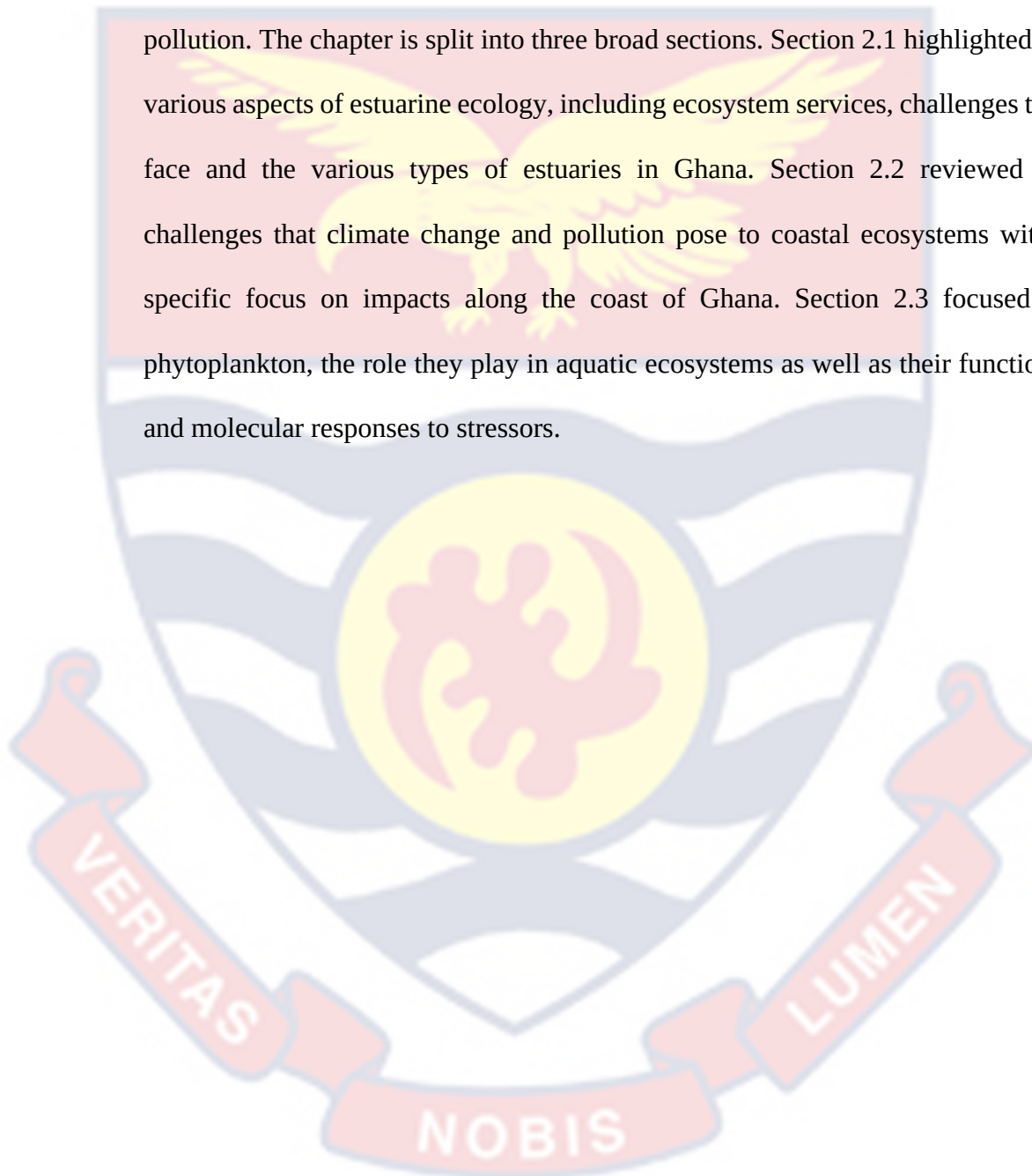


Figure 2: *Thalassiosira weissflogii* life cycle

Source: Chepurnov, Steigüber, and Siegel (2018)

2.4 Chapter Summary

This literature review section has covered the fundamental areas in the various multifaceted components of estuarine ecology, climate change and pollution. The chapter is split into three broad sections. Section 2.1 highlighted the various aspects of estuarine ecology, including ecosystem services, challenges they face and the various types of estuaries in Ghana. Section 2.2 reviewed the challenges that climate change and pollution pose to coastal ecosystems with a specific focus on impacts along the coast of Ghana. Section 2.3 focused on phytoplankton, the role they play in aquatic ecosystems as well as their functional and molecular responses to stressors.



CHAPTER THREE

MATERIALS AND METHODS

This chapter gives an account of the materials and methods used in this study. A detailed description of the field study area is provided, followed by sections on field sampling and sample preparation. The chapter continues to provide an exhaustive description of microcosm experiments involving laboratory exposure of *Thalassiosira weissflogii* to climate change and pollution-related stressors, as well as a description on the molecular techniques and analyses undertaken. Lastly, the different statistical analytical tools and software employed to make inferences are described in this section.

3.1 Field Study

3.1.1 Description of study area and sampling design

The field aspect of this study was carried out in the Whin Estuary, located between latitudes 1°46'47" W, 4°52'52" N and 1°46'04" W, 4°52'30" N, in the Western Region of Ghana (Figure 2). This is a shallow estuary with a funnel shape which facilitates more seawater-freshwater interchange. The estuary is narrower at the mouth when compared to further upstream where it spans over 350 m wide (Chuku et al., 2023). The Whin Estuary has previously been documented to be one of the most productive and pristine coastal ecosystems in Ghana (Atindana et al., 2020; CRC/FoN, 2010). The estuary supports a vibrant fishing, and particularly shellfish industry that supports the livelihoods of adjacent communities (Atindana et al., 2020; Osei et al., 2021). In recent years however, this estuary has been subject to multiple anthropogenic stressors ranging from increased population and

settlements, impacts from oil exploration activities and pollution (Aheto et al., 2011).

Field sampling was conducted twice in March and July 2022, at both high and low tides based on the timings for the dry and rainy seasons. The high tide and low tide sampling was conducted on the same day in order to give an accurate comparison between the two periods. The sampling followed a systematic design, with six stations between the upper and lower reaches of the estuarine waters. These sampling stations were based on the salinity ranges across the estuary, from the oligohaline conditions at the upper reaches to the euhaline conditions at the point where the estuary meets the sea (Montagna et al., 2013). At each station, three (3) replicate sampling points were selected across the estuary's width in order to account for the different environments within the same station. Sampling across the estuary was undertaken in non-motorised artisanal fishing boats.

Environmental parameters, water samples for chlorophyll-*a*, and phytoplankton samples were all collected at a depth of about 0.5 m to maintain uniformity for statistical comparisons. These depths were selected based on evidence from other studies that have shown no stratification (salinity, temperature and DO) within shallow estuaries in Ghana (Dzakpasu & Yankson, 2015), as well as preliminary investigations of the estuary prior to sampling.

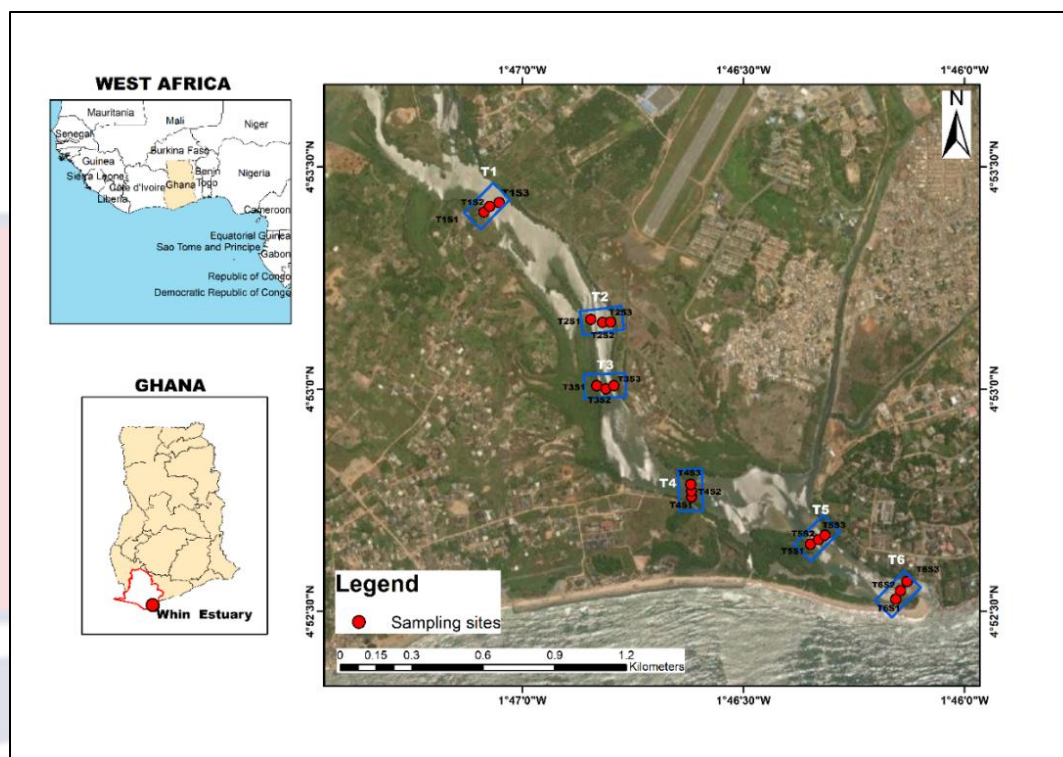


Figure 3: Location of sampling points at the six stations (T1-T6) along the transect on the Whin Estuary in Ghana, West Africa

3.1.2 Measurement of water physicochemical parameters

For each sampling point, environmental parameters, including water temperature, dissolved oxygen, pH, total dissolved solids, conductivity and salinity were measured using a pre-calibrated multi-parameter water quality checker (Horiba U-50 series) according to procedures described by Faseyi, Miyittah, Sowunmi, et al. (2022). The multiparameter water quality checker was dipped into the estuary waters in situ and the stable readings of the physicochemical parameters recorded. Transparency was also measured with a secchi disc (Preisendorfer, 1986). The depths (m) of the stations were determined using a depth sounder (sonar gun, PS-7FL) (Faseyi, Miyittah, Yafetto, et al., 2022).

3.1.3 Phytoplankton and chlorophyll-*a* and analysis

Water samples were also collected for the determination of chlorophyll-*a* concentration and phytoplankton composition. Water samples for these analyses were collected using Niskin water sampler, in line with previous research (Hernández Ruiz et al., 2021). For phytoplankton composition analysis, water samples were transferred into 500 mL of water bottles and fixed with an acidified Lugol Solution (1 mL of Lugol per every 100 mL of water samples) (Cloern, 2018). In contrast, samples for the estimation of Chlorophyll-*a* concentration were kept into 300 mL amber plastic bottles, stored in a cool box and analysed as soon as the samples arrived at the laboratory (2-4 hours between sampling and analysis).

Phytoplankton samples for each station were enumerated and identified according to methods described by Utermöhl, (1958). Water samples were first shaken gently to distribute phytoplankton cells evenly. Afterwards, 50 mL of the water samples were settled in Utermöhl chambers for 24 hours to allow for the cells to settle for enumeration and identification. The entire settled aliquots were examined under an inverted microscope (Olympus CK-2; X400 magnification) and identified using appropriate guides (Bainbridge 1972; Sournia, Chrétiennot-Dinet, and Ricard 1991; Chrétiennot-Dinet et al. 1993). The number of individuals per volume of water was calculated (individuals/ Litre) from the analysed sub-sample. Chlorophyll-*a* water samples were measured in the laboratory with a fluorometer (Trilogy, Turner Designs, USA), after 250 mL of water samples were filtered through Whatman GF/F and the chlorophyll extracted with 95% ethanol (Hernández Ruiz et al., 2021).

Environmental parameters, chlorophyll-*a* water samples, phytoplankton samples were all collected at a depth of about 0.5m to maintain uniformity for statistical comparisons. These depths were selected based on evidence from other studies that have shown no stratification (salinity, temperature and DO) within shallow estuaries in Ghana (Dzakpasu, 2019; Dzakpasu & Yankson, 2015), as well as preliminary investigation of the estuary, prior to sampling.

3.2 Laboratory Exposure of *Thalassiosira Weissflogii* TO Climate Change- and Pollution-Related Stressors

Microcosm experiments were conducted in order to assess the individual and combined effects of pollution and climate change stressors on *Thalassiosira weissflogii*, a diatom species selected for its abundance, ubiquitousness and being able to thrive under wide range of environmental conditions as observed during the field study and supported by Acheampong, Mantey, and Weremfo, (2021). Findings of the field study show that *T. weissflogii* occurred in dry and wet seasons as well as in low and high tides, account for 28% of all sampled phytoplankton genera. Another reason for choosing *T. weissflogii* in the microcosm experiments is that it has previously been used as a bioindicator to investigate the effects of stressors on aquatic organisms (Acheampong et al., 2021; Davis et al., 2006; Garcia et al., 2012; Harrison & Morel, 1986; Jiao et al., 2023).

The climate change related stressors used were temperature, salinity and pH, while the pollution related stressors used were pyrene and cadmium. Temperature, salinity and pH were used to represent climate change-related stressors because they are among the main indicators of climate change in coastal

ecosystems (Ankrah, 2018; IPCC, 2021, 2022). Pyrene and cadmium were used in the study due to their increasingly wide distribution in Ghana's coastal waters (Acheampong, Mantey, and Weremfo 2021; Asiedu 2020; Hernández Ruiz et al. 2021; Essumang 2010). For the study, the target organisms were exposed to five stressors in various levels and combinations to each other.

3.2.1 Culture and maintenance of *Thalassiosira weissflogii*

Thalassiosira weissflogii was obtained from running cultures at the National Institute of Aquatic Resources, Technical University of Denmark (DTU) and acclimatised under conditions similar to those observed in Whin Estuary for a period of two months. The cultures were kept in sterilized conical flasks and grown in seawater enriched with B1 media (preparation described in Appendix F) with vitamins and silicates in order to keep stability and provide natural trace substances. The seawater was passed through a membrane filter and sterilized using UV radiation for at least 30 minutes. The cultures were constantly aerated and maintained at $24 \pm 1^\circ\text{C}$ temperature, and 12:12 (light: dark) cycle artificial light provided by 2 pieces of Ostrum Lumilux cool white, fluorescent tubes positioned above the cultures.

3.2.2 Individual stressor exposure experiments

The experiments were set up by exposing *Thalassiosira weissflogii* to five different levels of climate change induced stressors of temperature, pH, salinity, cadmium and pyrene. Five temperature treatments (i.e., 24, 26, 28, 30 and 32°C) were used, with 28°C taken as the control temperature. This was based on the mean temperature observed at Whin Estuary, as well as being the mean average sea

surface temperature in coastal waters of Ghana during the stable hydrographic period (Asiedu, 2020). The experiment took place in water baths fitted with thermostatic heaters (EHEIM thermocontrol) to maintain constant temperatures (Figure 4). Temperatures were monitored every 15 minutes using digital temperature loggers (Onset UTBI-001, accuracy ± 0.21 °C). The salinity levels used for the individual experiments were 1, 5, 15, 25, and 35‰. These salinity ranges represented the different salinities found in estuarine environments ranging from freshwater, brackish and marine environments (Potapova, 2011). The different salinity concentrations were obtained from the ranges observed in Whin Estuary gradient, from the oligohaline to euhaline sections. The different salinity concentrations were obtained by diluting seawater with distilled water and adding artificial sea salt (Sera artificial marine and sea salt) when required.

Hydrogen ion concentrations (pH) treatments of 5, 6, 7, 8, and 9 were used to represent a range of neutral, acidic and alkaline waters found in aquatic ecosystems (Strickler et al., 2015). Similar to temperature and salinity, the different pH levels were obtained from the field observations in Whin Estuary for both the rainy and dry season. These pH ranges were achieved by adding either 1 M HCl or 0.5 M NaOH to the natural and artificial seawater mixture (Berge et al., 2010). A Sentron pH meter, equipped with a red line electrode, was used to measure pH.

The cadmium (Cd) in this study was presented in the form of cadmium chloride (CdCl_2) and stock solution was prepared as described by Asiedu, (2020). Briefly, 100 mg (0.1M) of CdCl_2 was prepared by dissolving the salt in 1 L of deionised 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. The experimental concentrations used were 0, 1, 3, 5, 7, 10 µg/L prepared by diluting a subsample of the stock solution with deionized water to achieve desired concentration. These concentrations of CdCl₂ were chosen after undertaking a range-finding test with higher concentrations of up to 100 µg/L (Appendix A).

The different concentrations of pyrene used for this study were 0, 10, 100, and 100+ nM (> 100 nM). Stock solutions were prepared by dissolving pyrene granules in acetone (Hernández Ruiz et al., 2021). Nominal stock solutions (0.1, 1 and 3 mM) were prepared weighing granulated pyrene (Sigma-Aldrich, purity N 99 %) on an analytical balance (Adventurer AX124, Ohaus, USA, readability 0.1 mg) and dissolving it into acetone (Appendix H). Stock solutions were kept in 50 ml amber glass bottles in dark at 4 °C and each bottle was protected with aluminium foil to avoid phototoxicity, since the toxicity of PAHs such as pyrene increase by exposure to natural or artificial sunlight via photosensitization and/ or photo-modification reactions (Hernández Ruiz et al., 2021). An additional solvent control was included in experiments to test whether acetone had any effect on the functional attributes of *T. weissflogii*.

For the different sets of experiments, *T. weissflogii* were maintained in fish bags filled with 1.5 L of enriched seawater. The fish bags were inoculated with stock cultures of the diatoms to a concentration of 2500 cells/mL that were determined from preliminary cell counts (Acheampong et al., 2021). The fish bags were then suspended into water baths containing the control (28 °C) or experimental

temperatures (for temperature experiments only). Each of the experimental treatment was replicated three times. Figure 4 below, gives an outline of the experimental set up.



Figure 4: Water baths used for the different experimental treatments

NB: The experimental set up involved large plastic containers filled with water under different temperature conditions. The fish transportation bags filled with sea water, diatoms and different levels of pollutants were suspended from wooden columns into the water bath. The wooden columns were fitted with fluorescent tubes.

3.2.3 Combined stressor experiments

The design of the experiments used for assessing the combined impact of the environmental stressors is described in Table 1. The organisms were exposed to combinations of cadmium and pyrene concentrations at different treatment levels

(10, 100, 1000 $\mu\text{g/L}$) against different climate change-related stressors (i.e., temperature, pH and salinity). The proportion of cadmium and pyrene in the combined exposure experiments were obtained from calculating the values of their effective concentrations (EC_{50}) values from the individual stressor experiments (Mensah et al., 2017). There were three different levels of climate change-related

Table 1: Experimental Design Indicating the Different Climate Change and Pollution Levels in the Combined Exposure Experiment

Environmental Stressor		Cadmium and Pyrene Concentrations ($\mu\text{g/L}$)			
	Levels	0	10	100	1000
Control (Control (temperature; 28 ± 1 , salinity; 25 ‰, pH; 8)		✓	X	X	X
Temperature ($^{\circ}\text{C}$)	24	X	✓	✓	✓
	28	X	✓	✓	✓
	32	X	✓	✓	✓
Salinity (‰)	15	X	✓	✓	✓
	25	X	✓	✓	✓
	35	X	✓	✓	✓
pH	5	X	✓	✓	✓
	7	X	✓	✓	✓
	9	X	✓	✓	✓

stressors, each of which was paired against the three levels of cadmium and pyrene combined concentrations (Table 1). The different treatments for temperature, salinity and pH were based on the results of the individual experiments as well as the projected impacts of climate change of these factors on Ghana's coastline (Ankrah, 2018; Asiedu, 2020; IPCC, 2021; Karikari et al., 2006; Nunoo et al., 2016; Salgado-Hernanz et al., 2021). The different conditions in the combined stressors were set according to the procedures described above in the individual stressor experiments.

3.2.4 Genotoxicity of combined stressors

Cells from the combined stressor experiments were used to determine the response of *Thalassiosira weissflogii* at the genetic molecular level. Temperature, pH and salinity levels selected for the molecular analysis were 32 °C, 5 and 35 ‰, respectively. These stressor levels were chosen because they represented warming, acidification and increased salinity, respectively, based on the climate change projections for the coastal ecosystems of Ghana (IPCC, 2021; Karikari et al., 2006; Nunoo et al., 2016). Procedures for genotoxicity measurements have been further described in subsequent sections of this chapter i.e., 3.4 on molecular analyses.

3.2.5 Determination of algal growth rate

Growth rate of the experimental diatom, *Thalassiosira weissflogii*, was determined through daily cell count of the culture, in line with recent investigations (Acheampong et al., 2021). The cells were collected at the same time interval after every 24 hours. Prior to the collection, samples containing the experimental setup were agitated several times to ensure uniform mixing of the cells. From each setup,

5 mL of the cultures was taken and fixed in 2% Lugol's solution for the cell count, after which 1 mL of the fixed sample was pipetted into a Sedgewick Rafter counting chamber and counted under an inverted microscope (Olympus CK-2). The Equation below was used to determine the specific growth rate (μ) of the cultures:

$$(\mu) = (\ln X - \ln X_0) / (t - t_0)$$

where X = cell count (cells mL⁻¹) at time of harvest (t); and X₀ = cell count at a time (t₀) before the time of harvest.

3.2.6 Determination of chlorophyll-*a* content

Chlorophyll-*a* concentrations were measured according to procedures previously described by Hernández Ruiz et al. (2021). Chlorophyll was extracted using 95% ethanol by filtering 100 mL of the cultured sample through 0.7 µm glass fibre filters (GF/F, Camlab). Chlorophyll-*a* concentrations were measured daily on a pre-calibrated fluorometer (Trilogy, Turner Designs, USA).

3.2.7 Determination of dry weight

The procedures for dry weight determination followed methods described in previous reports (Teoh, Phang, and Chu, 2013; Acheampong, Mantey, and Weremfo, 2021). A fixed volume (50 ml) of the culture with known density of cells was filtered onto pre-combusted (45°C, 5 h) glass fibre filters (GF/F, Camlab). The filter paper was then dried in an oven for 24 hours at a temperature of 100°C. The following equation was used to determine the algal dry weight (DW):

$$\text{Dry Weight (DW)} = \frac{\text{Weight of filter with algae} - \text{Weight of blank filter paper}}{\text{Volume of filtered algae (mL)} * \text{Density of algae filtered}}$$

3.3 Data Analysis

3.3.1 Statistical analysis of field observations

Descriptive statistics and analysis of variance were conducted using the Minitab Statistical Software Version 21.1.1 and Microsoft Excel version 2019. A two-way ANOVA was undertaken to compare the environmental and plankton parameters observed on different transects, tidal periods and sampling seasons. The diversity, richness and evenness of phytoplankton communities were evaluated based on indices proposed by Shannon & Weaver (1998), Margalef (1958) and Pielou, (1966) diversity indices, respectively (Hasan et al., 2022) in order to understand the variations in plankton composition and distribution between the two seasons and tidal periods. The relationship between phytoplankton distribution and environmental parameters were established with canonical correspondence analysis, using the Palaeontological Statistics (PAST) Package version 4.03.

3.3.2 Statistical analysis of laboratory observations

The effects of temperature, pH, salinity, cadmium and pyrene as well as their combinations on growth rate, chlorophyll-*a* content and biomass (dry weight) of *Thalassiosira weissflogii* were evaluated using multivariate ANOVA and Tukey's comparison test (post hoc test). A probit analysis was performed using the GraphPad Prism software version 9.5 to determine the median effective concentrations (EC₅₀), i.e., the concentration that has significant effect on 50% of test organisms.

Transcriptome reconstruction

Transcriptome reconstruction was conducted according to the procedures described by Haas et al., (2013), using the Trinity software (which was developed by the Broad Institute and the Hebrew University of Jerusalem). The Trinity software comprises three modules; Inchworm, Chrysalis, and Butterfly, whose workflow included: The inchworm constructs k-mer dictionary from all sequenced reads, the chrysalis clusters minimally overlapping contigs into sets of connected components and develops de Bruijn graphs for each component. Lastly, the Butterfly module reconciles the individual de Bruijn graphs to reconstruct plausible, full-length, linear transcripts with the original reads and paired end-reads.

Sequence alignment

After the transcriptome reconstruction process, the sequences were aligned using the Burrows-Wheeler Aligner software according to procedures described by Li & Durbin (2010).

Gene expression levels

The RNA-Seq by Expectation Maximization (RSEM) software was used to calculate the gene expression level (Li & Dewey, 2011). Each read count was converted into expected number of Fragments Per Kilobase of transcript sequence per Millions of base pairs sequenced (FPKM values.) These FPKM values were for estimating gene expression levels.

Analysis of gene expression difference

To analyse the gene expression difference, samples were initially grouped to identify the existing correlations (pairwise) between two groups and a control

treatment. For the analysis a Fold Change ≥ 2 and False Discovery Rate (FDR) < 0.01 were set as the screening criteria (Haynes et al., 2017). The Fold Change is used to indicate the ratio of expression levels between two samples or groups, while the False Discovery Rate (FDR) is obtained by correcting the p-value of the significant differences. To eliminate false positives and analyse the original hypothesis, a Benjamini-Hochberg calibration method was adopted for the differential expression analysis (Haynes et al., 2017).

Construction of volcano plot for differential expression genes

A volcano plot was used to visualise the expression of differential transcripts (Li 2012). The volcano plots allow for the visualisation of large magnitude changes in RNA transcripts that are also statistically significant and are constructed by plotting the expression fold change ($\log_2$) between the two experimental groups on the x-axis and the FDR ($-\log_{10}$) on the y-axis. Figure 6 below gives a summary of the workflow that was used in the molecular analysis.

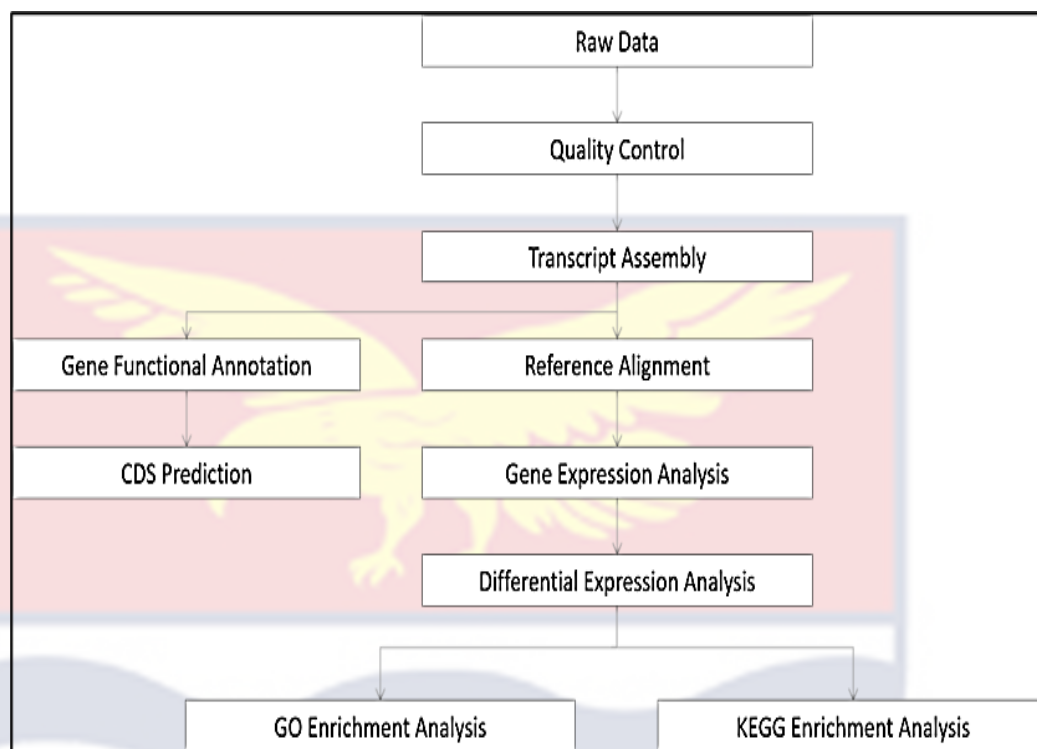


Figure 6: Molecular analysis workflow

3.5 Chapter Summary

The different materials and methods employed during the field and laboratory investigations were exhaustively described in this chapter of the thesis. In addition, the different statistical analysis tools and software that were used to analyse the data have been highlighted.

CHAPTER FOUR

RESULTS

This chapter presents the results obtained from field sampling, laboratory exposure experiments, and molecular analysis using relevant Tables and Figures.

4.1 Field Study

This section elaborates on the results of the field study undertaken between two different seasons and tidal periods, which includes data on abundance, distribution and composition of phytoplankton parameters at Whin Estuary as well as the pre-existing physicochemical parameters.

4.1.1 Physicochemical parameters

The observed physicochemical conditions in Whin Estuary have been summarised in Table 2. The highest salinity levels were recorded during high tide in the dry season (19.89 ± 3.01), while the lowest levels were observed during low tide in the rainy season (0.26 ± 0.02) (Table 2, Figure 7). Salinity differed significantly across the two seasons and tidal period, as well as across the six transects (distance across the length of Whin Estuary) ($p < 0.01$). The recorded water temperature ranged between 25.63°C - 33.19°C . Higher temperatures were observed at low tide in the dry season and high tide in the rainy season. The temperature varied significantly ($p < 0.01$) during seasons with the dry season recording a higher mean temperature than in the wet season (Figure 7). The pH of Whin estuary ranged between 3.82 - 8.33. pH differed significantly across the two seasons ($p < 0.01$), with the rainy season recording lower pH than the dry season.

However, there was no significant differences ($p > 0.05$) in pH across the different tidal periods and across the transects (distance).

DO values recorded along the Whin estuary gradient ranged between 1.4 - 10.4 mg/L and varied significantly ($p < 0.05$) across the seasons and tidal periods (Figure 8). However, no significant differences in DO levels were observed across the different transects (distance) ($p = 0.3$). Chlorophyll-*a* varied significantly ($p < 0.05$) between seasons, with the dry recording higher chlorophyll than the rainy season (Figure 8). Whin estuary is shallow with depths ranging from 0.21 - 2.6 m. Depth varied significantly ($p > 0.05$) across the different transects and different tidal periods, but not across seasons (Table 2). Transparency (light penetration) at the Whin Estuary ranged between 0.12 - 0.81 cm and varied significantly between the two seasons and tidal periods.

Table 2: Seasonal Physicochemical Parameters (+SE) Recorded Along the Whin Estuary Gradient

Parameter	Dry Season		Rainy Season	
	High Tide	Low Tide	High Tide	Low Tide
Temperature (°C)	29.084 ± 0.12	32.64 ± 0.1	26.69 ± 0.05	26.24 ± 0.08
pH	7.5 ± 0.008	7.91 ± 0.17	4.5 ± 0.15	4.6 ± 0.09
Conductivity (mS/cm)	40.32 ± 3.35	30.7 ± 2.25	6.47 ± 1.75	0.54 ± 0.04
DO (mg/l)	4.99 ± 0.32	7.789 ± 0.47	4.56 ± 0.2	3.6 ± 0.36
TDS (ppt)	24.5 ± 1.89	19.41 ± 1.44	4.11 ± 1.08	0.35 ± 0.02
Salinity (ppt)	19.89 ± 3.01	10.4 ± 1.62	3.87 ± 1.1	0.26 ± 0.02
Chlorophyll-a (mg/L)	6.2 ± 1.28	29.55 ± 2.53	2.169 ± 0.32	1.42 ± 0.19
Depth (cm)	86 ± 7.61	75.7 ± 10.4	111.7 ± 11.1	63.33 ± 7.8
Transparency(c m)	57.61 ± 3.45	38.36 ± 2.91	20.47 ± 1.05	18.18 ± 0.89

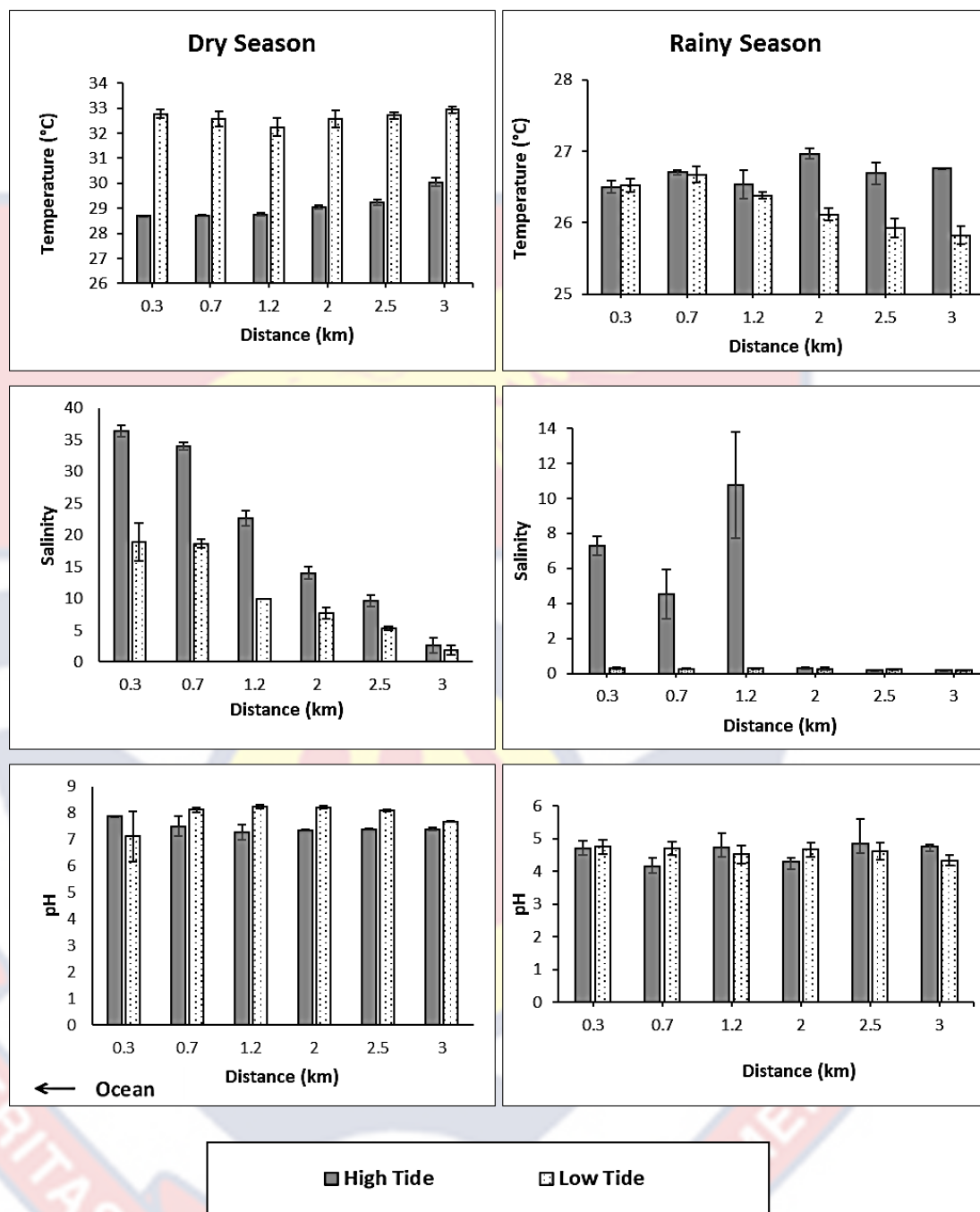


Figure 7: Seasonal variation of temperature, salinity, and pH along the Whin Estuary gradient at both high and low tide

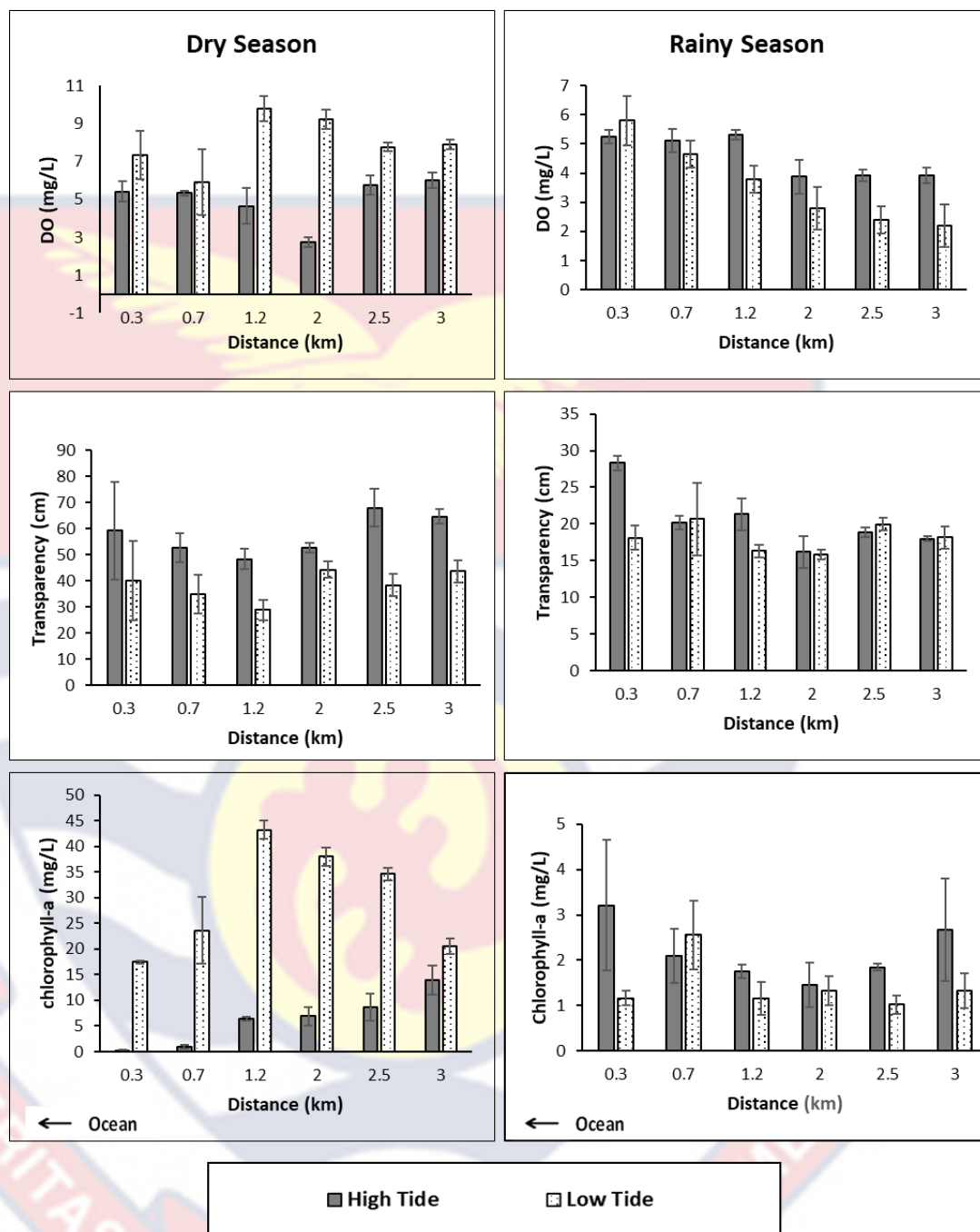


Figure 8: Seasonal variation of Dissolved Oxygen, Transparency and Chlorophyll-*a* along the Whin Estuary gradient at both high and low tide

4.1.2 Phytoplankton abundance and distribution

Seven (7) classes of phytoplankton communities were identified in Whin Estuary: Bacillariophyceae, Chlorophyceae, Coscinodiscophyceae, Cyanophyceae, Dinophyceae, Euglenophyceae, Zygnematophyceae (Table 3). Higher abundances were observed for class Bacillariophyceae and Dinophyceae with 29 and 19 genera, respectively. Phytoplankton genera including *Cymbella* spp., *Navicula* spp., *Nitzschia* spp, *Thalassiosira* spp, *Cylindrotheca* sp. belonging to the class Bacillariophyceae, and genera *Euglena* spp. belonging to Euglenophyceae were observed during all seasons and tidal periods (Tables 3). However, *Asterionellopsis* sp., *Phacus* sp., *Ceratium* spp., *Pseudo-nitzschia* sp., *Grammatophora* sp. and *Diploneis* sp. showed the lowest dispersion within the Whin estuary during the study period (Table 3).

Table 3: Comparison of Phytoplankton Abundance (cells/L) and Proportion (%) during Different Seasons and Tidal Periods in Whin Estuary, Ghana

Taxa	Dry Season		Rainy Season		Total
	High Tide	Low Tide	High Tide	Low Tide	
Bacillariophyceae (cells/L/ %)					
<i>Amphora</i> sp.	160 (0.1)	60 (0.014)	160 (0.822)	-	380 (0.061)
<i>Asterionellopsis</i> sp.	-	-	-	4520 (38.898)	4520 (0.727)
<i>Bacillaria</i> sp.	-	-	160 (0.822)	20 (0.172)	180 (0.029)
<i>Chaetoceros</i> spp.	320 (0.2)	-	20 (0.103)	20 (0.172)	360 (0.058)
<i>Cocconeis</i> sp.	160 (0.1)	660 (0.153)	120 (0.617)	-	940 (0.151)
<i>Cyclotella</i> sp.	20 (0.012)	40 (0.009)	180 (0.925)	-	240 (0.039)
<i>Cylindrotheca</i> sp.	45700 (28.541)	88540 (20.548)	3380 (17.369)	1880 (16.179)	139,500 (22.424)
<i>Cymatopleura</i> sp.	20 (0.012)	60 (0.014)	-	-	80 (0.013)
<i>Cymbella</i> spp.	760 (0.475)	520 (0.121)	320 (1.644)	180 (1.549)	1,780 (0.286)
<i>Denticula</i> sp.	-	-	-	20 (0.172)	20 (0.003)
<i>Detunola</i> sp.	-	20 (0.005)	-	-	20 (0.003)
<i>Diploneis</i> sp.	-	-	20 (0.103)	-	20 (0.003)
<i>Entomoneis</i> sp.	300 (0.187)	80 (0.019)	-	-	380 (0.061)
<i>Fragilaria</i> sp.	-	-	80 (0.411)	20 (0.172)	100 (0.016)
<i>Gomphonema</i> sp.	20 (0.012)	-	-	-	20 (0.003)
<i>Grammatophora</i> sp.	-	-	-	100 (0.861)	100 (0.016)
<i>Gyrosigma</i> spp.	60 (0.037)	120 (0.028)	120 (0.617)	-	300 (0.048)
<i>Hemiaulus</i> sp.	40 (0.025)	20 (0.005)	-	-	60 (0.01)

Table 3, Continued

<i>Licmophora</i> sp.	200 (0.125)	540 (0.125)	-	-	740 (0.119)
<i>Mastogloia</i> sp.	-	-	40 (0.206)	-	40 (0.006)
<i>Navicula</i> spp.	11060 (6.907)	15460 (3.588)	7640 (39.26)	2840 (24.441)	37,000 (5.948)
<i>Nitzschia</i> spp.	1320 (0.824)	1280 (0.297)	920 (4.728)	360 (3.098)	3,880 (0.624)
<i>Pinnularia</i> spp.	320 (0.2)	420 (0.097)	80 (0.411)	-	820 (0.132)
<i>Pleurosigma</i> spp.	140 (0.087)	20 (0.005)	140 (0.719)	-	300 (0.048)
<i>Pseudo-nitzschia</i> sp.	-	100 (0.023)	-	-	100 (0.016)
<i>Surirella</i> sp.	-	120 (0.028)	20 (0.103)	-	140 (0.023)
<i>Thalassionema</i> sp.	20 (0.012)	-	-	-	20 (0.003)
<i>Thalassiosira</i> spp.	32920 (20.56)	137100 (31.817)	5340 (27.441)	1480 (12.737)	176,840 (28.426)
<i>Ulnaria</i> sp.	-	-	20 (0.103)	-	20 (0.003)
Chlorophyceae					
<i>Dunaliella</i> spp.	80 (0.05)	100 (0.023)	-	-	180 (0.029)
<i>Pediastrum</i> sp.	-	20 (0.005)	100 (0.514)	-	120 (0.019)
Coscinodiscophyceae					
<i>Coscinodiscus</i> sp.	-	120 (0.028)	-	20 (0.172)	140 (0.023)
Cyanophyceae					
<i>Pseudanabaena</i> sp.	10320 (6.445)	-	-	-	10320 (1.659)
<i>Nostoc</i> sp.	38400 (23.982)	177860 (41.276)	-	-	216260 (34.763)
<i>Oscillatoria</i> sp.	-	-	20 (0.103)	-	20 (0.003)
Dinophyceae					
<i>Alexandrium</i> sp.	60 (0.037)	160 (0.037)	-	-	220 (0.035)
<i>Amphidium</i> sp.	40 (0.025)	-	20 (0.103)	-	60 (0.01)
<i>Ceratium</i> sp.	-	-	-	20 (0.172)	20 (0.003)

Table 3, Continued

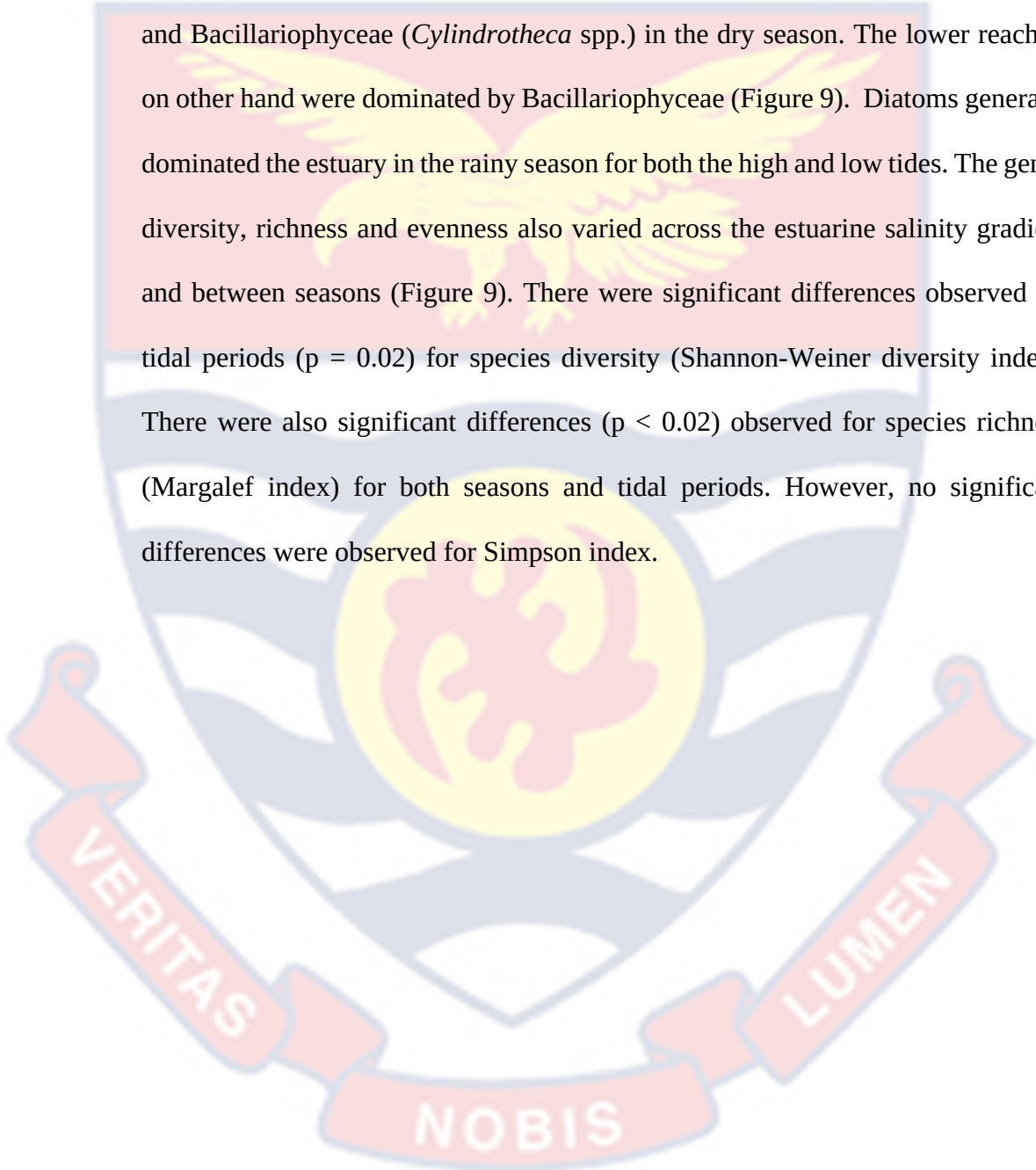
<i>Dinophysis</i> sp.	-	-	20 (0.103)	-	20 (0.003)
<i>Gymnodinium</i> sp.	80 (0.05)	160 (0.037)	-	-	240 (0.039)
<i>Gyrodinium</i> spp.	320 (0.2)	680 (0.158)	-	-	1000 (0.161)
<i>Heterocapsa</i> spp.	1200 (0.749)	620 (0.144)	-	-	1820 (0.293)
<i>Peridinium</i> spp.	5340 (3.335)	3680 (0.854)	-	-	9020 (1.45)
<i>Prorocentrum</i> spp.	2340 (1.461)	160 (0.037)	80 (0.411)	20 (0.172)	2600 (0.418)
<i>Scrippsiella</i> spp.	780 (0.487)	320 (0.074)	-	-	1100 (0.177)
Euglenophyceae					
<i>Euglena</i> spp.	380 (0.237)	860 (0.2)	200 (1.028)	20 (0.172)	1460 (0.235)
<i>Eutreptiella</i> spp.	6520 (4.072)	920 (0.214)	20 (0.103)	-	7460 (1.199)
<i>Phacus</i> sp.	-	-	20 (0.103)	-	20 (0.003)
Zygnematophyceae					
<i>Cosmaria</i> sp.	-	-	60 (0.308)	20 (0.172)	80 (0.013)
Other					
<i>Ciliophora</i> spp.	720 (0.45)	80 (0.019)	160 (0.822)	80 (0.688)	1040 (0.167)

(-) indicates absence of phytoplankton genera during the sampling period

() values in brackets indicate the proportion (%) of the different phytoplankton genera across the different season and tidal periods

Significantly ($p < 0.05$), higher phytoplankton abundance ($49,252 \pm 10,955$ cells/litre) was recorded in the dry season than in the rainy season ($2,590 \pm 357$

cells/litre). However, there were no significant differences between the high and low tide ($p > 0.05$) or along the different transects ($p > 0.05$) of the estuary. The estuary's upper reaches were dominated primarily by Cyanophyceae (*Nostoc* sp.), and Bacillariophyceae (*Cylindrotheca* spp.) in the dry season. The lower reaches, on other hand were dominated by Bacillariophyceae (Figure 9). Diatoms generally dominated the estuary in the rainy season for both the high and low tides. The genus diversity, richness and evenness also varied across the estuarine salinity gradient and between seasons (Figure 9). There were significant differences observed for tidal periods ($p = 0.02$) for species diversity (Shannon-Weiner diversity index). There were also significant differences ($p < 0.02$) observed for species richness (Margalef index) for both seasons and tidal periods. However, no significant differences were observed for Simpson index.



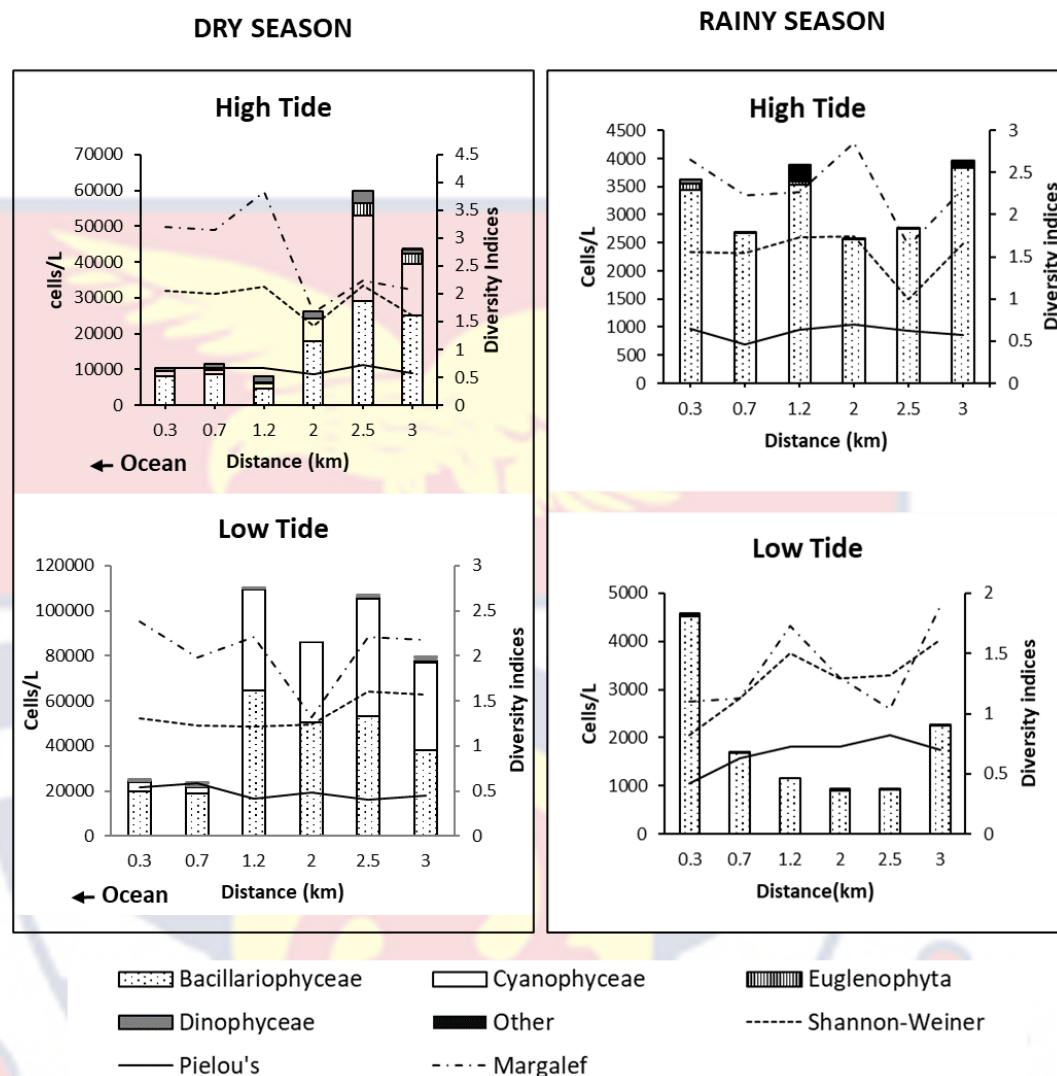


Figure 9: Phytoplankton abundance, diversity (Shannon-Weiner diversity index), richness (Margalef index) and evenness (Simpson index) and distribution along the Whin River Estuary

The scatterplot of the canonical correspondence analysis (CCA) for the main environmental parameters and phytoplankton genera in relation to the different sampling seasons and tidal periods is presented in Figure 10. The first axis which accounted for total variance of 51.36% was negatively correlated with all the environmental variables (temperature, pH, salinity DO and Chlorophyll-*a*

concentration). Axis 2 accounted for 38.52% of the variation and was positively correlated with all the environmental variables. Phytoplankton belonging to the genus *Nostoc* sp., had a positive correlation with all the physicochemical parameters and was mostly associated with chlorophyll-*a*. *Navicula* spp. Showed negative correlations with the environmental parameters. Phytoplankton genera i.e., *Eutreptiella* spp. and *Pseudanabaena* sp. had no correlation with the environmental parameters.

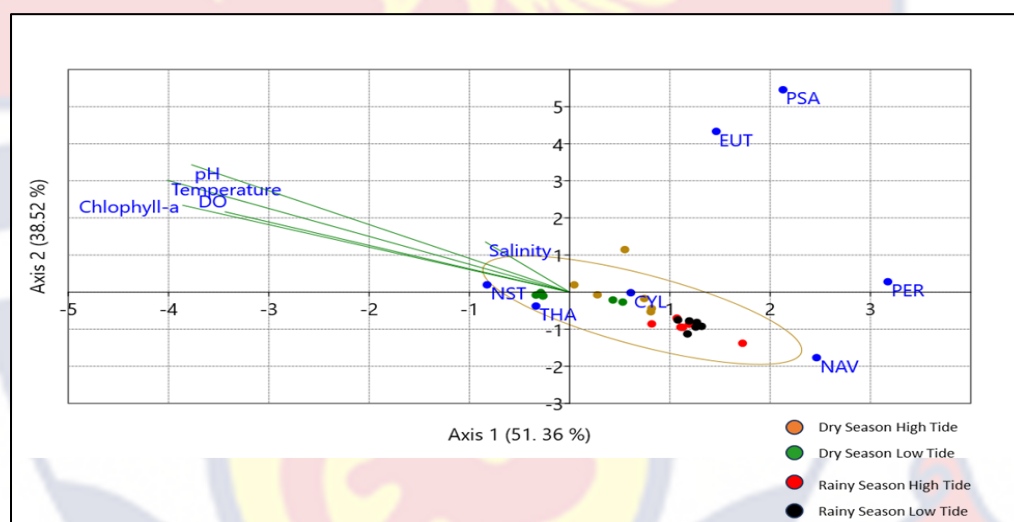


Figure 10: Canonical correspondence analysis between the major environmental parameters and phytoplankton species in Whin Estuary. NST: *Nostoc* sp., THA: *Thalassiosira* spp., CYL: *Cylindrotheca* sp., NAV: *Navicula* spp., PSA: *Pseudanabaena* sp., PER: *Peridinium* spp., EUT: *Eutreptiella* spp.

4.2 Laboratory Exposure of *Thalassiosira weissflogii* to Individual Climate Change and Pollution Related Stressors

Thalassiosira weissflogii were exposed to varying levels of temperature, pH, salinity, cadmium, and pyrene concentrations and their responses were measured by assessing their growth rate, chlorophyll-*a* concentration, and dry

biomass. Exponential growth was observed in the control treatments of all the individual experiments (Figure 11-15). The one-way ANOVA analyses for the different treatments have been summarised in Table 4. In comparison, information on total cell densities (cells/mL), chlorophyll-*a* densities ($\mu\text{g/L}$) and total biomass densities ($\mu\text{g/L}$) for the different treatments is presented in table 5.

Table 4: One-way ANOVA Comparing Growth Rates, Chlorophyll-*a*, and Dry Biomass Characteristics for the Different Individual Exposure Experiments

a) Growth-rate (d^{-1})

Stressor	24 hours		48 hours		72 hours	
	F	P-value	F	P-value	F	P-value
Temperature	2.97	0.07	4.84	0.02*	0.26	0.89
Salinity	6.24	0.01*	89.19	<0.01*	195.81	<0.01*
pH	1.63	0.25	4.4	0.03*	2.95	0.08
Pyrene	2.63	0.03*	1.73	0.15	1.08	0.38
Cadmium	5.06	0.01*	2.04	0.15	1.79	0.19

b) Chlorophyll-*a* ($\mu\text{g/cell}$)

Table 4: Continued

Stressor	24 hours		48 hours		72 hours	
	F	P-value	F	P-value	F	P-value
Temperature	0.32	0.86	0.7	0.611	0.17	0.38
Salinity	0.33	0.85	7.49	0.01*	15.5	<0.01*
pH	8.75	0.01	2.5	0.12	0.29	0.87
Pyrene	28	<0.01*	8.33	<0.01*	0.69	0.63
Cadmium	1.20	0.37	1.43	0.28	2.26	0.12
c) Dry biomass ($\mu\text{g}/\text{cell}$)						
Temperature	2.52	0.10	2.0	0.17	0.91	0.50
Salinity	1.39	0.32	22.95	<0.01*	21.2	<0.01*
pH	4.32	0.03	2.35	0.13	0.9	0.50
Pyrene	13.0	<0.01*	3.01	0.02*	1.68	0.15
Cadmium	0.47	0.79	0.24	0.94	12.61	<0.01*

The asterisk (*) indicate significantly different (<0.05) treatments

Table 5: Mean (+SE) Cell Densities, Chlorophyll-a($\mu\text{g/L}$), and Dry Biomass (mg/L) for the Different Individual (Climate Change And Pollution)

Exposure Experiments

Stressor	Stressor level	Cell Densities (Cells/mL)	Chlorophyll- <i>a</i> ($\mu\text{g/L}$)	Dry Biomass (mg/L)
<i>Temperature ($^{\circ}\text{C}$)</i>	24	4860 $\pm$ 577	15.16 $\pm$ 1.89	1304 $\pm$ 148
	26	4606 $\pm$ 502	15.15 $\pm$ 2.22	1281.8 $\pm$ 71.2
	28	4953 $\pm$ 481	17.64 $\pm$ 2.38	1269.1 $\pm$ 67.2
	30	5298 $\pm$ 614	20.68 $\pm$ 3.57	1191 $\pm$ 120
	32	5301 $\pm$ 614	15.88 $\pm$ 2.29	1194 $\pm$ 103
<i>Salinity (‰)</i>	1	1674 $\pm$ 377	4.562 $\pm$ 0.68	775 $\pm$ 148
	5	1863 $\pm$ 315	4.638 $\pm$ 0.94	646.5 $\pm$ 86.8
	15	5843 $\pm$ 761	9.05 $\pm$ 1.75	615 $\pm$ 112
	25	6248 $\pm$ 739	11.43 $\pm$ 1.89	656 $\pm$ 104
	35	6441 $\pm$ 905	10.74 $\pm$ 1.87	789 $\pm$ 159
<i>pH</i>	5	3738 $\pm$ 359	17.82 $\pm$ 1.6	1087 $\pm$ 121
	6	4108 $\pm$ 325	21.46 $\pm$ 2.07	1165 $\pm$ 106
	7	4837 $\pm$ 435	23.22 $\pm$ 2.04	1102 $\pm$ 106
	8	3707 $\pm$ 326	18.05 $\pm$ 2.07	1226 $\pm$ 188
	9	4098 $\pm$ 334	23.54 $\pm$ 2.91	1176 $\pm$ 108

Table 5, Continued

Pyrene (nM)	Control	5576 ± 640	7.662 ± 0.61	789.7 ± 44.3
	(no pyrene)			
	Control	5668 ± 724	7.223 ± 0.79	757.8 ± 38.8
	(Acetone)			
	1	4940 ± 437	7.306 ± 0.701	726.9 ± 29.6
	10	4815 ± 454	7.656 ± 0.88	713.6 ± 45.5
	100	4513 ± 367	8.4 ± 1.08	774.3 ± 38.5
	100	4441 ± 417	8.021 ± 0.9	751.5 ± 35.2
Cadmium (µg/L)	Control	4379 ± 463	9.46 ± 1.57	589 ± 90.2
	1	4082 ± 341	12.94 ± 1.66	691 ± 117
	3	4171 ± 329	9.04 ± 1.89	750.2 ± 96.7
	5	3933 ± 373	10.91 ± 1.92	695 ± 85.3
	7	3685 ± 260	10.28 ± 1.08	646.4 ± 88.1
	10	3636 ± 248	12.19 ± 2.12	647.4 ± 90.1

4.2.1 Effects of exposure of different temperature conditions on *Thalassiosira weissflogii*

Higher growth rates were observed at temperatures of 28, 30 and 32 °C within the first 48 hours (Figure 11). All temperature treatments showed exponential growth rates throughout the experiment, except for 30 and 32 °C, which

dropped after 72 hours of exposure. There was an increase in growth rate with increased temperature i.e., 0.58, 0.54, 0.62, 0.67 and 0.67 growth rate (d^{-1}) for the 24, 26, 28, 30 and 32°C of treatments respectively. There were no significant differences observed for the different treatments across the three days (Table 4) except for the second day after exposure ($p < 0.05$). Higher cell densities were also observed at higher temperatures throughout the exposure period (Table 5). Phytoplankton under the 30 and 32 °C treatments recorded about 10% increase in cell density above the control (28 °C). On the other hand, phytoplankton in the 24 and 26 °C treatments recorded a decrease in cell densities of 2.15% and 8.06%, respectively (Appendix C).

Chlorophyll-*a* and biomass concentrations in each cell decreased across the 72-hour period. There were, however, no significant differences ($p > 0.05$) in both chlorophyll-*a* and biomass concentrations across all treatments (Table 4). Cumulatively, the highest chlorophyll-*a* concentration and dry biomass ($\mu g/L$) were recorded in the control groups (28 °C) (Table 5).

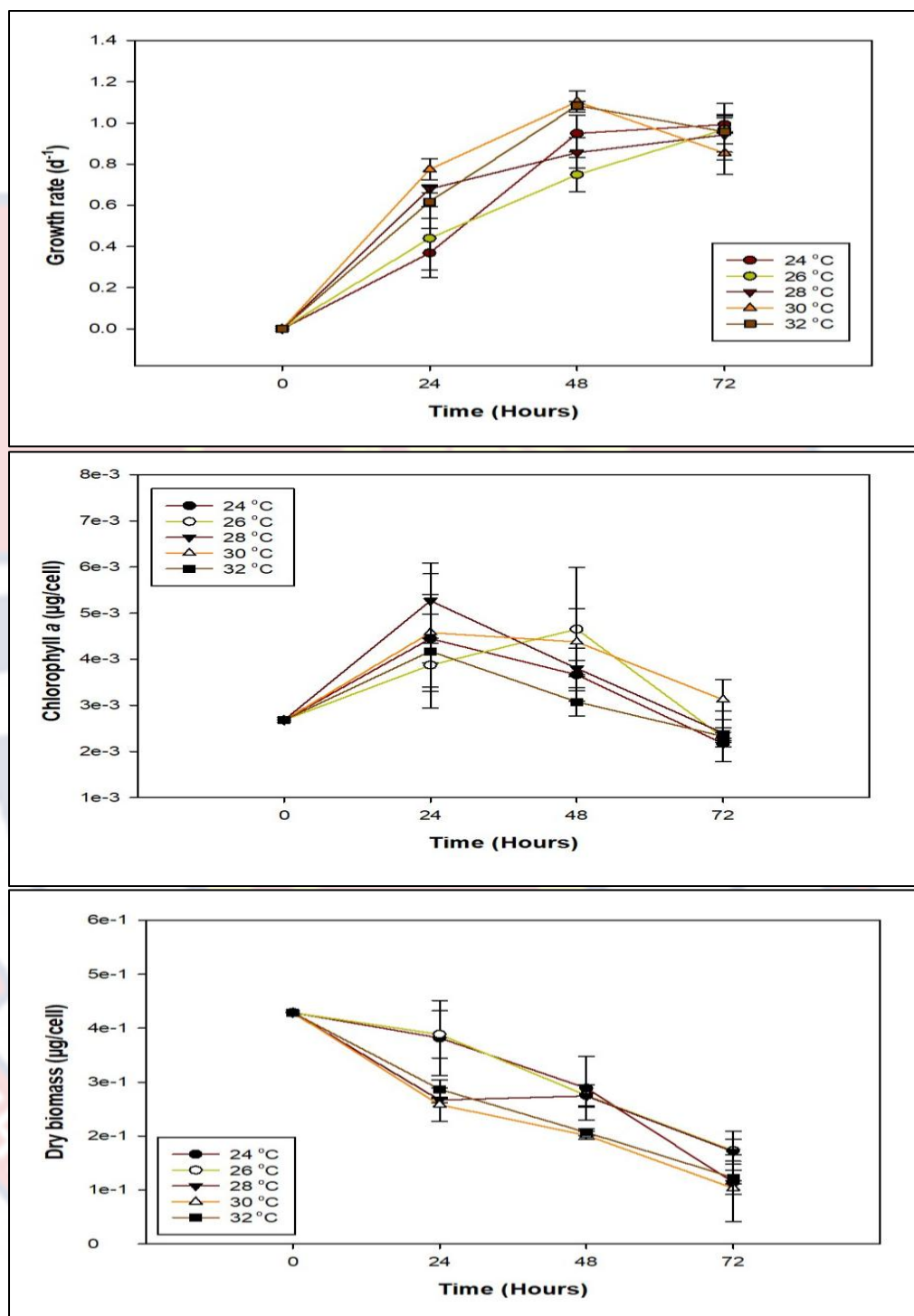


Figure 11: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different temperature conditions

4.2.2 Effects of exposure of different salinity conditions on *Thalassiosira weissflogii*

Salinity levels of 15, 25, and 35‰ showed exponential growth rates throughout the duration of the exposure experiments. However, the growth rates for 1 and 5‰ declined to below 0 after 24 hours of exposure (Figure 12). The mean growth rates (d^{-1}) observed in the experiments were 0.2 with minimum and maximum observed growth rates (d^{-1}) of -1.928 and 1.419, respectively. Significant differences ($p < 0.05$) in growth rates were observed for all treatments in all three days (Table 4). There were higher amounts of chlorophyll-*a* and dry biomass concentrations per cell in the 1 and 5‰ treatments. The highest cell densities were observed in the 25‰ (6248 ± 739 cells/mL) and 35‰ treatments (6441 ± 905 cells/mL), while the lowest treatments were observed in the 1‰ (1674 ± 377 cells/mL) and 5‰ (1863 ± 315 cells/mL) treatments, respectively (Table 8). Phytoplankton in the 1‰ and 5‰ treatments recorded more than 75% decrease in cell densities from the control (25 ‰).

Treatments with higher salinity levels had lesser amounts of chlorophyll-*a* and biomass concentrations per cell, despite the observed high growth rates (cell densities) (Table 5). There were no significant differences in chlorophyll-*a* concentrations and biomass ($p > 0.05$) for the first 24 hours. However, significant differences ($p < 0.05$) were observed after 48 and 72 hours respectively. Cumulatively, the highest value in chlorophyll-*a* ($\mu g/L$) was recorded in the 25‰ treatment group, while the highest dry biomass was recorded in the 35‰ treatment group (Table 5).

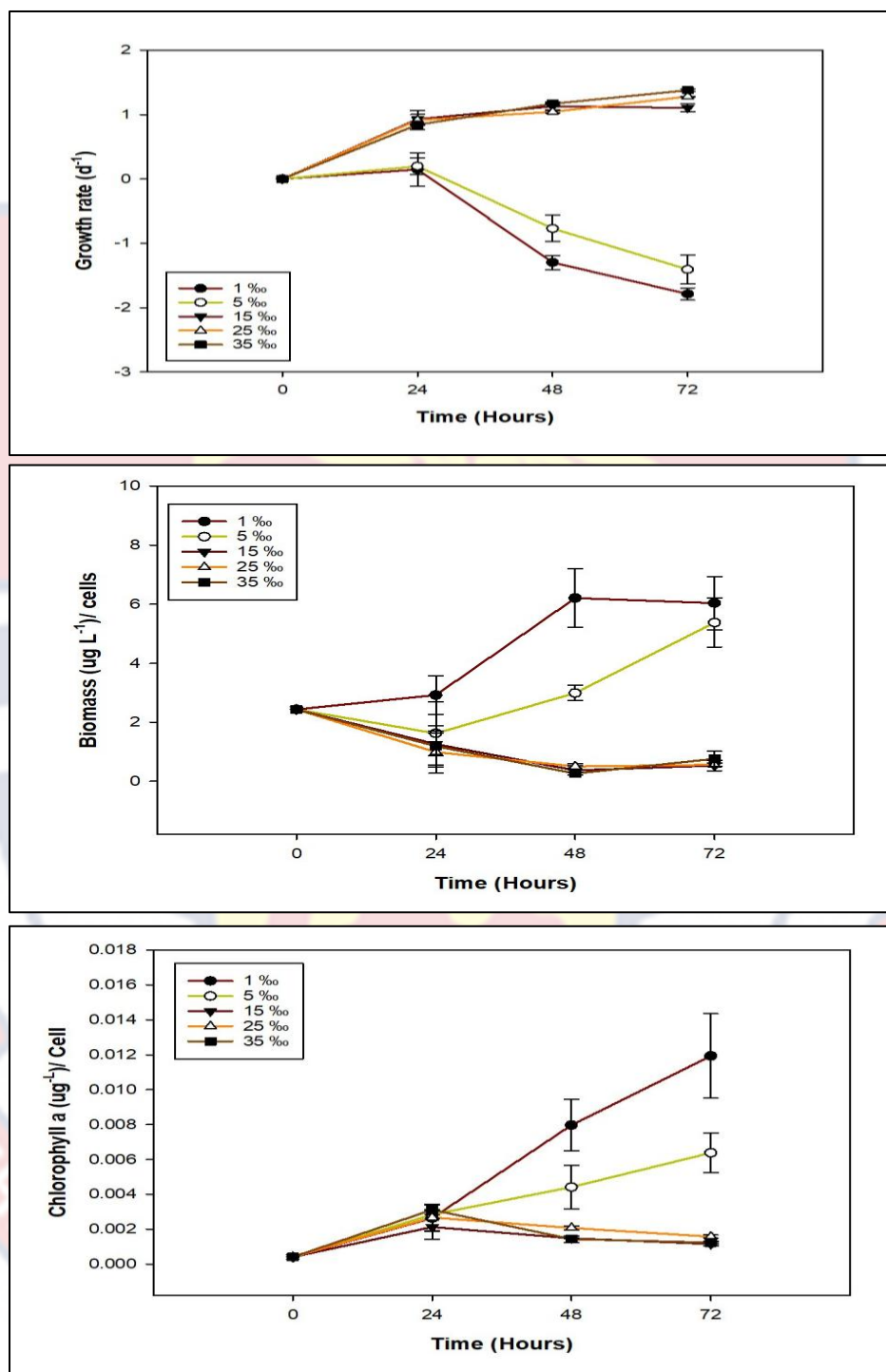


Figure 12: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different salinity conditions

4.2.3 Effects of exposure of different pH conditions on *Thalassiosira weissflogii*

Growth rate, chlorophyll-*a* and dry biomass concentrations per cell varied across the different days and pH treatments (Figure 13). Higher growth rate values were observed in pH 7 treatments ($0.602 \pm 0.109 \text{ d}^{-1}$). Growth rate under pH 5 peaked after 48 hours of exposure and then dropped significantly afterward and reached the least growth rate by the end of the 72 hours. Significant differences ($p < 0.05$) were observed for growth rate only during the second day (Table 4). The highest cell densities were observed in the treatments with the pH of 7 (4837 ± 435 cells/mL), which was also the control for the pH experiments (Table 5). All the treatments showed a percentage decrease in cell density from the control (-17.29% to -26.09%) (Appendix C).

Chlorophyll-*a* concentrations per cell dropped after the first day in all treatments except for pH 5 which showed an increase throughout the exposure duration. The highest biomass values per cell were observed at the end of the 24-hour duration for treatments under pH 8. Similar to chlorophyll-*a* concentrations, treatments under pH 5 also showed an increase in biomass throughout the duration of the experiments. Chlorophyll-*a* and dry biomass concentrations per cell across treatments only varied significantly during the first day (Table 4). The highest cumulative chlorophyll-*a* and dry biomass concentrations ($\mu\text{g/L}$) were observed in pH 9 and pH 8 treatments, respectively (Table 5). Treatments under pH 5 recorded approximately 30% decrease in chlorophyll-*a* and dry biomass from the control (Appendix C).

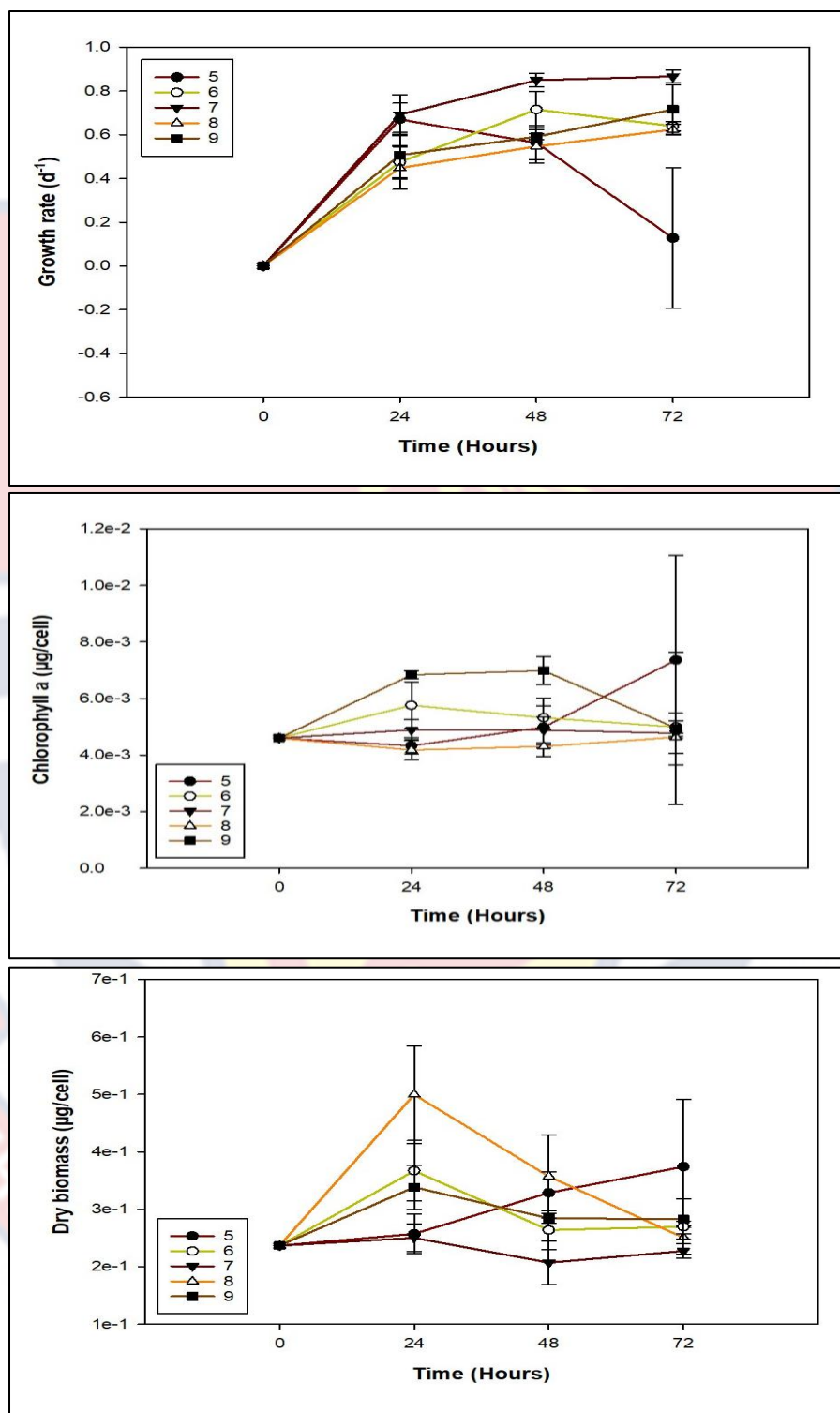


Figure 13: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different pH conditions

4.2.4 Effects of exposure of different pyrene conditions on phytoplankton

The two controls showed exponential growth and had the highest growth rates across all the days of the exposure (Figure 14). The least growth rates were recorded in the 100+ nM pyrene after 72 hours of experimentation. There were no significant differences ($p > 0.05$) observed across the different pyrene treatments after second and third days of exposure (Table 4). The highest cell densities were observed in the acetone control, followed by the no pyrene control with 5668 ± 724 cells/mL and 5576 ± 640 cells/mL, respectively (Table 5). There was a cumulative decrease in cell density from the control in all treatments except the acetone control, which exhibited 8.35% increase in cell densities (Appendix C).

Chlorophyll-*a* and biomass concentrations per cell decreased from the first day of the exposure to the last day of treatment groups. There was a notable increase in biomass in 100+ nM exposure to pyrene on the last day of experiments (Figure 14). Biomass and chlorophyll-*a* were found to be significantly different ($p < 0.05$) among the treatments in the first and second day, but no significant differences ($p > 0.05$) were observed for both biomass and chlorophyll-*a* on the last day (Table 4). Cumulatively, the experiments under 100 nM and 100+ nM of pyrene concentrations exhibited 12.13% and 5.89% increases in chlorophyll-*a* concentrations ($\mu\text{g/L}$) respectively, when compared with the control (0 nM of pyrene concentration). All the other treatments showed a slight decrease in chlorophyll-*a* concentrations, while all treatments exhibited a decrease in biomass ($\mu\text{g/L}$) in comparison to the control (0 nM of pyrene concentration) (Appendix C).

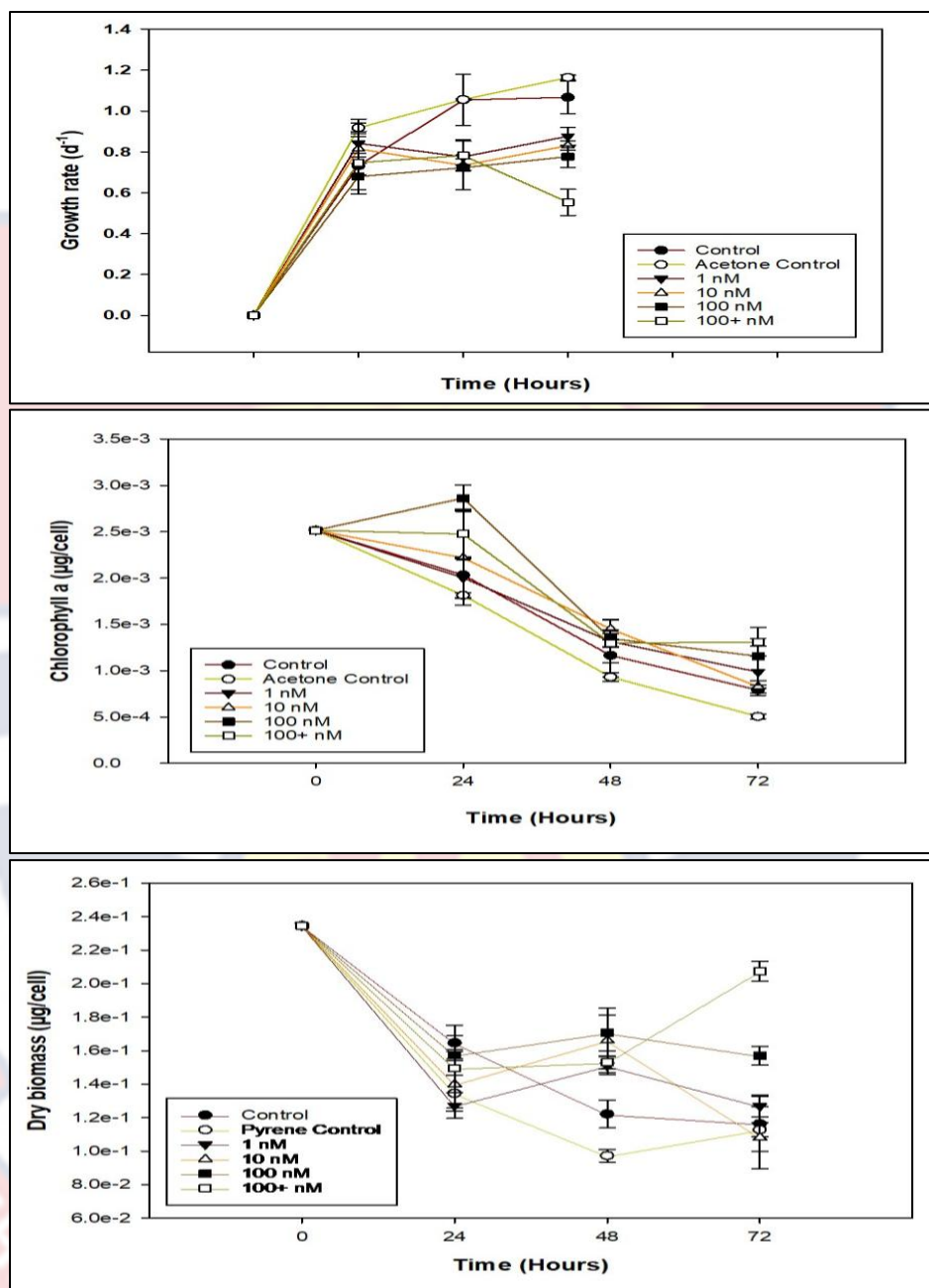


Figure 14: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different pyrene concentrations

4.2.5 Effects of exposure of different cadmium conditions on phytoplankton

The different cadmium treatments exhibited an exponential growth rate (Figure 15). An initial range-finding test was carried out with treatments ranging between 0 µg/L (control) to 200 µg/L (Appendix A). The treatments selected for the experiments ranged between 0 µg/L to 10 µg/L, which were primarily based on results of the range-finding test of cadmium concentrations within the Gulf of Guinea waters (Adokoh et al., 2011).

All the experimental treatments exhibited exponential growth rates with highest in the control group and reduces with increasing cadmium concentrations (Figure 16). Significant differences ($p < 0.05$) in growth rates were only observed on the first day of experiments (after 24 hours) (Table 4). The highest cell densities were recorded in the control group (4379 ± 463 cells/mL) followed by 1 µg/L (4171 ± 329 cells/mL) and 3 µg/L (3933 ± 373 cells/mL) treatments, respectively (Table 5). There was a decrease in cell densities across all treatments in comparison to the no cadmium control (Appendix C).

Chlorophyll-*a* and biomass concentrations dropped after 24 hours of exposure but increased after 72 hours. The highest concentration of biomass per cell was observed in the highest concentration of cadmium (10 µg/L) after 72 hours of exposure. There were no significant differences observed for chlorophyll-*a* concentration across the different treatments and days ($p > 0.05$). There was, however, a significant difference ($p < 0.05$) in dry biomass after 72 hours of exposure (Table 4).

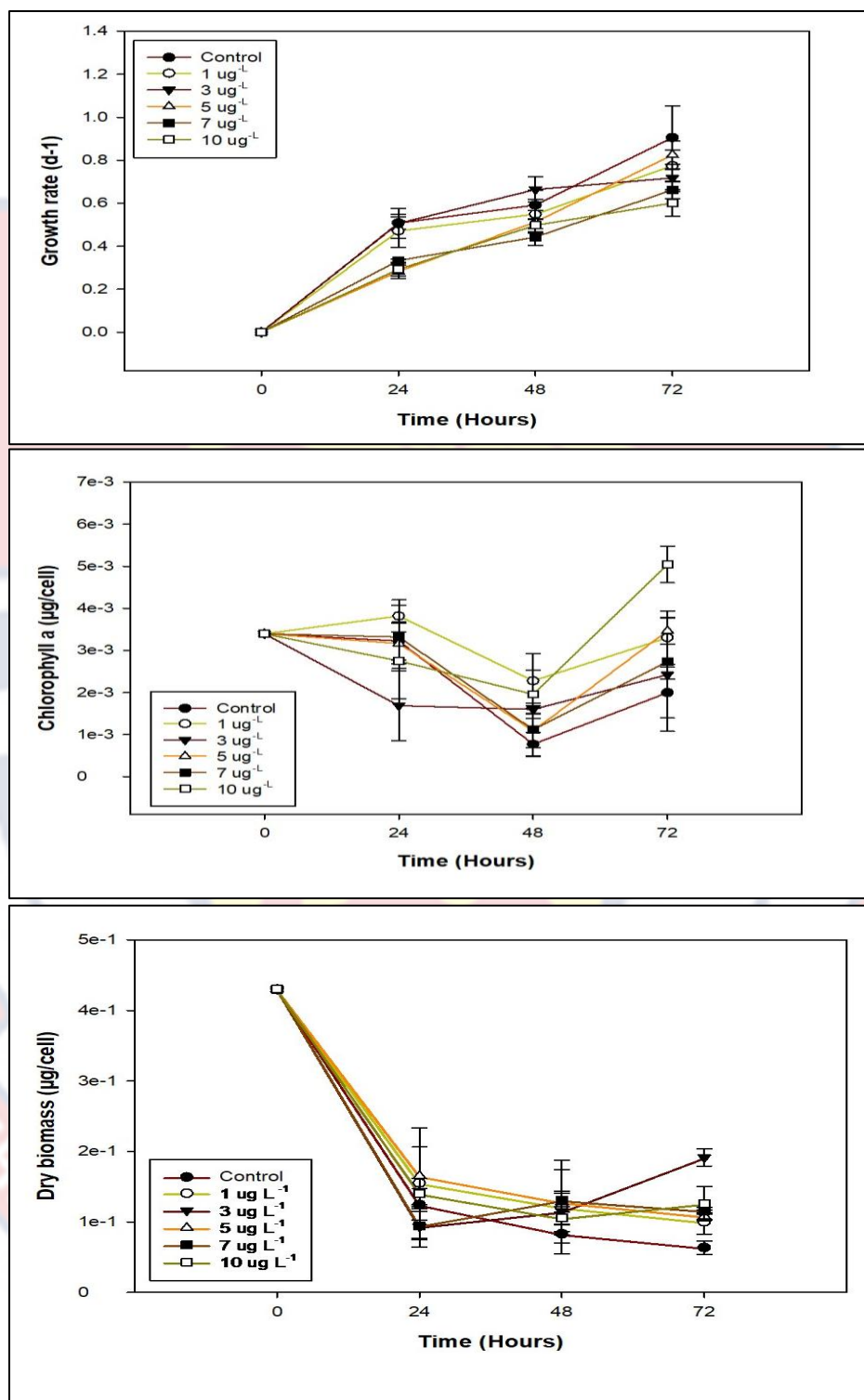


Figure 15: Growth rate, chlorophyll-a, and biomass concentration of *Thalassiosira weissflogii* under different cadmium concentrations

4.2.6 Toxicity effects of individual climate change and pollution related stressors exposure to *Thalassiosira weissflogii*

The different effective mediation concentration (EC_{50}) values for the individual pyrene and cadmium exposure experiments are presented in Table 6. The mean EC_{50} concentrations for individual pyrene and cadmium stressors were 0.56 (nM) and 2.9 ($\mu\text{g/L}$) respectively.

Table 6: Effective Concentration (EC_{50}) for each Stressor at each Time Interval

Stressor	Day 1 (24 hours)	Day 2 (48 Hours)	Day 3 (72 Hours)
Pyrene (nM)	0.3342	0.4793	0.871
Cadmium ($\mu\text{g/L}$)	3.525	3.080	2.131

4.3 Laboratory Exposure of *Thalassiosira Weissflogii* to Combined Climate Change and Pollution Related Stressors

The control group of the combined stressor exposure experiment had the highest growth rate, exhibiting exponential growth until 72 hour (Figures 16-24). *Thalassiosira weissflogii* cultures responded differently to the different combinations of climate change and pollution stressors.

4.3.1 Effects of combined temperature and pollution related stressor exposure experiments to *Thalassiosira weissflogii*

The functional attributes (i.e., growth rate, chlorophyll-*a* and dry biomass densities per cell) varied across the different days and temperatures. There was a decrease in growth rate across treatments with higher pyrene and cadmium

concentrations (100 and 1000 $\mu\text{g/L}$) across all the temperature treatments, particularly after 72 hours of exposure (Figures 16-18). There was a significant interacting effect between temperature treatments and pollution stressors in the first 24 hours of exposure (temperature treatments, cadmium and pyrene; $p < 0.05$). However, the growth rate of *Thalassiosira weissflogii* was not affected by the different combinations of temperature, cadmium and pyrene treatments or the interaction between the two in the second after 48 and 72 hours of exposure (Table 7).

Chlorophyll-*a* and dry biomass concentrations per cell showed a sharp decrease in all temperature treatments and control (Figures 16-18), although there was an increase for majority of the treatments at the end of exposure period. There were however no significant differences ($p > 0.05$) in chlorophyll-*a* and dry biomass concentrations for the combined temperature and pollution (cadmium and pyrene) treatments or their interactions (Table 7).

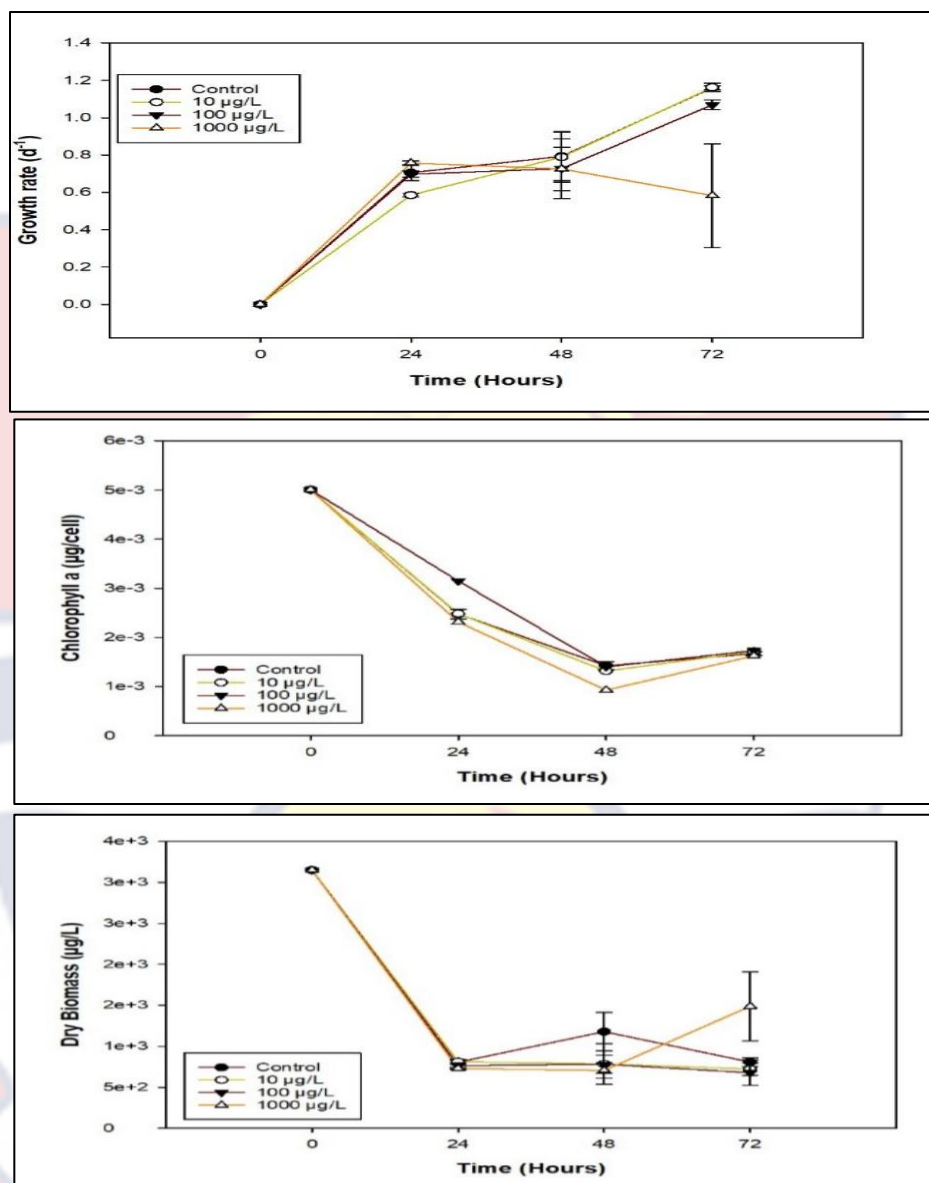


Figure 16: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at 24 °C with time (h)

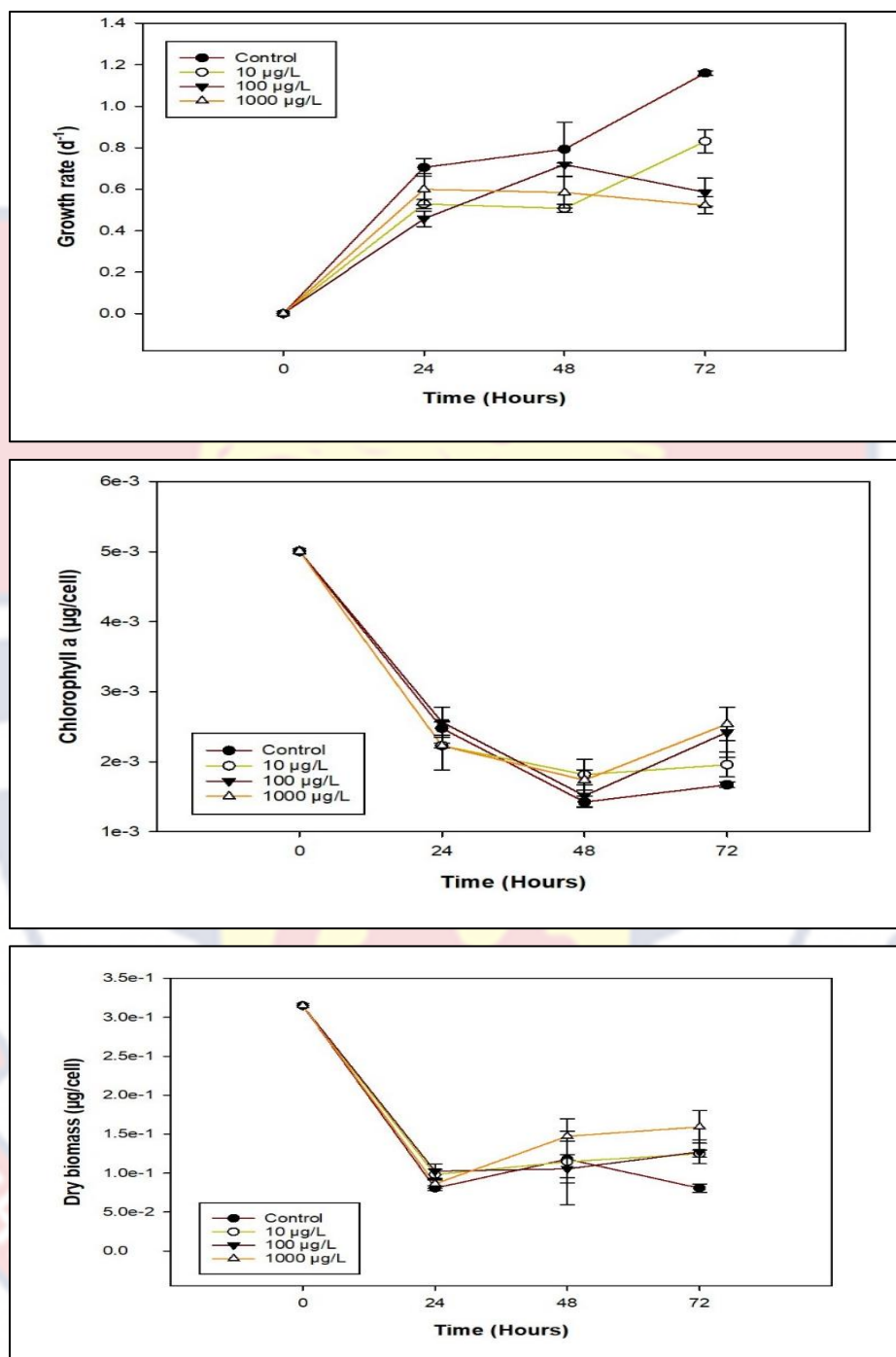


Figure 17: Growth rate, chlorophyll-a, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at 28 °C with time (h)

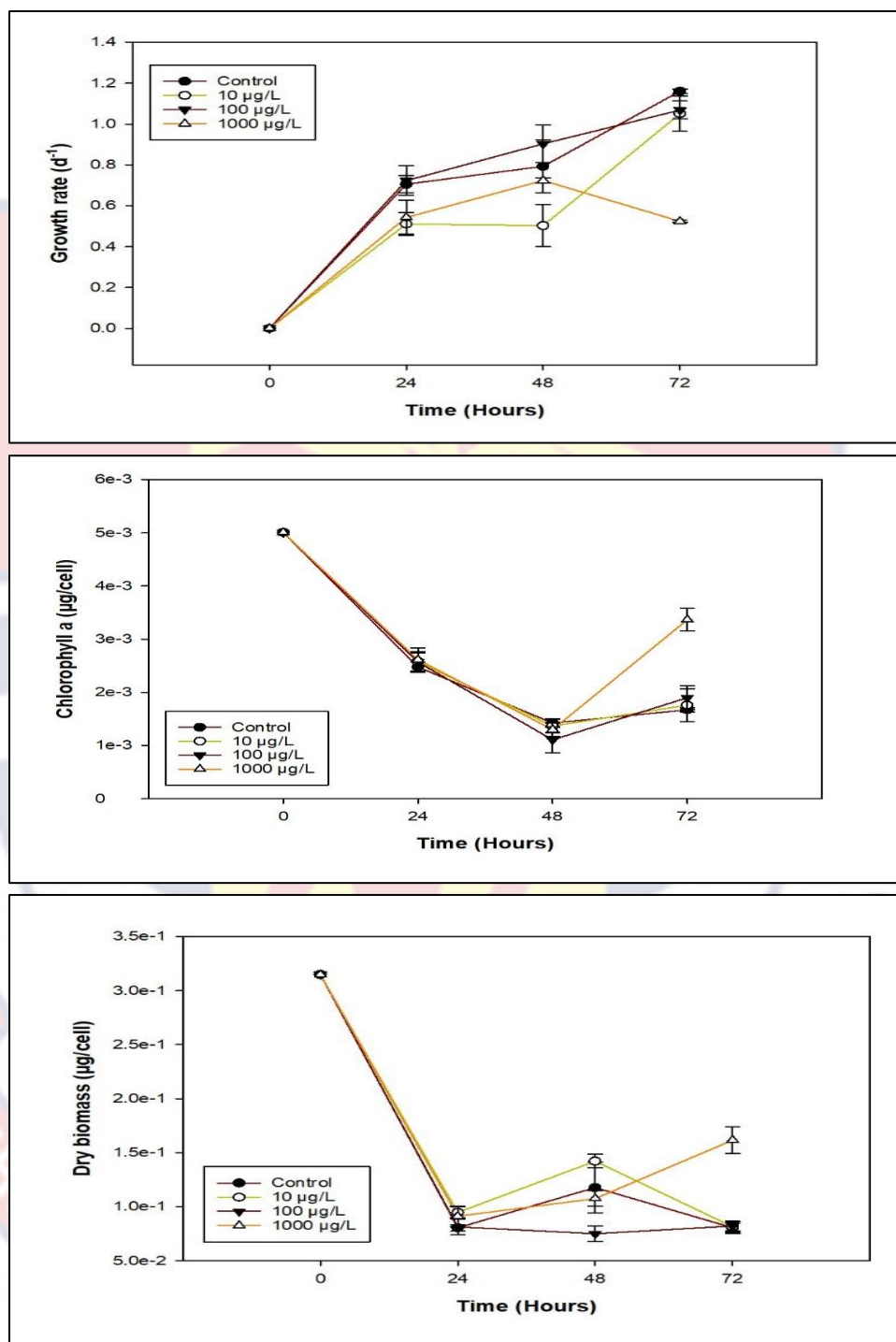


Figure 18: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at 32 °C with time (h)

4.3.2 Combined salinity and pollution related stressor exposure experiments

The Figures 19-21 describe the observed growth rates, chlorophyll-*a*, and biomass concentrations. Similar to the observations in the combined temperature and pollution experiments, there was a decrease in growth rate with increased pyrene and cadmium concentrations (Figures 19-21). For salinity at 15‰, all treatments showed exponential growth rates except for those having 10 µg/L of pyrene and cadmium (Figure 19). There was a significant interacting effect between combined salinity and pollution stressors for growth rate in the first 24 hours of exposure ($p < 0.05$). However, the growth rate of *T. weissflogii* was not affected by the different combined salinity, cadmium and pyrene treatments, or the interaction between the two after 48 and 72 hours of exposure, respectively (Table 7).

Chlorophyll-*a* concentrations per cell showed a sharp decrease after the first day, followed by an increase in the last day (72 hours) in nearly all the salinity combination experiments (Figures 19-21). There was a significant interaction effect between salinity treatments and cadmium and pyrene concentrations on chlorophyll-*a* concentrations only after 48 hours of exposure ($p < 0.05$), with no significant differences observed for the first (24 hours) and last (72 hours) days, respectively. Biomass concentrations per cell also showed the same trends as those of chlorophyll- *a* concentrations per cell, except for the 15‰ treatments, which showed a decrease on the first day, followed by an increase on the second day (Figures 19-21). There was a significant interacting effect between combined salinity, cadmium and pyrene concentrations on the biomass concentrations after

24 hours of exposure ($p < 0.05$), with no significant differences observed after 48- and 72-hours, respectively.

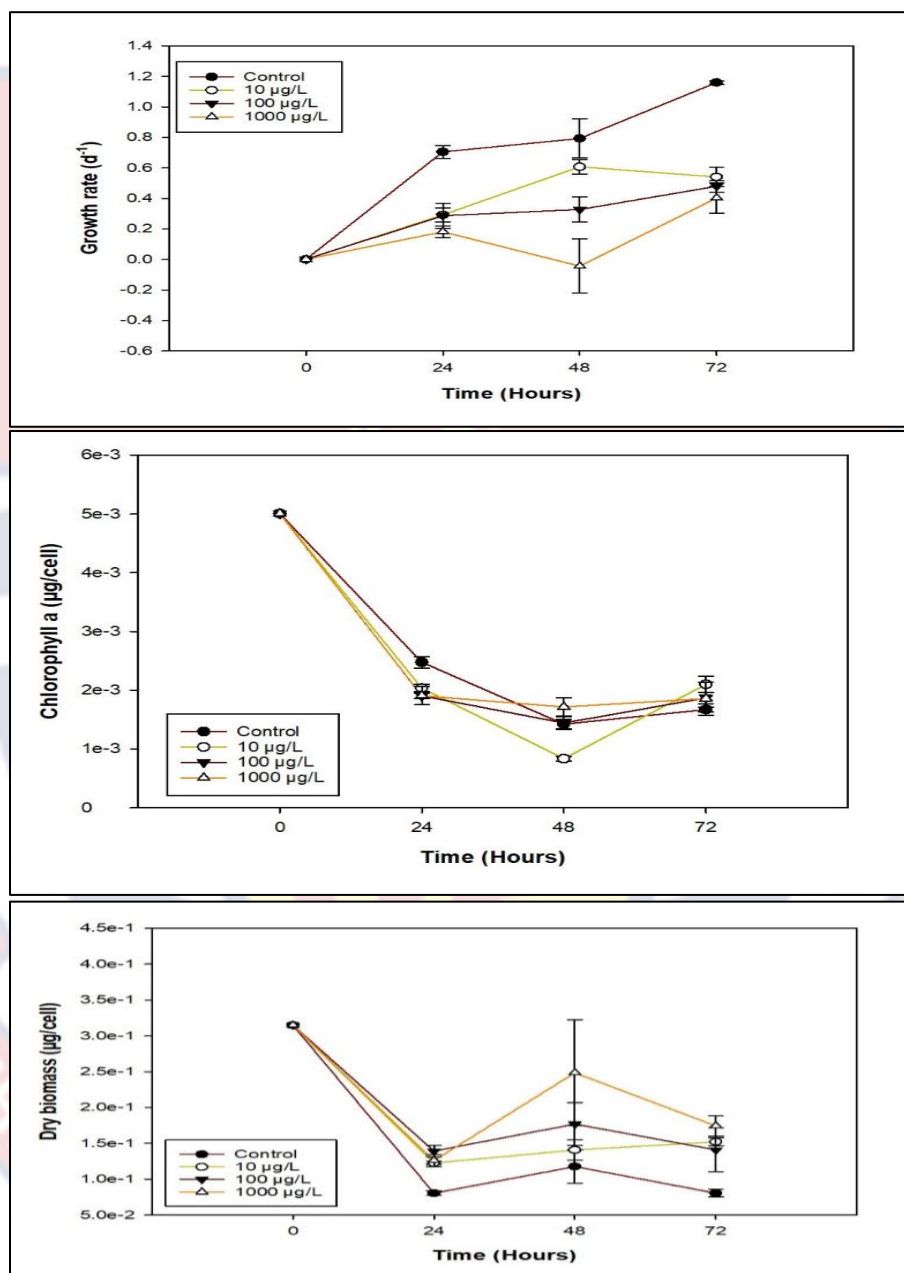


Figure 19: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at 15‰ with time (h)

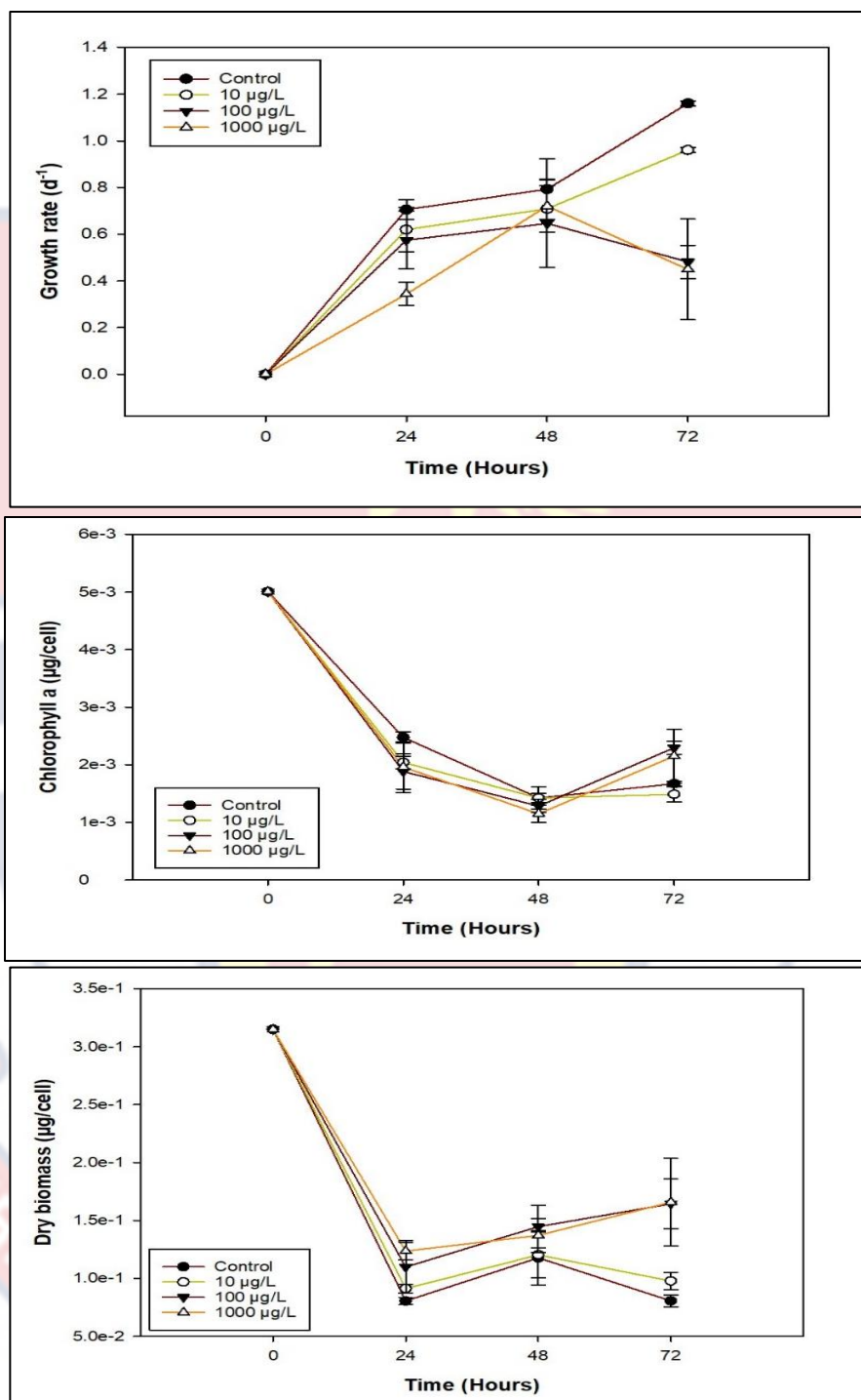


Figure 20: Growth rate, chlorophyll-a, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at 25‰ with time (h)

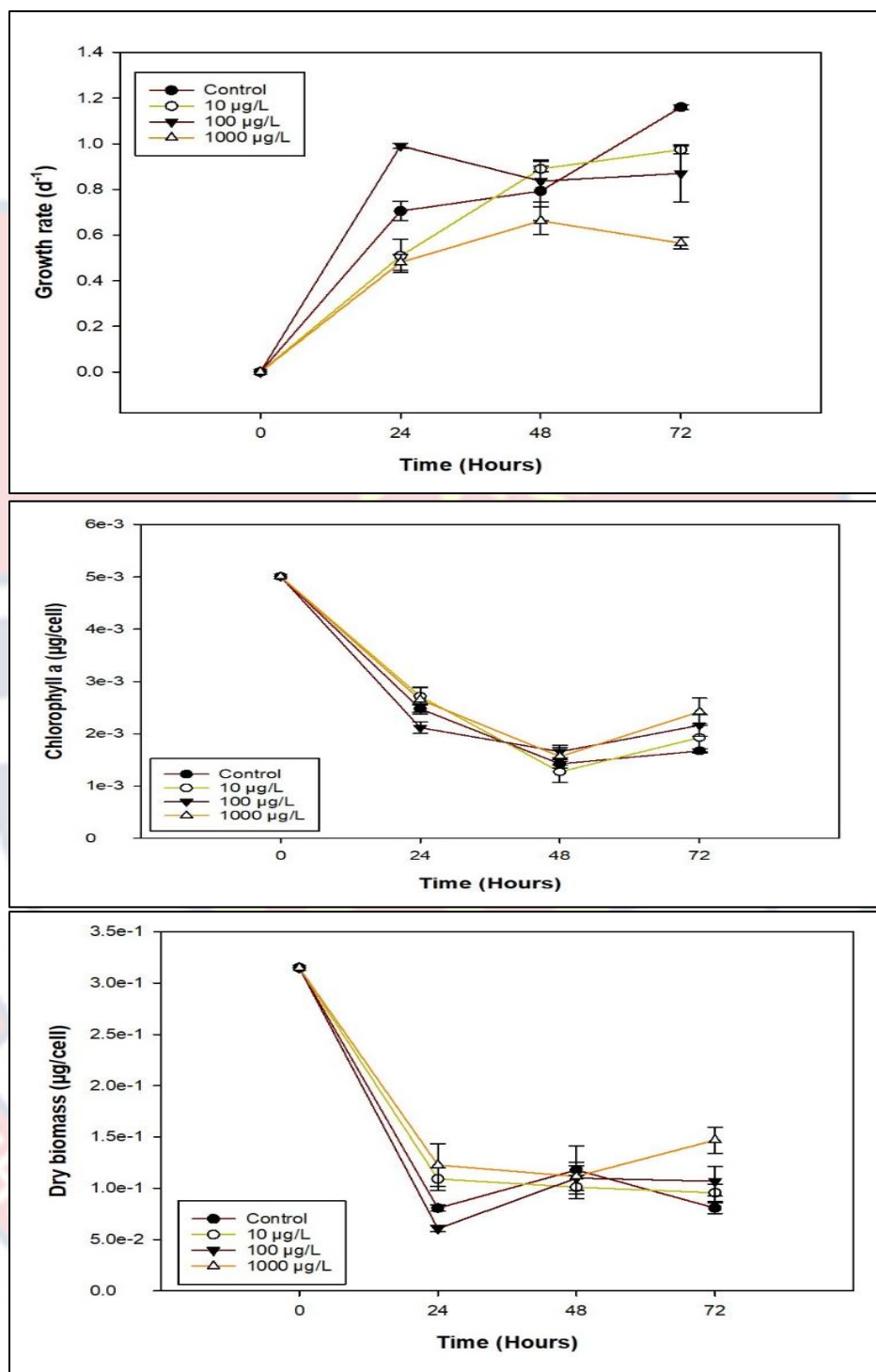


Figure 21: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at 35‰ with time (h).

4.3.3 Combined pH and pollution related stressor exposure experiments

The different growth rates, chlorophyll-*a*, and biomass concentrations per cell are described in Figures 22-24. Growth rates varied across the different days, pH and cadmium and pyrene concentrations. The control group and 10 µg/L cadmium and pyrene concentration in all the pH treatments exhibited exponential growth rates until the 72-hour period. However, pyrene and cadmium concentrations of 100 µg/L and 1000 µg/L respectively showed a decrease in growth rates, particularly after 72 hours of exposure (Figures 22-24). There were no significant differences in growth rates of the interacting effects between pH and pollution stressors after 24 and 48 hours of exposure. However, significant differences in growth rates were observed after 72 hours of exposure (combined pH, cadmium x pyrene treatments; $p < 0.05$,) (Table 7).

Chlorophyll-*a* and biomass exhibited similar trends as those observed in the temperature and salinity treatments, where there was a sharp decrease in their concentrations per cell after 24 hours of exposure. There were no significant differences in chlorophyll-*a* and biomass concentration per cell (Table 7) across the different days.

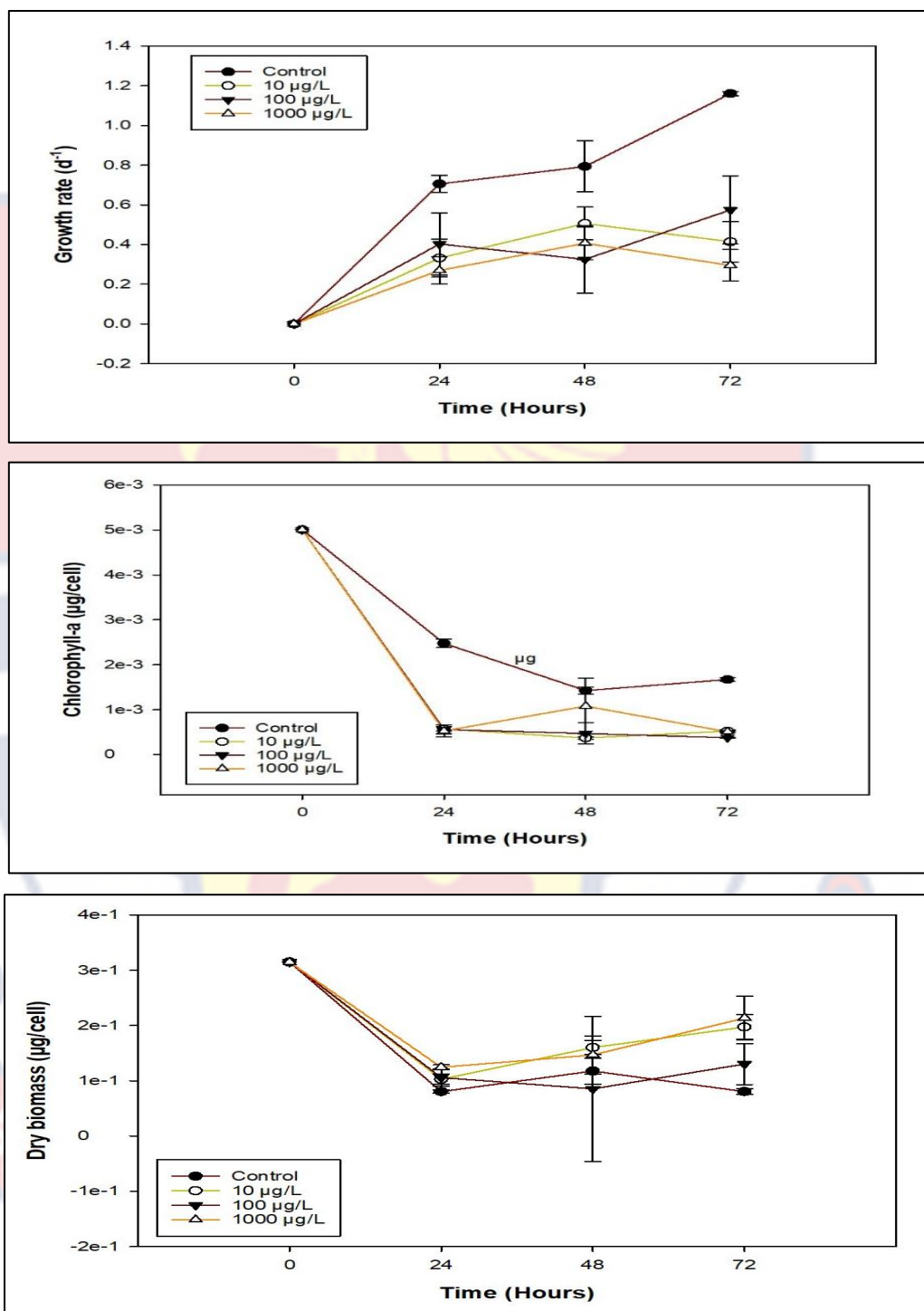


Figure 22: Growth rate, chlorophyll-a, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at pH 5 with time (h)

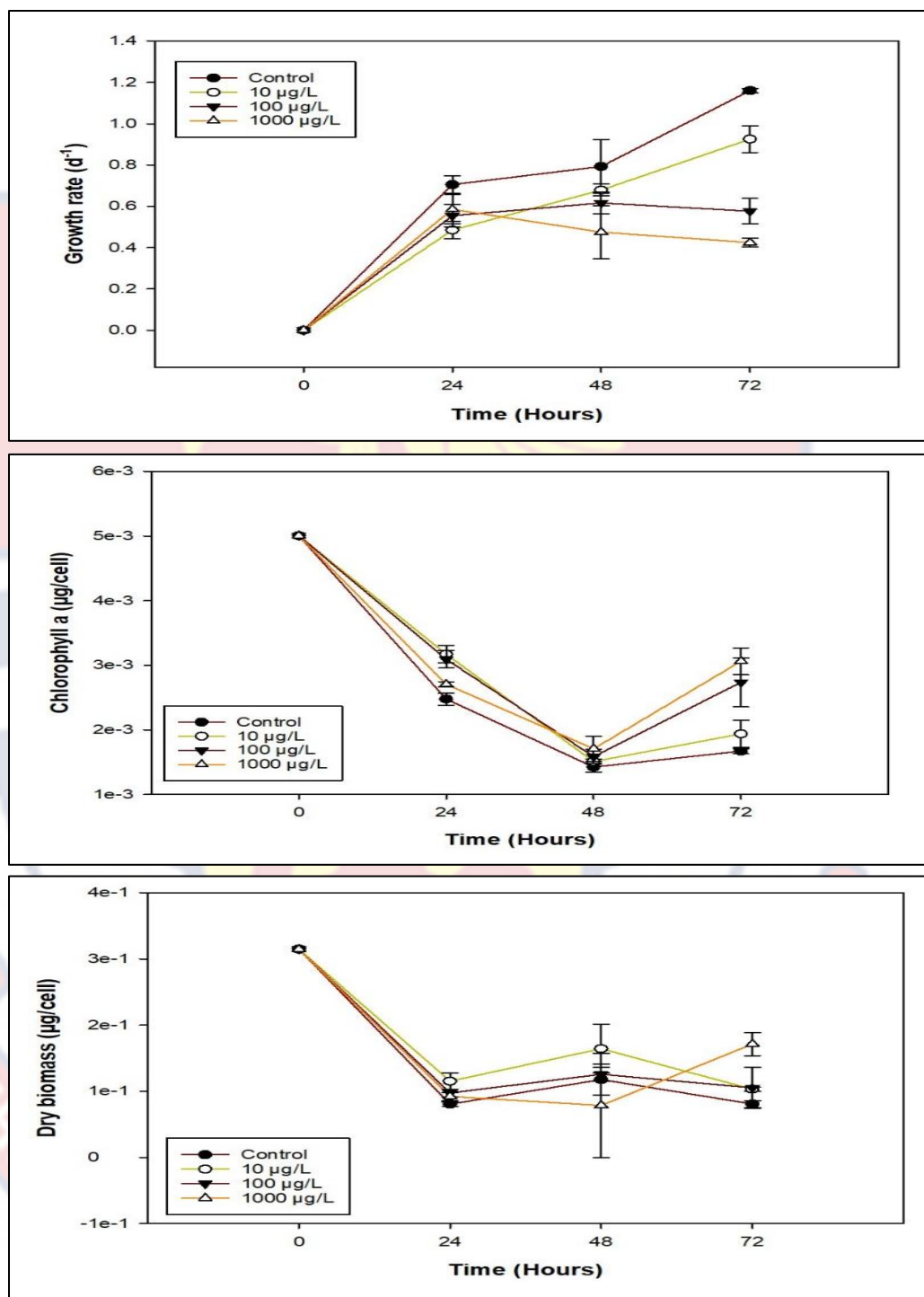


Figure 23: Growth rate, chlorophyll-*a*, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at pH 7 with time (h)

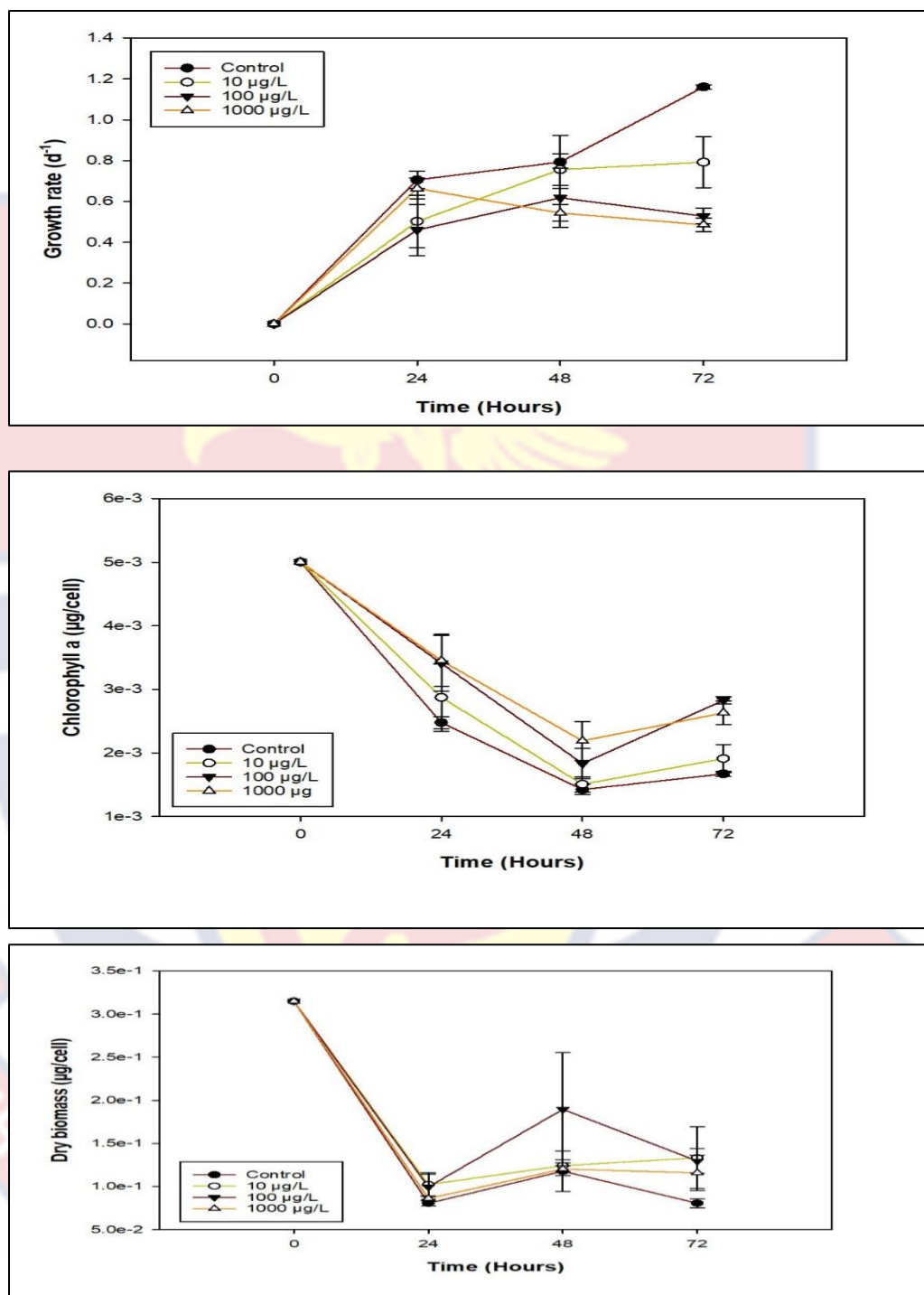


Figure 24: Growth rate, chlorophyll-a, and biomass concentration of *Thalassiosira weissflogii* under different concentrations of combined pollution stressors (pyrene and cadmium) at pH 9 with time (h)

Table 7: Two-way ANOVA Comparing Growth Rates, Chlorophyll-a, and Dry Biomass Characteristics for the Different Combination Exposure Experiments

Functional Attribute	Stressor combinations	24 hours		48 hours		72 hours	
		F	P-value	F	P-value	F	P-value
Growth rate (d ⁻¹)	Temperature, cadmium and pyrene treatments	3.5	0.028*	1.58	0.224	1.66	0.203
	Salinity, cadmium and pyrene treatments	4.95	0.007*	2.41	0.087	2.0	0.138
	pH, cadmium and pyrene treatments	0.9	0.485	0.38	0.822	3.13	0.042*
Chlorophyll-a (µg/L)	Temperature, cadmium and pyrene treatments	1.44	0.265	0.21	0.926	3.13	0.042*
	Salinity, cadmium and pyrene treatments	0.35	0.842	4.06	0.016	1.49	0.246
	pH, cadmium and pyrene treatments	0.87	0.504	0.34	0.845	2.82	0.058
Biomass (µg/L)	Temperature, cadmium and pyrene treatments	1.15	0.364	1.04	0.417	0.72	0.586
	Salinity, cadmium and pyrene treatments	3.78	0.022	0.95	0.459	1.09	0.391
	pH, treatments cadmium and pyrene	1.5	0.246	0.5	0.737	1.52	0.241

The asterisk (*) indicate significantly different (<0.05) treatments

4.3.4 Cell densities, total chlorophyll-*a* and biomass concentrations for the different combination experiments (climate change and pollution)

In the previous sections 4.3.1- 4.3.3, we have documented the growth rate (d^{-1}), chlorophyll-*a* ($\mu g/L$) and biomass ($\mu g/L$) concentration across the different combination (climate change related stressors and pollution). In this section, data for the same combination experiments (temperature and pollution, salinity and pollution and pH and pollution) will be presented in the form of total cell densities (cells/mL), total chlorophyll-*a* ($\mu g/L$) concentrations and total biomass ($\mu g/L$). A summary of this data has been presented in Table 8.

The highest cell densities (cells/mL) were observed in the control group (5291 ± 607 cells/mL), followed by the $24\text{ }^{\circ}C$ and 35‰ treatments with 4736 ± 337 cells/mL and 4694 ± 291 cells/mL, respectively. The lowest cell densities were observed in the pH 5 treatments with 3422 ± 150 cells/mL. Similarly, cell densities decreased with increased concentrations of cadmium and pyrene across all climate change-related stressor treatments.

All the treatments exhibited a decrease in cell densities when compared to the control except for cultures under the $32\text{ }^{\circ}C$ and $100\text{ }\mu g/L$ (pyrene and cadmium) concentrations, which recorded an increase of 0.13%. The highest decrease in cell densities was 50.47%, which was recorded in the treatments with 15‰ salinity and $1000\text{ }\mu g/L$ (cadmium and pyrene concentration) (Appendix D). The total chlorophyll-*a* concentrations range between 0.9-21.75 $\mu g/L$ across the different treatments. The lowest chlorophyll-*a* levels were observed in the pH 5 treatments

(4.48 ± 0.87 $\mu\text{g/L}$), while the highest level were observed under the pH 9 (11.71 ± 0.45 .) (Table 8).

For all the combination climate change-related (temperature, salinity and pH) and pollution (cadmium and pyrene) experiments, majority of the treatments exhibited a decrease in chlorophyll-*a* concentrations when compared to the control treatments except for cultures under 24 °C (10 and 100 $\mu\text{g/L}$ of pyrene and cadmium), salinity 35‰ (100 $\mu\text{g/L}$ pyrene and cadmium), and pH 9 (100 and 1000 $\mu\text{g/L}$ of pyrene and cadmium).

The highest biomass values were observed in 25‰ (814.9 ± 70.2 $\mu\text{g/L}$) treatment group, followed by the control group (797 ± 128 $\mu\text{g/L}$). Both chlorophyll-*a* and biomass concentrations decreased with increase in cadmium and pyrene concentrations in majority of the treatments. Similar to observations made for chlorophyll-*a* concentrations, majority of the treatments exhibited a decrease in dry biomass when compared to the control. The highest decrease in dry mass concentrations was recorded in pH 5 (100 $\mu\text{g/L}$ of cadmium and pyrene) with a proportion decrease of 29.9%. cultures in the 24 °C (10 and 100 $\mu\text{g/L}$ of cadmium and pyrene) treatment groups recorded a decrease of 28.48% and 23.99%, respectively, the second and third highest decreases observed in all experiments.

Table 8: Mean (+SE) (a) Cell Densities, (b) Chlorophyll-a ($\mu\text{g/L}$), and (c) Dry Biomass (mg/L) for the Different Combination of Climate Change (Temperature, Salinity, Ph) and Cadmium x Pyrene Stressors

a) Cell densities (cells/ML)

Climate Treatments		Cadmium and pyrene stressor level		
		10 μg	100 μg	1000 μg
Temperature °C	24	5147 $\pm$ 619	5031 $\pm$ 548	4031 $\pm$ 574
	28	4164 $\pm$ 355	4025 $\pm$ 303	3953 $\pm$ 278
	32	4514 $\pm$ 538	5297 $\pm$ 560	4053 $\pm$ 302
Salinity (‰)	15	3692 $\pm$ 262	3348 $\pm$ 192	2936 $\pm$ 203
	25	4714 $\pm$ 463	4003 $\pm$ 357	3836 $\pm$ 376
	35	4847 $\pm$ 497	5281 $\pm$ 537	3953 $\pm$ 278
pH	5	3410 $\pm$ 250	3615 $\pm$ 328	3236 $\pm$ 169
	7	4458 $\pm$ 431	3994 $\pm$ 278	3731 $\pm$ 265
	9	4414 $\pm$ 422	3875 $\pm$ 311	3937 $\pm$ 274
Control	-			5291 $\pm$ 607

b) Chlorophyll-a concentrations ($\mu\text{g/L}$)

Climate Treatments		Cadmium and pyrene stressor level		
		10 μg	100 μg	1000 μg
Temperature °C	24	11.96 $\pm$ 0.65	11.65 $\pm$ 0.8	11.45 $\pm$ 0.98
	28	10.18 $\pm$ 0.61	10.31 $\pm$ 0.64	10.28 $\pm$ 0.64
	32	10.32 $\pm$ 0.93	11.53 $\pm$ 0.94	11.16 $\pm$ 0.88
Salinity (‰)	15	8.048 $\pm$ 0.96	7.898 $\pm$ 0.95	7.32 $\pm$ 0.97
	25	9.71 $\pm$ 0.63	9.02 $\pm$ 0.71	8.36 $\pm$ 0.86
	35	11.05 $\pm$ 0.76	12.28 $\pm$ 0.56	10.39 $\pm$ 0.66
pH	5	4.48 $\pm$ 1.76	4.87 $\pm$ 1.49	5.06 $\pm$ 1.46
	7	11.24 $\pm$ 0.7	11.35 $\pm$ 0.78	10.8 $\pm$ 0.76
	9	10.64 $\pm$ 0.61	11.59 $\pm$ 0.63	12.39 $\pm$ 1.06
Control	-			11.57 $\pm$ 0.68

Table 8, Continued

c) *Biomass densities ($\mu\text{g/mL}$)*

Climate Treatments		Cadmium and pyrene stressor level		
		10 μg	100 μg	1000 μg
Temperature °C	24	677 $\pm$ 152	696 $\pm$ 144	717 $\pm$ 141
	28	766 $\pm$ 140	755 $\pm$ 134	807 $\pm$ 128
	32	768 $\pm$ 131	750 $\pm$ 135	783 $\pm$ 131
Salinity (‰)	15	805 $\pm$ 127	790 $\pm$ 131	771 $\pm$ 132
	25	796 $\pm$ 129	830 $\pm$ 122	818 $\pm$ 125
	35	801 $\pm$ 126	794 $\pm$ 128	796 $\pm$ 127
pH	5	804 $\pm$ 162	671 $\pm$ 182	789 $\pm$ 132
	7	859 $\pm$ 126	743 $\pm$ 137	737 $\pm$ 157
	9	822 $\pm$ 127	814 $\pm$ 134	728 $\pm$ 137
Control	-			797 $\pm$ 128

4.4 RNA Sequencing and Downstream Analysis

4.4.1 Pearsons correlation coefficient across different treatments

To reveal the differences in gene expressions across sample treatments, Pearson's correlation coefficient was calculated. The correlation between samples indicated the reliability of the experiment with a coefficient of 1 implying greater correlation. ENCODE was used to suggest the square of the Pearson correlation coefficient which should be larger than 0.92, under ideal experimental conditions (Weinstock, 2007). The scatter diagram (Figure 25) demonstrates the correlation

coefficient between samples. The scatter diagrams demonstrate the correlation coefficient between samples.

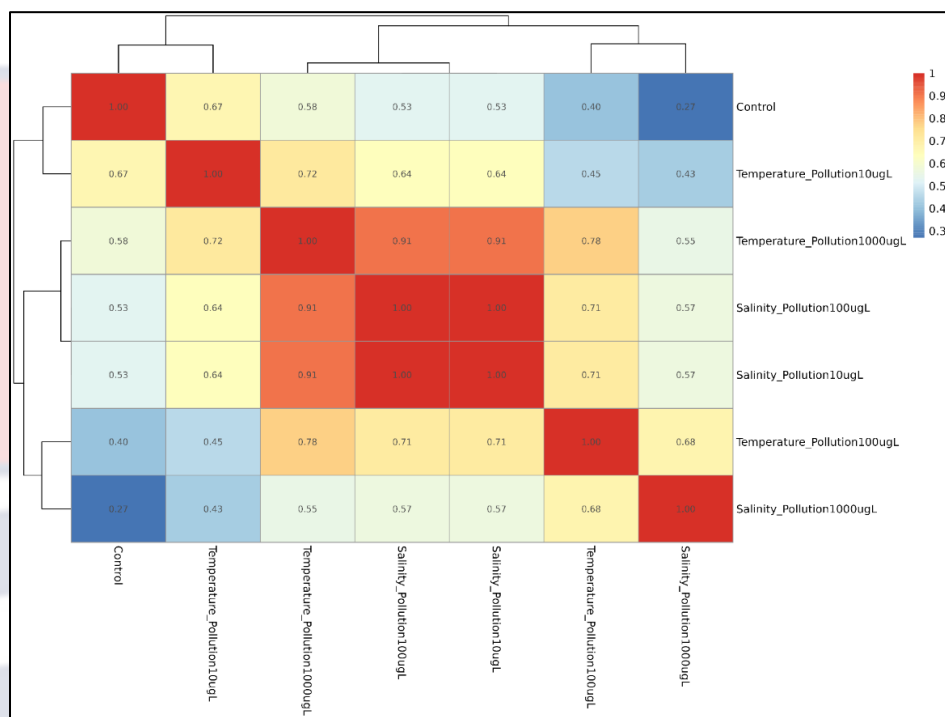


Figure 25: Correlation analysis between the different temperature and salinity combined sample treatments

4.4.2 Gene expression analysis

Salinity vs. control treatments

Based on the RNA-seq analysis, a total of 52, 452, 62,395, and 58,703 differently expressed genes (Fold Change ≥ 2 , and FDR < 0.05) in relation to the control for the 10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, and 1000 $\mu\text{g/L}$ cadmium and pyrene treatments respectively. The number of genes that were upregulated for the 10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, and 1000 $\mu\text{g/L}$ cadmium and pyrene treatments were 30,044, 34, 223, and 24,827 respectively. The number of downregulated genes for the 10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, and 1000 $\mu\text{g/L}$ cadmium and pyrene treatments were 22,408, 28,172, and 33,876 (Figures 26 and 27).

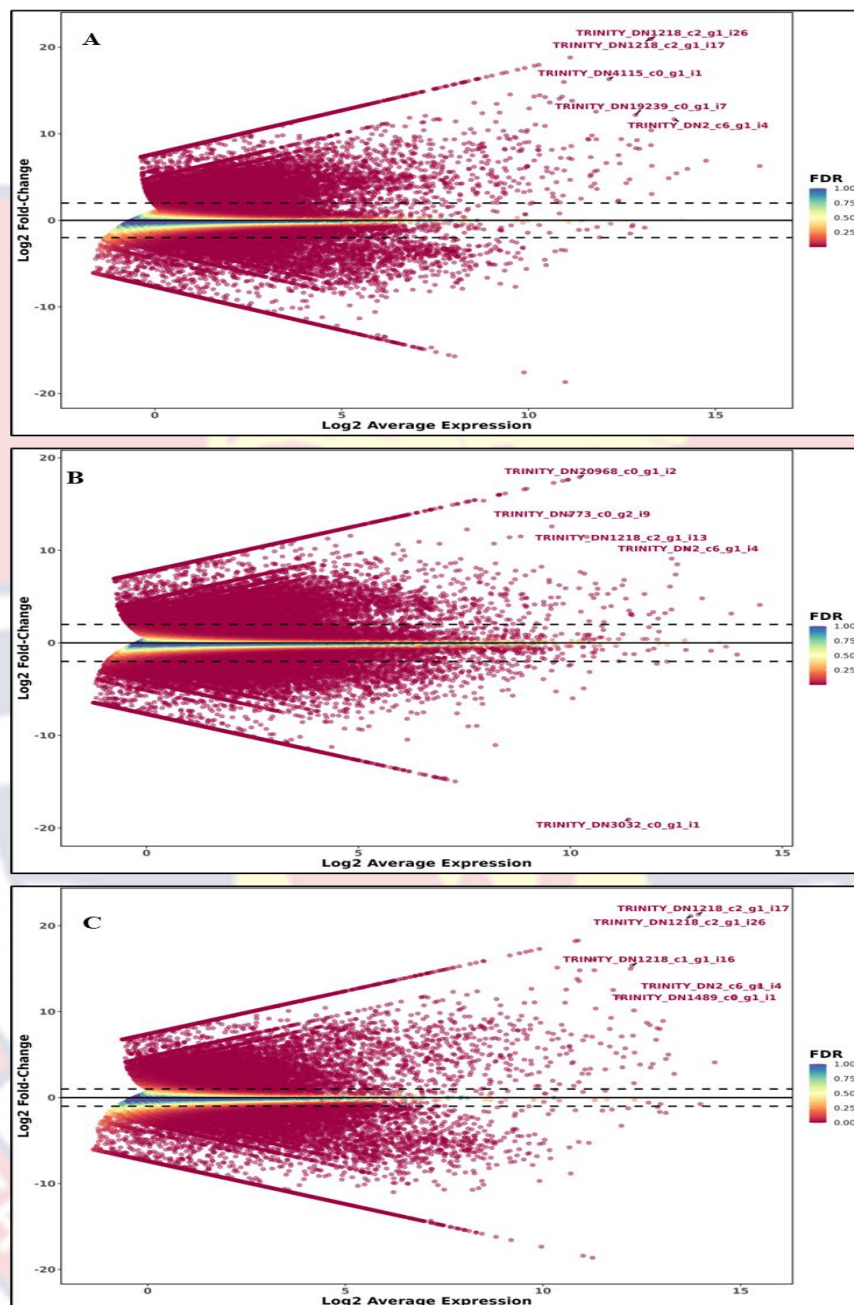
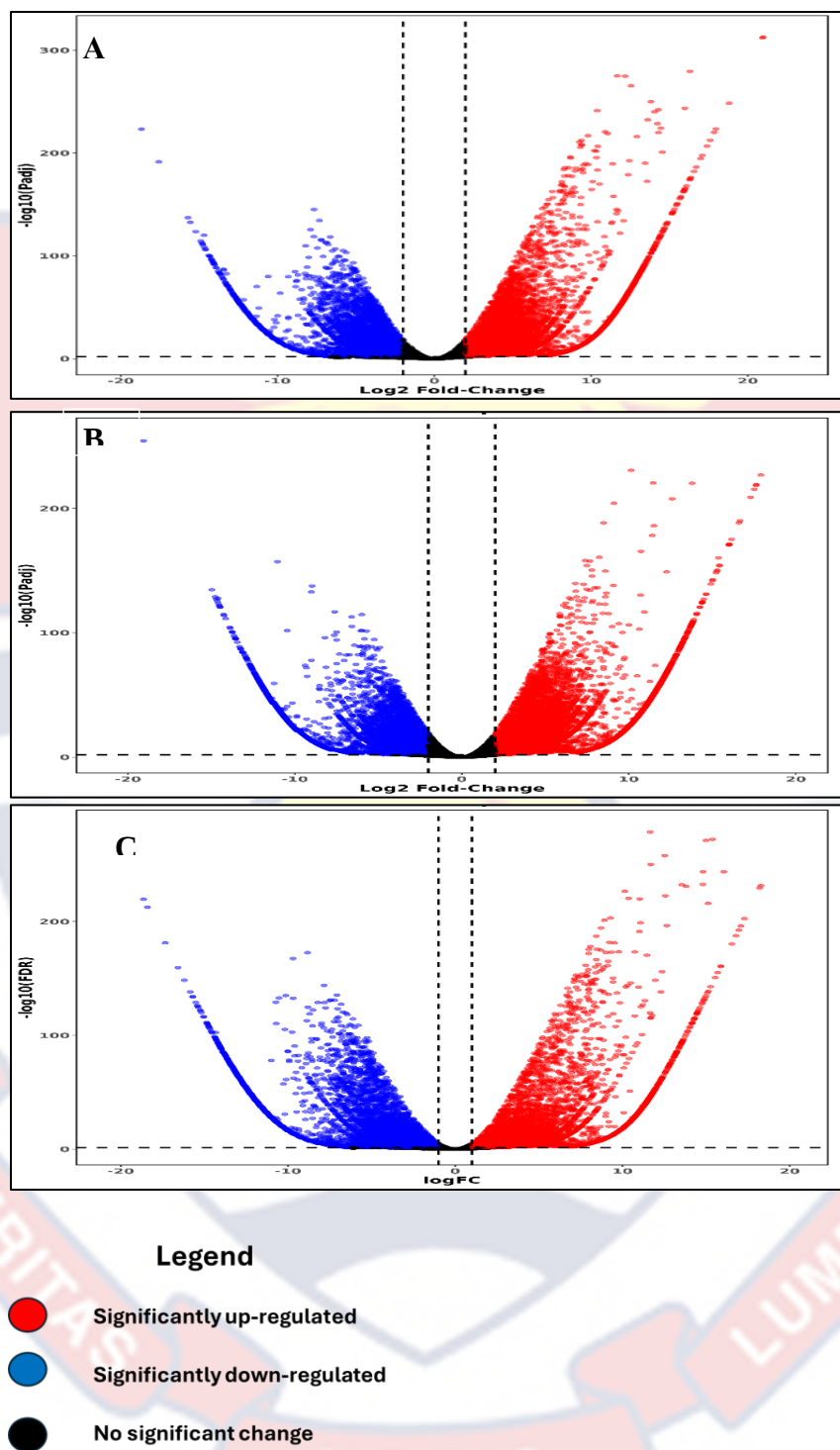
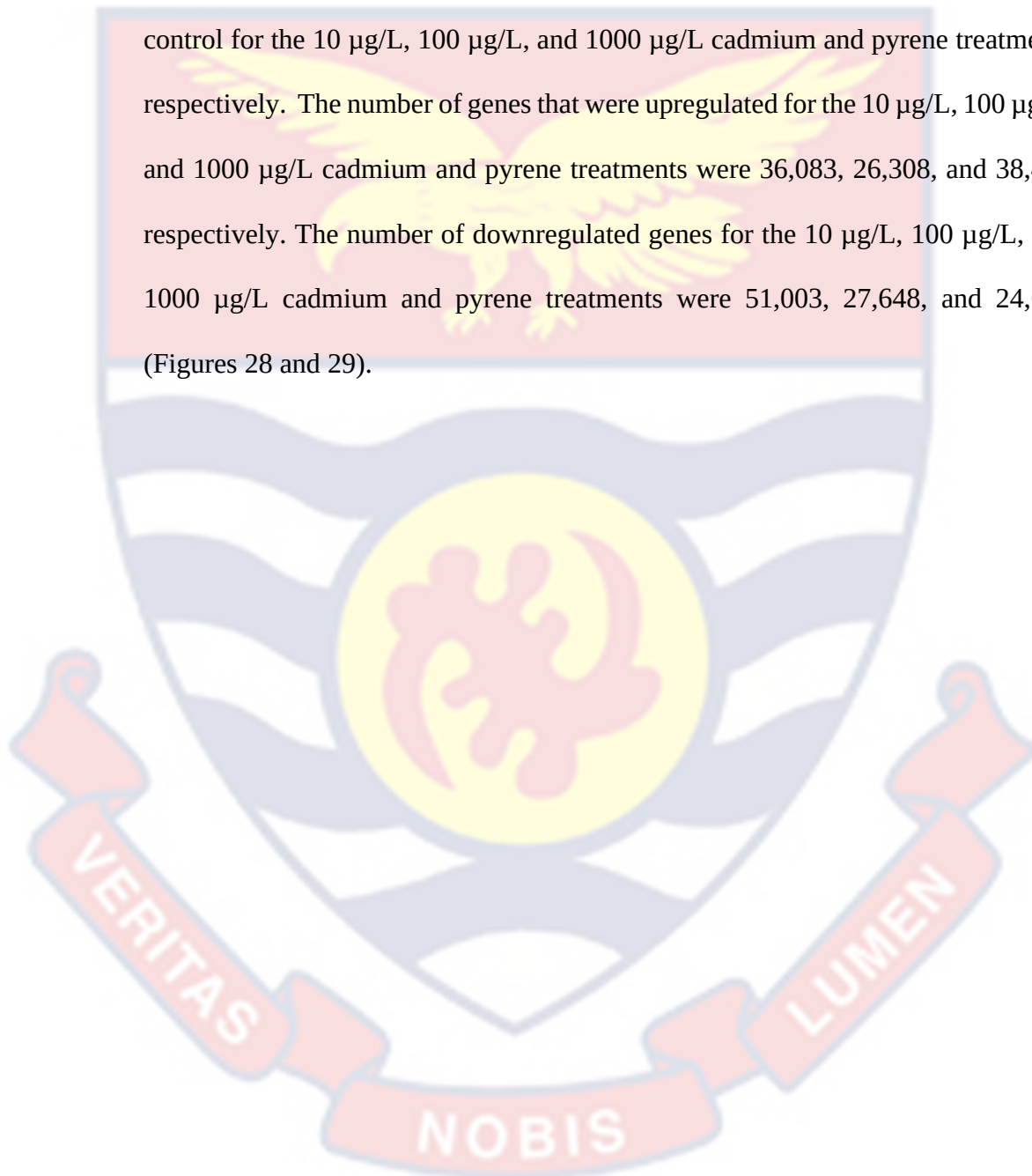


Figure 26: MA plot on the different salinity mixture treatments in comparison to the control. A; Salinity, Cadmium and pyrene (10 $\mu\text{g/L}$), B; Salinity, Cadmium and pyrene (100 $\mu\text{g/L}$), C; Salinity, Cadmium and pyrene (1000 $\mu\text{g/L}$)



Temperature vs. Control treatments

Based on the RNA-seq analysis, a total of 87, 086, 53,956, and 63,129 differently expressed genes (Fold Change ≥ 2 , and FDR < 0.05) in relation to the control for the 10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, and 1000 $\mu\text{g/L}$ cadmium and pyrene treatments respectively. The number of genes that were upregulated for the 10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, and 1000 $\mu\text{g/L}$ cadmium and pyrene treatments were 36,083, 26,308, and 38,485 respectively. The number of downregulated genes for the 10 $\mu\text{g/L}$, 100 $\mu\text{g/L}$, and 1000 $\mu\text{g/L}$ cadmium and pyrene treatments were 51,003, 27,648, and 24,644 (Figures 28 and 29).



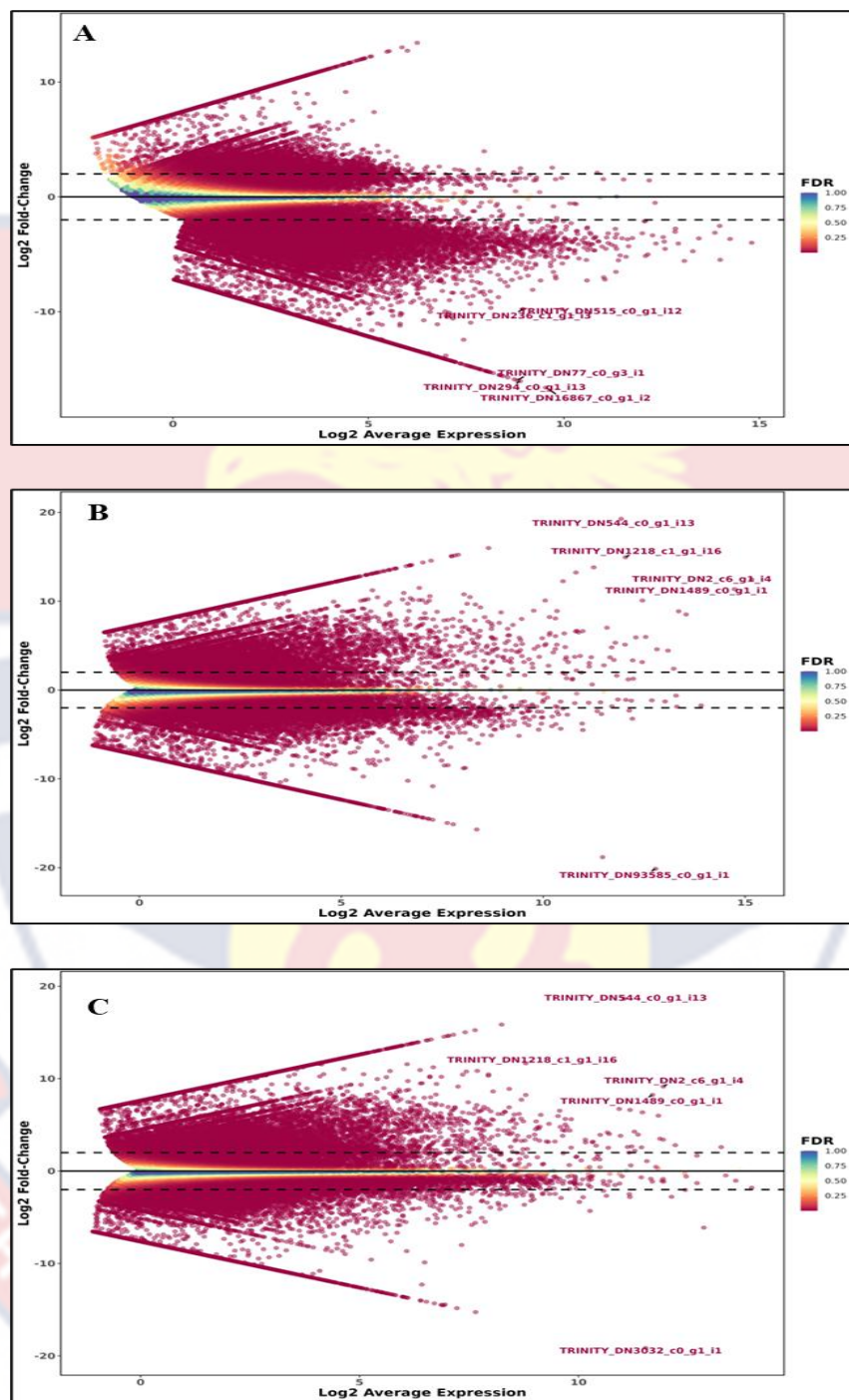


Figure 28: MA plots on the different temperature mixture treatments in comparison to the control. A; Temperature, Cadmium and pyrene (10 $\mu\text{g/L}$), B; Temperature, Cadmium and pyrene (100 $\mu\text{g/L}$), C; Temperature, Cadmium and pyrene (10 $\mu\text{g/L}$)

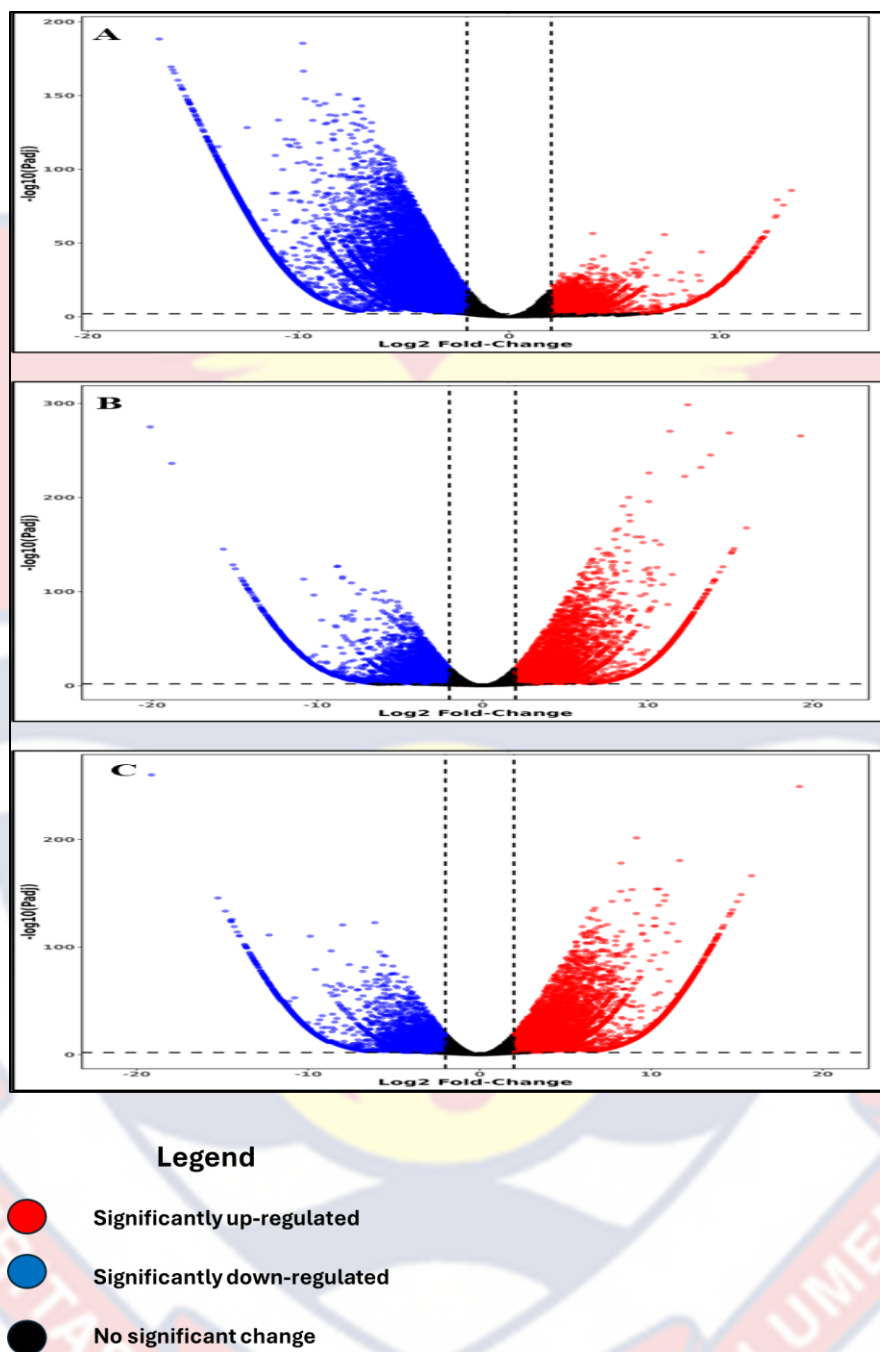


Figure 29: Volcano plot on the different temperature mixture treatments in comparison to the control. A; Temperature, Cadmium and pyrene (10 $\mu\text{g/L}$), B; Temperature, Cadmium and pyrene (100 $\mu\text{g/L}$), C; Temperature, Cadmium and pyrene (10 $\mu\text{g/L}$)

4.4.3 Functional annotations

Gene ontology (GO) enrichment analyses for the differently expressed genes (DEGs) were conducted. The results outlined in Figures 30 and 31 show the GO functional annotations and classifications that explain the distributions of DEGs i.e., biological processes (BP), molecular functions (MF), and cellular components (CC). In BP, the highest number of genes were involved in metabolic processes while in MF, the greatest number of genes were involved in catalytic or binding processes. Due to the large number of DEGs involved in many pathways, the visual results (Figures 10 and 13) only showed the terms involved in 30 differential genes (the highest p-value).

Within the salinity, cadmium and pyrene treatments, biological processes dominated the 30 visually represented GO functional annotations, particularly in the 100 µg/L cadmium and pyrene treatments, while more cellular and molecular functions were observed in the 10 µg/L (Figure 30). The major biological processes recorded across the different salinity, cadmium and pyrene treatments were mitochondrion organization, mitochondrial genome maintenance, and DNA strand replication. The top 30 GO annotations for the temperature, cadmium and pyrene treatments were majorly dominated by biological processes including mitochondrion organisation, organelle inheritance, and mitochondrial genome maintenance (Figure 31).

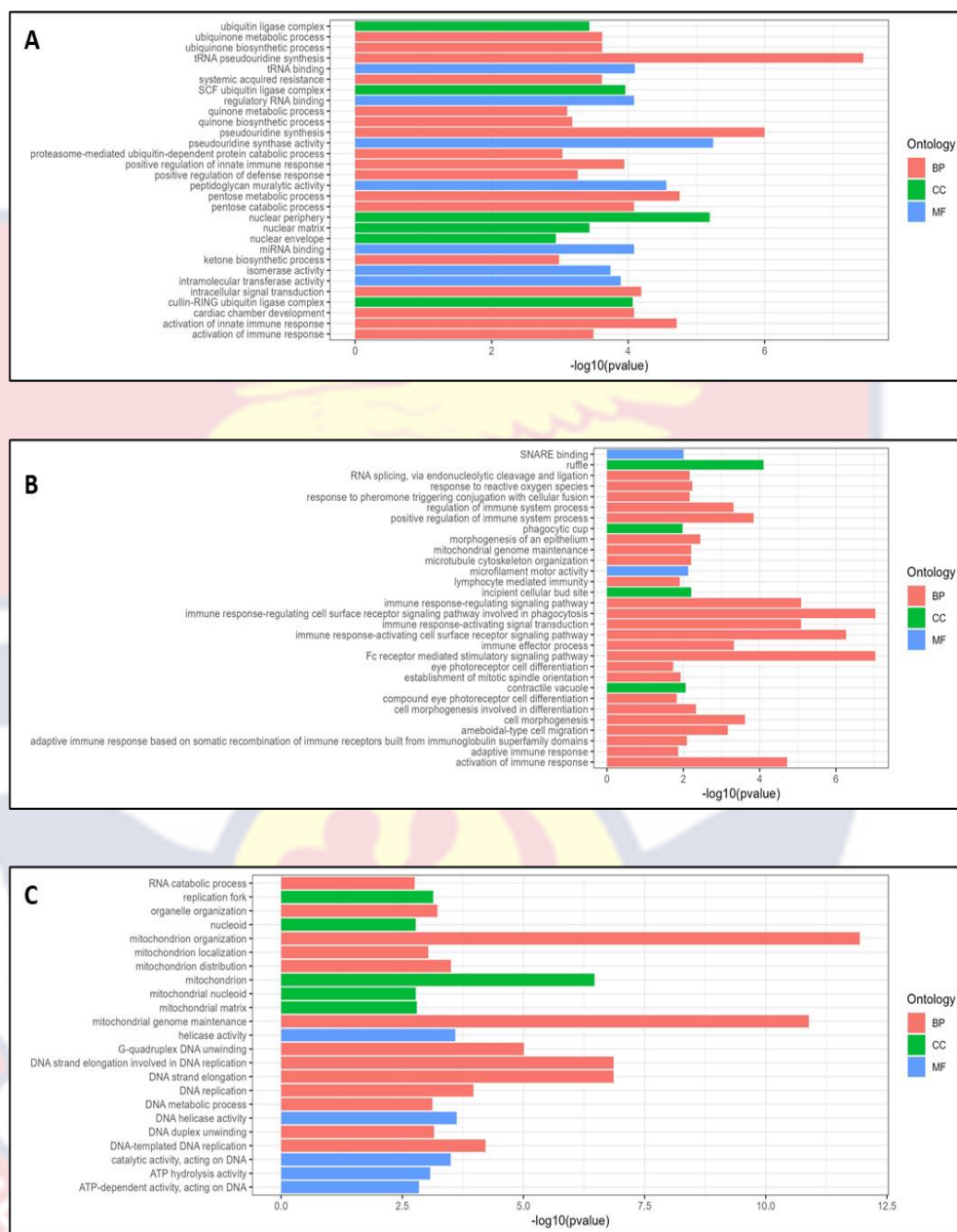


Figure 30: Gene Ontology annotation of differentially expressed genes after a mixture of A: Salinity + Cadmium_ pyrene (10 $\mu\text{g/L}$), B; Salinity + Cadmium_ pyrene (100 $\mu\text{g/L}$), C; Salinity + Cadmium_ pyrene (1000 $\mu\text{g/L}$). Bar graphs representing GO annotation into three categories, biological processes (BP), molecular functions (MF), and cellular components (CC)

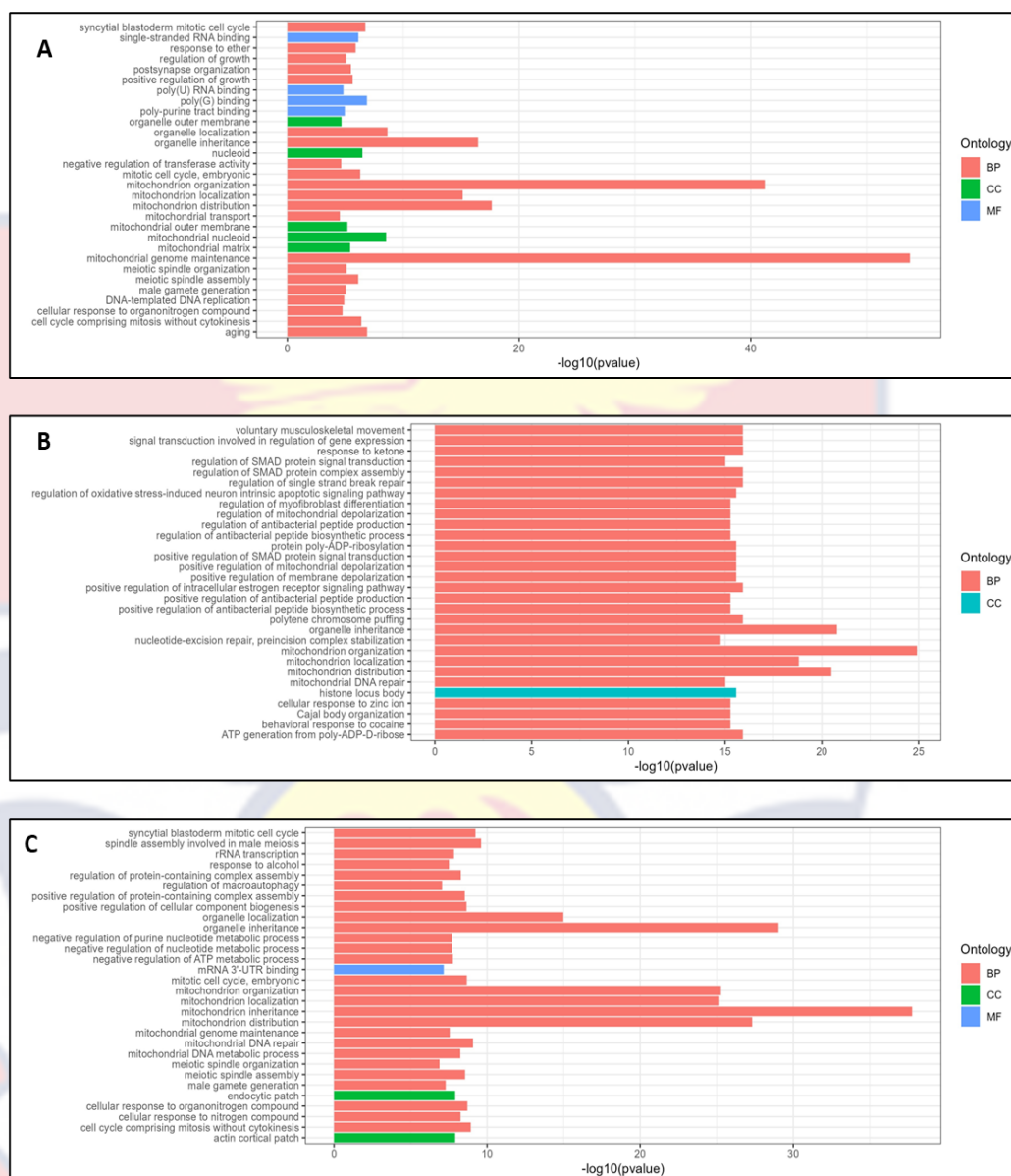


Figure 31: Gene Ontology annotation of differentially expressed genes after a mixture of A; Temperature, Cadmium and pyrene (10 $\mu\text{g/L}$), B; Temperature, Cadmium and pyrene (100 $\mu\text{g/L}$), C; Temperature, Cadmium and pyrene (10 $\mu\text{g/L}$). Bar graphs representing GO annotation into three categories, biological processes (BP), molecular functions (MF), and cellular components (CC)

CHAPTER FIVE

DISCUSSION

Phytoplankton represent the basis of coastal and marine productivity and support food-web structures and biochemical cycles. The occurrence, abundance, diversity, and physiological characteristics of these important organisms are highly influenced by environmental growth conditions such as temperature, nutrient, pH, and salinity (Bonachela et al., 2016; Boyce et al., 2010, Boyce et al., 2014), which are also under significant threat from climate change and pollution impacts. As a result of their strong interaction with environmental conditions, phytoplankton functional attributes such as chlorophyll-*a* and growth rate are used as indicators of water quality conditions in aquatic ecosystems (Sathicq et al., 2017). Many coastal ecosystems are dominated by diatoms which can produce blooms by multiplying their biomass within a few days (Acheampong et al., 2021; Vincent & Bowler, 2020). Particularly, diatoms of the *Thalassiosira* genus have been found to dominate blooms in many eutrophic waters since they are tolerant to ecosystems with poor water quality (Ishida et al., 2000). The focus of this study was to assess the dynamics of tropical coastal phytoplankton communities using the Whin Estuary in Ghana as a case study. Based on the findings of this field study, *Thalassiosira weissflogii*, a cosmopolitan diatom adapted to the coastal water of Ghana, was employed as a test organism to investigate its functional and molecular responses to individual and combined impacts of climate change (temperature, pH, salinity) and pollution (pyrene, cadmium) related stressors. This section discusses

the results of these field and laboratory studies, offering explanations to the findings by drawing on appropriate literature.

5.1 Physicochemical and Phytoplankton Characteristics of Whin Estuary

Estuarine ecosystems by their nature are characterised by high variability of environmental conditions (Miththapala, 2013). Organisms in these environments must therefore be adapted to daily and seasonal fluctuations in environmental conditions. Similarly, Whin Estuary exhibited significant variations in physicochemical parameters across different seasons and tidal periods (Figure 2). The physicochemical parameters recorded in this study, particularly for the dry season, are similar to those reported by Aheto et al., (2011) and CRC/FoN, (2010) for the same ecosystem.

Temperature is perhaps one of the most important environmental parameters in aquatic ecosystems (Pliński & Józwiak, 1999), which influences physiological and behavioural attributes in plankton organisms such as growth, reproduction, and survival (Fatema et al., 2015). It also controls the solubility of gases and salts in water, further influencing the distribution of organism in aquatic environments. Expectedly, higher temperatures were observed during the dry season than in the rainy season, which reflects the climate conditions in southern Ghana for both the dry and rainy seasons (Dzakpasu, 2019). The high temperatures during the dry season could have been as a result of high solar radiation as well as the influence from tidal currents and inflow of freshwater with higher temperatures (Hasan et al., 2022). The recorded temperature ranges in Whin is similar to those observed in other estuarine ecosystems during similar sampling periods. For

instance, studies carried out by Dzakpasu, (2019) on shallow estuarine ecosystems in Ghana recorded temperature ranging between 23 – 34 °C. Other studies undertaken by Abdul et al., (2016) also recorded similar temperature ranges within a coastal ecosystem along the Gulf of Guinea coastline.

Salinity is the primary factor influencing the distribution of organisms across an estuary (Elliott & Whitfield, 2011). In a typical estuarine ecosystem, freshwater organisms will be limited to the upper reaches while marine organisms will inhabit the estuarine mouth. However, majority of the estuary associated species are highly euryhaline (Bulger et al., 1993). In addition, the high temperatures observed particularly during the dry season could have facilitated higher rates of evaporation (Gadhia et al., 2013). Contrastingly, high precipitation levels, coupled with increased inflow of freshwater into the estuary could explain the lower levels of salinity recorded during the rainy season (Hasan et al., 2022). It is also possible that other anthropogenic factors such as increasing urbanisation and destruction of coastal wetland could have further facilitated higher freshwater inputs into the estuary during the rainy season (Dadzie-Paintsil & Mensah, 2022).

A pH of about 6.5-8.5 is considered optimal for the growth and survival of estuarine organisms (Fisher, 1993). For the present study, the pH for the dry season was within these optimal levels, while that of the rainy season was below these values. The high pH levels observed in the dry season could be attributed to the high levels of salinity recorded, which provided a natural buffering from the carbonate and bicarbonate dissolved in water (Fisher, 1993). Contrastingly, lower pH levels were observed during the rainy season with mean values of 4.59 being

recorded. Fisher, (1993) asserts that pH values below 5 and above 10 are often an indication of industrial pollution or cataclysmic event. He further indicated that several factors determine pH levels of estuarine ecosystems i.e., chemical constituents in run-off, water turbulence, bacterial activity and anthropogenic activities such as acid mine drainage and accidental spills (Fisher, 1993). Studies carried out on the Long Island sound reported pH below 5, particularly in summer and fall, which was attributed mainly to the high rates of microbial respiration fuelled by nutrient enriched organic matter (Wallace et al., 2014). Similar observations have also been reported in other studies i.e., Howarth et al. (2011) and Cai et al. (2021) in other coastal ecosystems. Another cause of low pH in aquatic ecosystems is drainage from acid soils, which increase oxidation and leaching of acidity of metals into aquatic ecosystems, particularly during high run-off periods (Saarinen & Kløve, 2012). Whin estuary is characterised by a great diversity of industrial, domestic and agricultural discharges (Dadzie-Paintsil & Mensah, 2022), which may have contributed to the observed significant wide pH variations across the seasons. However, it is impossible to conclude on this because of the limited data available on Whin River hydro-morphology, or the anthropogenic factors that influence its parameters.

Another important indicator of water quality and pollution in estuarine ecosystems is dissolved oxygen (DO) (Liu et al., 2020). DO concentration of 5 mg/L is considered the minimum for survival of organisms in lotic waters (Okyere 2019). For the study, high levels of DO were observed during the low tide in the dry season. This correlates with the relatively high number of phytoplankton genera

observed during the same period, signalling high levels of photosynthetic activities (Hasan et al., 2022). Further, the very low levels in DO observed during the rainy season could be attributed to increased rates of run-off that potentially introduced higher levels of oxygen-depleting organic compounds from domestic, industrial, and agricultural sources (Li et al. 2020). Similar to DO, the high chlorophyll-*a* levels observed during the high tide could be associated with the relatively high phytoplankton abundances during this season and tidal period (Fatema et al., 2015; Hasan et al., 2022).

Secchi disk observation have shown that the Whin Estuary is shallow, with high levels of transparency particularly during the dry season. Generally, estuaries in the Gulf of Guinea are shallow with transparency levels similar to those observed in Whin (Abdul et al. 2016; Abdul et al. 2018; Biney 1990; Simier et al. 2021).

The study identified 50 genera of phytoplankton belonging to seven (7) classes, each varying in distribution across the different sampling stations, tidal periods, and season. This study recorded higher diversity of phytoplankton groups in estuarine ecosystems than that of earlier study on the Whin Estuary (CRC/FoN, 2010) that reported 25 genera. Studies on phytoplankton distribution in Ghana have primarily focused on freshwater ecosystems and coastal lagoons (Addico et al., 2017; Agyekum et al., 2008; Alhassan, 2015) with very little consideration being given to estuaries. Studies undertaken in other tropical coastal aquatic ecosystems such as in Nigeria (Abdul et al. 2018) and Bangladesh (Hasan et al., 2022) reported fewer number of phytoplankton genera. Whin has been reported to be one of

Ghana's most productive estuaries (CRC/FoN, 2010), which explains the relatively high abundance of phytoplankton groups observed.

Generally, phytoplankton groups tend to become dominant when the environmental conditions of the aquatic ecosystems match its growth requirements (Hasan et al., 2022). During the dry season, the upper reaches of the estuary were particularly inhabited by freshwater and brackish water organisms such as the *Pseudanabaena* sp. and *Cylindrotheca* spp., while the mouth was dominated by diatoms. Salinity is the most important determinant for diatoms distribution and composition in estuarine ecosystems (Taffs et al., 2017; Vos & de Wolf, 1993). There are even a number of schemes that have been developed to associate diatom groups to specific salinity levels (Potapova, 2011). In the current study, Bacillariophyceae and Dinophyceae had the highest abundance of observed genera, which could be attributed to suitable environmental conditions in terms of salinity, temperatures, pH and dissolved oxygen that optimised their growth and productivity (El Gammal et al., 2017), particularly within the dry season. Additionally, the relatively stable stream flow and environmental conditions further explain the high numbers of diatoms observed (Varela & Prego, 2003).

Diatom genera such as *Coscinodiscus* and *Pseudo-nitzschia* have been found to form blooms which are caused by combination of elevated factors such as high concentrations of nutrients and elevated temperatures (Torres Palenzuela et al., 2019). These blooms are characterised by high biomass accumulation which could in turn be harmful to the organisms in estuarine ecosystems by facilitating conditions such as hypoxia, anoxia and decreased penetration of sunlight to

submerged vegetation (Tsikoti & Genitsaris, 2021). The high phytoplankton abundances coupled with low diversity, richness and evenness observed in the Whin Estuary during the dry seasons could be an indication of the potential for blooms to be formed, particularly when the favourable conditions are facilitated.

Diatom genera such as *Navicula*, *Nitzschia*, *Thalassiosira*, were observed across all the seasons and tidal periods, which could mean that they are adapted to the conditions within the estuary. This could also be an indication of their euryhaline and eurythermal nature as highlighted by previous studies by Bharathi et al. (2022). These organisms also occupy wide ranges of habitats from freshwater, brackish and marine ecosystems, which could explain their presence throughout the different periods. In contrast, the rainy season had very low phytoplankton abundance which could be attributed to factors such as reduced salinity, low pH and high levels of flushing associated with heavy rainfall (Hasan et al., 2022).

The results from the canonical correspondence analysis (CCA) for the main environmental parameters and phytoplankton genera showed that organisms belonging to the genus *Nostoc* had a positive correlation with all the physicochemical parameters (Figure 10). *Nostoc* is a cosmopolitan cyanobacteria associated with fresh, brackish and marine waters (Fidor et al., 2019). Cyanobacteria such as *Nostoc* have been known to form harmful blooms that threaten human and environmental health and release toxins and secondary metabolites that alter biodiversity, food webs and water quality (Fidor et al., 2019; Liu et al., 2020). Studies carried out by Yadav et al. (2022) have shown that *Nostoc* growth rates increased in high temperatures ($> 25^{\circ}\text{C}$) and alkalinity (19 – 78 mg

$\text{CaCO}_3 \text{ L}^{-1}$ /7-9). Perhaps, this explains why a high abundance of *Notsoc* was similarly recorded in the dry season for the current study.

5.2 Effects of Individual Stressors on the Functional Attributes of

Thalassiosira weissflogii

This study assessed the impacts of varying degrees of individual stressors (i.e., temperature, pH, salinity, cadmium, and pyrene concentrations) on functional attributes (i.e., growth rates, chlorophyll-*a*, and biomass concentrations) (per cell and cumulatively per Liter) of the model cosmopolitan diatom *Thalassiosira weissflogii*. In nature, these stressors do not exist individually, but often in combination with each other. Nevertheless, it is important for laboratory experiments to assess diatom responses to individual stressors before carrying out combined stressor tests. In this study, individual stressor experiments were also used to determine the proportions of individual stressors needed to form the combined stressor concentrations.

Temperature does not only influence cell processes in all organisms; it is also one of the most studied stressors in relation to diatom physiological functions (Acheampong, Mantey, and Weremfo 2021; Agawin, Duarte, and Agustí 2000; Boyd et al. 2013; Pliński and Józwiak 1999; Walter et al. 2015; Thomas et al. 2017). Studies have shown that when diatoms are removed from other competitive factors (i.e., from the natural environment to unicellular cultures in the laboratory), their growth rates increase with increasing temperatures (Anderson, 2000). Results of the current study indicates that higher temperatures (28-32 °C) caused increased growth rates in *T. weissflogii* within the first 48 hours of exposure but decreased

after 72 hours (Figure 11). Previous studies carried out by Walter et al., (2015) found that above a critical temperature of 8°C, growth rates of *T. weissflogii* increased with increasing temperature under experimental conditions. However, other studies carried out by Acheampong, Mantey, and Weremfo (2021), highlights that those high levels of warming conditions significantly reduced the growth rates in *T. weissflogii* and *Gymnodinium* sp. The conclusion for our observation could be that high temperatures spur high rates of reproduction *T. weissflogii*, however, at the same time, prolonged exposure to these high warming conditions may hamper their growth rate. Again, this study did not find any significant differences in growth rates between the different temperatures (24-32 °C), which concurs with Chen et al. (2021) who also reported consistent growth rates of *T. weissflogii* irrespective of temperatures, which ranged between 20-28 °C. This observation could be attributed to the fact that in natural ecosystems, *T. weissflogii* is a widely distributed cosmopolitan diatom with wide thermal range capacities (Chen et al. 2021; Kent, Whyte, and LaTrace 1995). Thus, it is possible *T. weissflogii* and similar diatoms could adapt to warmer sea-surface temperatures as projected by the IPCC (2022).

In addition to their cosmopolitan nature, diatoms such as those belonging to the genus *Thalassiosira* have also demonstrated the ability to acclimate and adapt to gradual increases in sea surface temperatures (Irwin et al., 2015). Phytoplankton in general are characterised by short generation times and large population sizes, which shorten the processes of natural selection required for evolutionary change (Acheampong et al., 2021; Schaum et al., 2016). For instance, studies undertaken

by Schaum et al., (2016) and Schaum et al., (2018) demonstrated that *T. pseudonana* can adapt to warming after 300 generations, whereby warming scenarios were realistic and temperature increases were moderate. However, extreme warming events such as those associated with marine heatwaves can lead to adverse consequences that exceed the ability of phytoplankton to adapt (Acheampong, Mantey, and Weremfo, 2021). Similarly, Schaum et al. (2018) found that evolutionary rescue was slow (> 1 year) under severe warming conditions.

Arguably, salinity is the most important environmental factor limiting the distribution of organisms in coastal ecosystems. As highlighted in the previous sections of this chapter, salinity changes can affect phytoplankton communities due to species-level differences in salinity optima (D'ors et al., 2016; Muylaert et al., 2009). For instance, rapid and large salinity changes can subject phytoplankton cells to osmotic stress, leading to the disturbance of cellular homeostasis, suppress physiological processes such as photosynthesis, carbon fixation, cell division and nitrogen metabolism, all of which are related to growth rate (Sew & Todd, 2020). In this study, *T. weissflogii* cultures were exposed to varying salinity conditions (1 - 35 ppt) and their functional attributes measured. Lower salinities (1 and 5 ppt) had the least growth rates, which declined to below 0 after 24 hours of exposure (Figure 12). This could be due to the acclimation conditions that were set based on the ranges observed for the Whin Estuary (approximately 25‰). Balzano, Sarno, and Kooistra (2011) have also shown that factors such as the area of sampling and conditions of acclimation had an impact on phytoplankton response to different

salinities. They highlighted that phytoplankton strains that show no growth below a salinity of 7.5 tend to be sampled from either plain seawater or mildly brackish sea water.

Salinity decreases below 15‰ are considered to be within the range of hypotonic stress for phytoplankton (Hernando et al., 2015; Kirst, 1977). For the current study, growth rates for *T. weissflogii* showed a slight increase in cell density in the first 24 hours of exposure, followed by declines in growth rates in the subsequent periods. Despite this decrease in growth rates, the diatoms proved capable of surviving in varied salinity conditions including those with high levels of hypotonic stress. The decrease in the number of cells after the first day of exposure to low salinity conditions could be attributed to the loss of diatom membrane integrity as outlined in other studies (Radchenko & Il'Yash, 2006).

In contrast, higher growth rates were documented in high salinities (15-35‰) across all the three days of exposure. *Thalassiosira* sp. is generally considered to be a coastal diatom with adaptations suited for mild to higher salinities (>15‰). Vrieling et al. (2007) found similar growth responses in two diatoms (*T. punctigera* and *T. weissflogii*) at 20 and 33 ‰, respectively. In a separate study, Baek, Jung, and Shin (2011) also found that diatoms such as *T. pseudonana* became more dominant when salinity was increased (>25‰) because of their wide salinity ranges. Studies have shown that future coastal ecosystems such as estuaries will likely become more saltier because of the increase in climate change drivers such as sea-level rise, reduced freshwater inflows and droughts (Havens & Steinman, 2015). In contrast, climate change driven storms and flooding

will result in sudden decreases in salinities in coastal ecosystems (Radchenko & Il'Yash, 2006). The results of this study show that these projected global changes will have significant and lasting impacts on phytoplankton, their growth and their ability to carry out primary production and support coastal food webs.

Increased CO₂ in the atmosphere as a result of fossil fuel combustion, land use changes and deforestation have resulted in the decrease of global water pH (Gattuso et al., 2015). For this study, *T. weissflogii* cultures were exposed to five levels of pH (5-9), which were obtained from the values observed in the Whin estuary. Higher growth rates were observed for pH 7, while the least growths were observed under pH 5 treatments. These results are similar those recorded in other studies by Berge et al., (2010) and Chen & Durbin, (1994), who reported higher growth rates in higher pH values of up to 8.6. in addition, a study carried out (Zaher & Helal, (2020), also reported maximum cell growth and chlorophyll-*a* production in pH ranging between 7-9. Just like in this current study, the general consensus among all these studies is that lower pH (< 7) significantly reduces the growth rate in phytoplankton (Falkowski 1997; Chen and Durbin 1994; Berge et al. 2010; Zaher and Helal 2020). Generally, few studies have been conducted to highlight the impacts of low pH on phytoplankton (Berge et al., 2010), particularly in tropical ecosystems. This could be attributed to the fact that pH was previously not considered an important chemical parameter influencing biotic processes in marine and coastal ecosystems (Chen and Durbin 1994). However, this study has highlighted that pH changes have significant impacts on phytoplankton growth rates, with even more significant risks to coastal productivity.

Coastal environments are highly susceptible to crude oil contamination and their subsequent impacts on ecosystem and human health (Botwe et al., 2017; Duran & Cravo-Laureau, 2016). In the Gulf of Guinea, around 12,000 oil spills have been reported from 1976 to 2014, resulting in the spillage of approximately 3 million barrels of oil into the Niger Delta (Chinedu & Chukwuemeka, 2018; Salgado-Hernanz et al., 2021). In recent times, Ghana has continued to intensify petroleum oil exploration, particularly along the coast, which have resulted in severe impacts on the environment. For this study, the impacts of pyrene on *Thalassiosira weissflogii* were evaluated. The results showed significant differences in growth rates across the different pyrene concentrations just after day 1 of exposure. However, exponential growth rates were observed in control groups (no-pyrene and acetone control) across all the days of exposure. This suggests that the acetone solvent (i.e., as a carrier of pyrene) did not affect growth rates of *T. weissflogii* cultures during exposure, and therefore, any observed effects were purely as a result of pyrene concentrations. The estimated effective median concentrations (EC50) values of pyrene to *T. weissflogii* in this study are similar to those reported by Bopp and Lettieri (2007) in *T. pseudonana*. In another study, Hjorth et al. (2007) found a 25% reduction in phytoplankton activity in the first 24 hours exposure to 25 nmol L⁻¹ of pyrene compared to the control (no pyrene concentration), although the differences were not statistically different. The lower sensitivity of *T. weissflogii* to pyrene observed in these studies could be attributed to the fact that cosmopolitan diatoms isolated from estuarine conditions exhibits lower sensitivity to chemical contamination than similar species isolated in the open

ocean where there is low fluctuations in chemical conditions (Bopp & Lettieri, 2007).

Cadmium pollution of coastal ecosystems is of great concern because of its potential to bioaccumulate across food chains (Xu & Morel, 2013). In the current study, *T. weissflogii* were exposed to different cadmium concentrations (1- 10 µg/L), and the results showed that growth rate significantly decreased with increasing cadmium concentrations after 24 hours of exposure. These observations are consistent with report by Wahyu, Hindarti, and Permana (2020) whose study assessed the impacts of cadmium toxicity on *Thalassiosira* sp. and its alteration on the diatoms chlorophyll-*a* and carotenoid contents. Their study concluded that the growth inhibition of *Thalassiosira* sp. and cadmium concentration are correlated; the greater the concentration of cadmium, the less cell densities of *Thalassiosira* sp. obtained. In a separate study, Lee, Roberts, and Morel (1995) opined that cadmium acts as a nutrient in Zn-limited environments, with very low concentrations even stimulating growth.

The total chlorophyll-*a* and biomass (µg/L) concentrations of *T. weissflogii* obtained for the various individual experiments followed a similar trend as that of the growth rate. Generally, higher chlorophyll-*a* and biomass concentrations were observed under conditions similar to those prevailing in the natural environment (Whin Estuary), or during acclimation period. However, across the different treatments, *T. weissflogii* chlorophyll-*a* and biomass concentrations per each individual cell increased in higher levels of stress. For instance, the highest values for chlorophyll-*a* per cell was observed in the following treatments across the

different exposure experiment; 1‰ for salinity, 30 °C for temperature, 5 for pH, 100+nM for pyrene and 10 µg/L for cadmium (Figure 11-15). These observations are important in highlighting the disparities between what happens at the broader ecosystem level and cellular levels and the need to assess phytoplankton responses to stressors beyond just the loss of cells, but more towards the processes that occur within the cell. Similar observations have been documented in other studies that evaluated diatoms under stress conditions such as CO₂, temperature, nutrients and pH (Gao et al., 2019; Gao & Campbell, 2014; G. Li & Campbell, 2013a, 2013a; Sun et al., 2011; Torstensson et al., 2012; Wu et al., 2010).

5.3 Effects of Combined Stressors on the Functional Attributes of

Thalassiosira Weissflogii

Traditionally, ecotoxicological studies determine the toxicity of chemicals using single species and a limited set of apical endpoints such as survival, growth and reproduction (Segner et al., 2014). However, many studies have also shown that in their natural environments, organisms are always exposed to multiple environmental hazards at the same time (Crain et al., 2008; Griffen et al., 2016; Mensah et al., 2017). Coastal ecosystems face a number of multiple stressors, including changes in salinity, nutrients, sea-level rise, temperature, CO₂, acidification, UV radiation, toxins, invasive species, diseases, hypoxia, disturbance, fishing pressure, among others (Griffen et al., 2016). Many studies on multiple stressors usually combine two or three of these stressors in orthogonal experiments (Crain et al., 2008). However, there still exist huge gaps in data when it comes to multiple stressor experiments, which has limited the ability to predict

the impact of multiple stressors on organisms, particularly under novel conditions (Griffen et al., 2016). In addition, impacts from multiple stressors have not been fully understood, particularly in tropical ecosystems (Asiedu, 2020; Griffen et al., 2016). In this context, this study evaluated three levels of combined stressors, i.e., temperature, cadmium and pyrene; salinity, cadmium and pyrene; and pH, cadmium and pyrene in separate experiments. Results of these experiments show that growth rates of the control groups were higher compared to all the treatment groups after 72 hours of exposure. However, growth rates decreased in higher combinations of cadmium and pyrene treatments (100 and 1000 µg/L) across all climate stressors (temperature, salinity and pH). Likewise, other studies have reported reduction in algal growth parameters in response to combined stressors (G. Li and Campbell 2013b; Li et al. 2021; Su et al. 2022; Ben Othman et al. 2023; Page et al. 2022). For instance, studies carried out by Aksmann and Tukaj, (2004) found that the combination of seawater acidification and 10 µg/L benzo(a)pyrene concentration decreased growth rate of the diatom *Skeletonema costatum* by about 70 % compared to the control group. In addition, their study further highlights that exposure to high CO₂ exacerbated the negative impacts of anthracene on growth and photosynthesis of *Scenedesmus armatus*, a green alga. Contrastingly, Dong et al. (2020) reported that combined ocean acidification and cadmium stress promoted growth in *Phaeodactylum tricornutum*.

In the current study, highest decrease in cell densities was 50.47 % recorded in 15‰ salinity and 1000 µg/L (cadmium and pyrene concentration) exposure (Appendix D). The mean cell density reductions across the different stressors were

30.84 %, 26.22 % and 17.64 % for the pH, cadmium and pyrene mixture; salinity, cadmium and pyrene mixture; and temperature, cadmium and pyrene mixture treatments respectively. These values are significantly lower than those obtained by Aksmann and Tukaj (2004) who assessed impacts of two PAH pollutants (anthracene and phenanthrene) in combination with different levels of PAR Irradiance and CO₂ level on the growth rate of *Scenedesmus armatus*. The effects of combined concentrations on phytoplankton differs depending on the type of species and the concentrations of the stressors, making it difficult to draw comparison between their observations with that of our own study.

Phytoplankton generally tend to be more affected by higher concentrations of pollutant. Our study recorded cell densities of 19.92%, 21.79% and 33.23% in the three pollution (cadmium and pyrene) stressor concentrations, 10 µg/L, 100 µg/L and 1000 µg/L respectively. The highest reduction of cell density was observed in the higher concentration pollution treatments (1000 µg/L) across all the different experiments. This observation could be attributed to the fact that organisms generally tend to be greatly affected by higher concentrations of environmental stressors. In addition, the combination of climate change and pollution stressors has been found to result in synergistic effects in coastal ecosystems (Cabral et al., 2019).

5.4 Effects of Combined Climate Change and Pollution Stressors on *Thalassiosira Weissflogii* At Molecular Level

Transcriptome analysis of phytoplankton response to stressors in their environment, provides an indication of the metabolic processes that occur within

the cell and most likely, entire populations (Amato et al., 2017). Various studies have utilized molecular techniques to understand the effects of exposure to heavy metals and organic pollutants and nutrient starvation on phytoplankton (Zhao et al., 2020). For the current study, *Thalassiosira Weissflogii* cultures were exposed to temperature (32 °C) and salinity (35‰) in different concentrations of pyrene and cadmium combined. The temperature and salinity levels chosen for this study were based on the expected climate change impacts on estuarine ecosystems within the Gulf of Guinea (increased temperatures and salinities) (Acheampong et al., 2021; Amlalo & Oppong-Boadi, 2015; Ankrah, 2018).

Exposure to different environmental stressors results in the expression of genes differently as shown by this study (Zhao et al., 2020). Within the different salinity treatments, more genes were upregulated than downregulated in the 10 µg/L and 100 µg/L cadmium and pyrene mixtures. Oppositely, more genes were downregulated in the 10 µg/L and 100 µg/L cadmium and pyrene mixtures within the temperature treatments. This could mean that these genes were suppressed under the temperature, cadmium and pyrene mixtures treatments but induced under the salinity, cadmium and pyrene mixture treatments (Fu et al., 2020).

Various studies have reported different types of responses to environmental stressors on phytoplankton. For instance, studies carried out by Jian et al., (2017) found that nitrogen depleted *Thalassiosira pseudonana* cells involved in the carbon fixation process were downregulated, while genes from the same organisms were upregulated when exposed to polycyclic aromatic hydrocarbons (PAHs), heat stress, high light intensity and Polybrominated

diphenyl ethers (PBDEs) (Carvalho et al., 2011; Dong et al., 2016; Fu et al., 2020; Zhao et al., 2020). Unlike these studies however, the current study did not target specific biological processes, hence the overwhelmingly high number of genes mapped (> 50,000).

There were more differently expressed genes in the temperature (32 °C) than in the salinity (35‰) treatments across the three cadmium and pyrene concentrations (10 µg/L, 100 µg/L, and 1000 µg/L). Previous studies by (Acheampong et al., 2021) have shown that higher temperatures impaired the growth rates and protein contents of *Thalassiosira weissflogii*. On the other hand, *T. weissflogii* is a cosmopolitan diatom that thrives in marine environments (Heudre et al., 2021), which could explain, the lesser differently expressed genes (DEGs) observed in the salinity treatments in comparison to the temperature treatments, in combination with cadmium and pyrene.

The results from our studies show Gene Ontology (GO) annotations and classifications that explain the distributions of the top 30 DEG's in our different experiments. In organisms, one gene could be annotated into several categories due to its multiple functions (Yon Rhee et al., 2008; Zhao et al., 2020). Biological processes comprised the highest amount of DEG's recorded in the top 30 GO annotations. Studies carried out by Fu et al., (2020) on the red *algae Agarophyton vermiculophyllum*, indicate that majority of the observed biological functions were activated under heat stress. Generally, biological functions are controlled by temperature (Rowbury, 2003). This could explain why the temperature, cadmium and pyrene mixture treatments had more biological processes in their

GO annotations than the salinity, cadmium and pyrene mixture treatments. Additionally, the greatest number of functional annotations under molecular functions (MF) were involved in catalytic or binding processes. These results are synonymous with those obtained Zhao et al., (2020) on the molecular response mechanisms of a diatom *Thalassiosira pseudonana* to the toxicity of Polybrominated diphenyl ethers (PBDEs).



CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The main aim of the study was to assess the response of *Thalassiosira weissflogii* to the individual and combined climate change and pollution related stressors. Which were achieved through four specific objectives. Field studies were carried out in the Whin Estuary in order to determine the existing phytoplankton community structure and physicochemical parameters. Species belonging to the genus *Thalassiosira* were found to be the most dominant in terms of abundance and availability, while temperature, salinity and pH were found to be important climate change-related environmental stressors that significantly impacts the estuary's ecology. Literature review also suggested that pyrene and cadmium were important pollution-related stressors often present in coastal ecosystems of Ghana that could impact phytoplankton communities as individual stressors and in combination with climate change-related stressors. Therefore, *T. weissflogii* was selected and exposed to different levels of temperature, salinity, pH, cadmium and pyrene as individual and combined stressors in laboratory ecotoxicological experiments. The endpoints measured as responses of *T. weissflogii* to the environmental stressors were growth rate, chlorophyll-*a*, dry biomass (functional attributes) and a whole transcriptome analysis (genetic molecular attributes). The field studies highlighted the relationship between phytoplankton environmental variables, and climate change in coastal ecosystems. Conversely, the microcosm experiments showed that

climate change and pollution could have significant impact on phytoplankton at the functional and genetic molecular levels, whether acting individually or in combination to each other. These findings have provided insight into understanding the synergistic effects of climate change and pollution on coastal phytoplankton and productivity.

6.2 Conclusions

The following conclusions have been made, based on the findings from the present study:

- i. Differences in environmental parameters during dry and rainy seasons along the gradient of Whin Estuary strongly influenced phytoplankton abundance, composition, diversity and distribution. The Genus *Thalassiosira* was the most abundant genera recorded across all the seasons and tidal periods, accounting for nearly 30% of all the organisms recorded in Whin Estuary.
- ii. The model diatom, *Thalassiosira weissflogii*, used in the ecotoxicological investigations exhibited different responses to varying levels of climate change and pollution related stressors, either individually or in combination with each other. For the individual exposure experiments, significant differences were observed for salinity, pH and cadmium treatments. The highest decreases in cell densities, chlorophyll-*a* and biomass concentrations were observed in lower salinity and pH treatments.
- iii. The combination of pH and pollution stressors (cadmium and pyrene mixtures) had the highest effect on phytoplankton functional attributes. The

highest reductions in cell densities, chlorophyll-*a* and biomass concentrations were recorded in higher cadmium and pyrene levels, irrespective of the climate change-related stressor (temperature, pH, and salinity) with which they were combined.

- iv. The molecular response of *Thalassiosira weissflogii* using transcriptomics demonstrates that a combination of climate change and pollution stressors have a significant effect on their molecular processes. Higher numbers of differently expressed genes (DEGs) were recorded in the temperature, cadmium and pyrene mixture treatments in comparison to the salinity, cadmium and pyrene mixture treatments. In terms of functional annotation of the DEGs, more biological processes were observed in the temperature, cadmium and pyrene treatments when compared to the salinity, cadmium and pyrene treatments.

6.3 Recommendations

- i. The data presented in this study could be used as a baseline information to mitigate climate change and pollution impacts on Whin Estuary and other coastal ecosystems in Ghana.
- ii. The data generated from the study could be used to provide species-specific parameters for modelling the impacts of global change in tropical coastal ecosystems.
- iii. Future studies could attempt to understand the impacts of climate change and pollution related stressors on community interactions in coastal ecosystems to complement single-species effects as were done in this current study.

- iv. Further studies should be undertaken to understand the pathways for the major biological and molecular processes identified in *Thalassiosira weissflogii* during the transcriptome analysis.



REFERENCES

- Abdul, W. O., Adekoya, E. O., Ademolu, K. O., Omoniyi, I. T., Odulate, D. O., Akindokun, T. E., & Olajide, A. E. (2016). The effects of environmental parameters on zooplankton assemblages in tropical coastal estuary, South-west, Nigeria. *The Egyptian Journal of Aquatic Research*, 42(3), 281–287. <https://doi.org/10.1016/j.ejar.2016.05.005>
- Abdul, W. O., Oguntuase, K. E., Omoniyi, I. T., Bada, S. B., Adekoya, E. O., Bashir, A. O., Ibebuikwe, L. C., & Opajobi, G. A. (2019). Dynamics of heavy metal pollution in tropical lagoon of Gulf of Guinea, West Africa. *Journal of Applied Sciences and Environmental Management*, 23(5), 985–993.
- Abdul, W. O., Omoniyi, I. T., Oguntuase, K. E., Bada, S. B., Adekoya, E. O., & Braide, A. F. (2018). Influence of Environmental Variables on the Abundance and Distribution of Phytoplankton: A case Study of Lekki Lagoon, Sub-Saharan Africa. *Egyptian Journal of Aquatic Biology and Fisheries*, 21(4), 97–110. <https://doi.org/10.21608/ejabf.2018.7724>
- Acheampong, E., Mantey, P., & Weremfo, A. (2021). Potential impact of marine heatwaves on selected phytoplankton adapted to the Gulf of Guinea during stable hydrographic periods. *African Journal of Marine Science*, 43(1), 77–86. <https://doi.org/10.2989/1814232X.2021.1879267>
- Adams, S. M., & Greeley, M. S. (2000). Ecotoxicological Indicators of Water Quality: Using Multi-response Indicators to Assess the Health of Aquatic Ecosystems. *Water, Air, and Soil Pollution*, 123(1/4), 103–115. <https://doi.org/10.1023/A:1005217622959>

- Addico, G., Amu-Mensah, F. K., Akrong, M., Amu-Mensah, M., & Darko, H. (2017). Phytoplankton species diversity and biomass and its impact on the sustainable management of Lake Bosomtwe in the Ashanti Region of Ghana. *African Journal of Environmental Science and Technology*.
- Addo, K. A., & Adeyemi, M. (2013). Assessing the impact of sea-level rise on a vulnerable coastal community in Accra, Ghana. *Jàmbá: Journal of Disaster Risk Studies*, 5(1), 1–8.
- Adhiambo, R., Mensah, P. K., & Acheampong, E. (2023). Widespread Geographical Disparities in Phytoplankton Ecology Research in the Face of Climate Change: A Review. *Water*.
- Adhiambo, R., Mensah, P. K., Acheampong, E., & Krampah, E. A. (2024). Plankton assemblages in a tropical West African estuary. *Aquatic Sciences*, 86(3), 70. <https://doi.org/10.1007/s00027-024-01085-8>
- Adokoh, C. K., Obodai, E. A., Essumang, D. K., Serfor-Armah, Y., Nyarko, B. J. B., & Asabere-Ameyaw, A. (2011). Statistical Evaluation of Environmental Contamination, Distribution and Source Assessment of Heavy Metals (Aluminum, Arsenic, Cadmium, and Mercury) in Some Lagoons and an Estuary Along the Coastal Belt of Ghana. *Archives of Environmental Contamination and Toxicology*, 61(3), 389–400. <https://doi.org/10.1007/s00244-011-9643-5>
- Agawin, N. S., Duarte, C. M., & Agustí, S. (2000). Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnology and Oceanography*, 45(3), 591–600.

- Agyekum, K. A., Wiafe, G., & Appiah, K. A. (2008). *Diversity and spatial distribution of phytoplankton in a tropical coastal lagoon*. 10(1).
- Aheto, D., Mensah, E., Aggrey-Fynn, J., Obodai, E. A., Mensah, C. J., Okyere, I., & Okyere, S. P. K. (2011). Spatio-temporal analysis of two coastal wetland systems in Ghana: Addressing ecosystem vulnerability and implications for fisheries development in the context of climate and land use changes. *Archives of Applied Science Research*, 3(3), 499–513.
- Aksmann, A., & Tukaj, Z. (2004). The effect of anthracene and phenanthrene on the growth, photosynthesis, and SOD activity of the green alga *Scenedesmus armatus* depends on the PAR irradiance and CO₂ level. *Archives of Environmental Contamination and Toxicology*, 47, 177–184.
- Alemzadeh, E., Haddad, R., & Ahmadi, A.-R. (2014). Phytoplanktons and DNA barcoding: Characterization and molecular analysis of phytoplanktons on the Persian Gulf. *Iranian Journal of Microbiology*, 6(4), 296.
- Alhassan, E. H. (2015). *Seasonal variations in phytoplankton diversity in the Bui dam area of the Black Volta in Ghana during the pre- and post-impoundment periods*. 63(1).
- Alloway, B. J. (2013). Sources of heavy metals and metalloids in soils. *Heavy Metals in Soils: Trace Metals and Metalloids in Soils and Their Bioavailability*, 11–50.
- Almeda, R., Wambaugh, Z., Wang, Z., Hyatt, C., Liu, Z., & Buskey, E. J. (2013). Interactions between zooplankton and crude oil: Toxic effects and

bioaccumulation of polycyclic aromatic hydrocarbons. *PloS One*, 8(6), e67212.

Amato, A., Dell'Aquila, G., Musacchia, F., Annunziata, R., Ugarte, A., Maillet, N.,

Carbone, A., d'Alcala, M. R., Sanges, R., & Iudicone, D. (2017). *Marine diatoms change their gene expression profile when exposed to microscale turbulence under nutrient replete conditions*. *Sci Rep* 7: 3826.

Amlalo, D. S., & Oppong-Boadi, K. Y. (2015). *Ghana's Third National Communication Report to the UNFCCC: 2015 Climate Change Report*. Ministry of Environment, Science and Technology.

Anang, E. R. (1979). The seasonal cycle of the phytoplankton in the coastal waters of Ghana. *Hydrobiologia*, 62(1), 33–45.

<https://doi.org/10.1007/BF00012560>

Anderson, N. J. (2000). Diatoms, temperature and climatic change. *European Journal of Phycology*, 35(4), 307–314.

Ankrah, J. (2018). Climate change impacts and coastal livelihoods; an analysis of fishers of coastal Winneba, Ghana. *Ocean and Coastal Management*, 161, 141–146.

Appeaning Addo, K., Jayson-Quashigah, P.-N., Codjoe, S. N. A., & Martey, F. (2018). Drone as a tool for coastal flood monitoring in the Volta Delta, Ghana. *Geoenvironmental Disasters*, 5(1), 1–13.

Armstrong, B., Hutchinson, E., Unwin, J., & Fletcher, T. (2004). Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: A review and meta-analysis. *Environmental Health Perspectives*, 112(9), 970–978.

Asante, F. A., & Amuakwa-Mensah, F. (2014). Climate change and variability in Ghana: Stocktaking. *Climate*, 3(1), 78–101.

Asiedu, D. A. (2020). *Copepod abundance and impact of selected human induced stressors on calanoid copepod in the coastal sea of Ghana* [PhD Thesis]. University of Cape Coast.

Assmy, P., & Smetacek, V. (2009). *Encyclopedia of Microbiology*. Elsevier, chap. Algal Blooms.

Atindana, S. A., Fagbola, O., Ajani, E., Alhassan, E. H., & Ampofo-Yeboah, A. (2020). Coping with climate variability and non-climate stressors in the West African Oyster (*Crassostrea tulipa*) fishery in coastal Ghana. *Maritime Studies*, 19(1), 81–92. <https://doi.org/10.1007/s40152-019-00132-7>

Atindana, S. A., Ofori-Danson, P. K., & Brucet, S. (2019). Modelling the effects of climate change on shellfish production in marine artisanal fisheries of Ghana. *AAS Open Research*, 2, 16. <https://doi.org/10.12688/aasopenres.12956.1>

Awortwi, F. E., Frempong, E., Aikins, S. A., Hecky, R. E., Hall, R., & Puchniak, M. (2015). The relationship between mixing and stratification regime on the phytoplankton of Lake Bosomtwe (Ghana), West Africa. *West African Journal of Applied Ecology*, 23(2), 43–62.

Awortwi, F., Frempong, E., Aikins, S., & Hecky, R. (2022). Areal Light Utilization Efficiency of a Natural Community of Pelagic Phytoplankton in a Tropical

Lake (Lake Bosomtwe, Ghana). *Journal of Science and Technology (Ghana)*, 40(3), 77–93.

Ayoubi, H. E., & Failler, P. (2012). *Fishery and aquaculture industry in Ghana*.

<https://doi.org/10.13140/RG.2.1.1624.3362>

Azhikodan, G., & Yokoyama, K. (2016). Spatio-temporal variability of phytoplankton (Chlorophyll-*a*) in relation to salinity, suspended sediment concentration, and light intensity in a macrotidal estuary. *Continental Shelf Research*, 126, 15–26.

Baek, S. H., Jung, S. W., & Shin, K. (2011). Effects of temperature and salinity on growth of *Thalassiosira pseudonana* (Bacillariophyceae) isolated from ballast water. *Journal of Freshwater Ecology*, 1–6.

<https://doi.org/10.1080/02705060.2011.582696>

Bailey, A., De Wit, P., Thor, P., Browman, H. I., Bjelland, R., Shema, S., Fields, D. M., Runge, J. A., Thompson, C., & Hop, H. (2017). Regulation of gene expression is associated with tolerance of the Arctic copepod *Calanus glacialis* to CO₂-acidified sea water. *Ecology and Evolution*, 7(18), 7145–7160.

Bainbridge, V. (1972). The zooplankton of the Gulf of Guinea. *Bull. Mar. Ecol*, 8, 61–97.

Balzano, S., Sarno, D., & Kooistra, W. H. C. F. (2011). Effects of salinity on the growth rate and morphology of ten *Skeletonema* strains. *Journal of Plankton Research*, 33(6), 937–945. <https://doi.org/10.1093/plankt/fbq150>

- Bandowe, B. A. M., Bigalke, M., Boamah, L., Nyarko, E., Saalia, F. K., & Wilcke, W. (2014). Polycyclic aromatic compounds (PAHs and oxygenated PAHs) and trace metals in fish species from Ghana (West Africa): Bioaccumulation and health risk assessment. *Environment International*, 65, 135–146.
- Barnes, B. D., & Wurtsbaugh, W. A. (2015). The effects of salinity on plankton and benthic communities in the Great Salt Lake, Utah, USA: A microcosm experiment. *Canadian Journal of Fisheries and Aquatic Sciences*, 72(6), 807–817. <https://doi.org/10.1139/cjfas-2014-0396>
- Bauder, A. G., Cembella, A. D., Bricelj, V. M., & Quilliam, M. A. (2001). Uptake and fate of diarrhetic shellfish poisoning toxins from the dinoflagellate *Prorocentrum lima* in the bay scallop *Argopecten irradians*. *Marine Ecology Progress Series*, 213, 39–52.
- Beaugrand, G. (2005). Monitoring pelagic ecosystems using plankton indicators. *ICES Journal of Marine Science*, 62(3), 333–338. <https://doi.org/10.1016/j.icesjms.2005.01.002>
- Behrenfeld, M. J., Boss, E., Siegel, D. A., & Shea, D. M. (2005). Carbon-based ocean productivity and phytoplankton physiology from space: phytoplankton growth rates and ocean productivity. *Global Biogeochemical Cycles*, 19(1). <https://doi.org/10.1029/2004GB002299>
- Ben Othman, H., Pick, F. R., Sakka Hlaili, A., & Le Boulanger, C. (2023). Effects of polycyclic aromatic hydrocarbons on marine and freshwater microalgae – A review. *Journal of Hazardous Materials*, 441, 129869. <https://doi.org/10.1016/j.jhazmat.2022.129869>

- Berge, T., Daugbjerg, N., Balling Andersen, B., & Hansen, P. (2010). Effect of lowered pH on marine phytoplankton growth rates. *Marine Ecology Progress Series*, 416, 79–91. <https://doi.org/10.3354/meps08780>
- Bergström, A.-K., & Karlsson, J. (2019). Light and nutrient control phytoplankton biomass responses to global change in northern lakes. *Global Change Biology*, 25(6), 2021–2029.
- Bharathi, M. D., Venkataramana, V., & Sarma, V. (2022). Phytoplankton community structure is governed by salinity gradient and nutrient composition in the tropical estuarine system. *Continental Shelf Research*, 234, 104643.
- Bindoff, N. L., Willebrand, J., Artale, V., Cazenave, A., Gregory, J. M., Gulev, S., Hanawa, K., Le Quere, C., Levitus, S., & Nojiri, Y. (2007). *Observations: Oceanic climate change and sea level*.
- Biney, C. A. (1990). A review of some characteristics of freshwater and coastal ecosystems in Ghana. *Hydrobiologia*, 208, 45–53.
- Bonachela, J. A., Klausmeier, C. A., Edwards, K. F., Litchman, E., & Levin, S. A. (2016). The role of phytoplankton diversity in the emergent oceanic stoichiometry. *Journal of Plankton Research*, 38(4), 1021–1035. <https://doi.org/10.1093/plankt/fbv087>
- Bopp, S. K., & Lettieri, T. (2007). Gene regulation in the marine diatom *Thalassiosira pseudonana* upon exposure to polycyclic aromatic hydrocarbons (PAHs). *Gene*, 396(2), 293–302.

- Botwe, B. O., Kelderman, P., Nyarko, E., & Lens, P. N. (2017). Assessment of DDT, HCH and PAH contamination and associated ecotoxicological risks in surface sediments of coastal Tema Harbour (Ghana). *Marine Pollution Bulletin*, 115(1–2), 480–488.
- Boyce, D. G., Dowd, M., Lewis, M. R., & Worm, B. (2014). Estimating global chlorophyll changes over the past century. *Progress in Oceanography*, 122, 163–173. <https://doi.org/10.1016/j.pocean.2014.01.004>
- Boyce, D. G., Lewis, M. R., & Worm, B. (2010). Global phytoplankton decline over the past century. *Nature*, 466(7306), 591–596. <https://doi.org/10.1038/nature09268>
- Boyd, P. W., Rynearson, T. A., Armstrong, E. A., Fu, F., Hayashi, K., Hu, Z., Hutchins, D. A., Kudela, R. M., Litchman, E., Mulholland, M. R., Passow, U., Strzepek, R. F., Whittaker, K. A., Yu, E., & Thomas, M. K. (2013). Marine Phytoplankton Temperature versus Growth Responses from Polar to Tropical Waters – Outcome of a Scientific Community-Wide Study. *PLoS ONE*, 8(5), e63091. <https://doi.org/10.1371/journal.pone.0063091>
- Brown, C. A., Sharp, D., & Mochon Collura, T. C. (2016). Effect of climate change on water temperature and attainment of water temperature criteria in the Yaquina Estuary, Oregon (USA). *Estuarine, Coastal and Shelf Science*, 169, 136–146. <https://doi.org/10.1016/j.ecss.2015.11.006>
- Bulger, A. J., Hayden, B. P., Monaco, M. E., Nelson, D. M., & McCormick-Ray, M. G. (1993). Biologically-Based Estuarine Salinity Zones Derived from a

Multivariate Analysis. *Estuaries*, 16(2), 311.

<https://doi.org/10.2307/1352504>

Burian, R. M. (1993). How the Choice of Experimental Organism Matters: Epistemological Reflections on an Aspect of Biological Practice. *Journal of the History of Biology*, 26(2), 351–367. JSTOR.

Cabral, H., Fonseca, V., Sousa, T., & Costa Leal, M. (2019). Synergistic effects of climate change and marine pollution: An overlooked interaction in coastal and estuarine areas. *International Journal of Environmental Research and Public Health*, 16(15), 2737.

Cai, W.-J., Feely, R. A., Testa, J. M., Li, M., Evans, W., Alin, S. R., Xu, Y.-Y., Pelletier, G., Ahmed, A., & Greeley, D. J. (2021). Natural and anthropogenic drivers of acidification in large estuaries. *Annual Review of Marine Science*, 13, 23–55.

Carneiro, F. M., Nabout, J. C., & Bini, L. M. (2008). Trends in the scientific literature on phytoplankton. *Limnology*, 9(2), 153–158.
<https://doi.org/10.1007/s10201-008-0242-8>

Carvalho, R. N., Burchardt, A. D., Sena, F., Mariani, G., Mueller, A., Bopp, S. K., Umlauf, G., & Lettieri, T. (2011). Gene biomarkers in diatom *Thalassiosira pseudonana* exposed to polycyclic aromatic hydrocarbons from contaminated marine surface sediments. *Aquatic Toxicology*, 101(1), 244–253.

Chakraborty, P., Acharyya, T., Babu, P. R., & Bandyopadhyay, D. (2011). Impact of salinity and pH on phytoplankton communities in a tropical freshwater

system: An investigation with pigment analysis by HPLC. *Journal of Environmental Monitoring*, 13(3), 614–620.

Chen, C. Y., & Durbin, E. G. (1994). Effects of pH on the growth and carbon uptake of marine phytoplankton. *Marine Ecology-Progress Series*, 109, 83–83.

Chen, J., Guo, K., Thornton, D. C., & Wu, Y. (2021). Effect of temperature on the release of transparent exopolymer particles (TEP) and aggregation by marine diatoms (*Thalassiosira weissflogii* and *Skeletonema marinoi*). *Journal of Ocean University of China*, 20, 56–66.

Chepurnov, V. A., Steigüber, C. G., & Siegel, P. (2018). Diatoms as hatchery feed on-site cultivation and alternatives. *Hatcheryfeed*, 6(3), 23–27.

Chinedu, E., & Chukwuemeka, C. K. (2018). Oil spillage and heavy metals toxicity risk in the Niger Delta, Nigeria. *Journal of Health and Pollution*, 8(19), 180905.

Chizhova, T., Koudryashova, Y., Prokuda, N., Tishchenko, P., & Hayakawa, K. (2020). Polycyclic aromatic hydrocarbons in the estuaries of two rivers of the sea of Japan. *International Journal of Environmental Research and Public Health*, 17(17), 6019.

Chrétiennot-Dinet, M. J., Sournia, A., Ricard, M., & Billard, C. (1993). A classification of the marine phytoplankton of the world from class to genus. *Phycologia*, 32(3), 159–179.

Christiansen, S., Salter, M. E., Gorokhova, E., Nguyen, Q. T., & Bilde, M. (2019). Sea spray aerosol formation: Laboratory results on the role of air

entrainment, water temperature, and phytoplankton biomass.

Environmental Science & Technology, 53(22), 13107–13116.

Chuku, E. O., Yankson, K., Obodai, E. A., Acheampong, E., & Aheto, D. W.

(2023). Spatiotemporal spatfall dynamics and prevailing estuarine conditions for optimal oyster (*Crassostrea tulipa*) spat availability in selected Gulf of Guinea brackish systems. *Frontiers in Marine Science*, 10, 1075313. <https://doi.org/10.3389/fmars.2023.1075313>

Cloern, J. E. (2018). Why large cells dominate estuarine phytoplankton: Large cells dominate in estuaries. *Limnology and Oceanography*, 63(S1), S392–S409. <https://doi.org/10.1002/lno.10749>

Collins, S., Rost, B., & Rynearson, T. A. (2014). Evolutionary potential of marine phytoplankton under ocean acidification. *Evolutionary Applications*, 7(1), 140–155. <https://doi.org/10.1111/eva.12120>

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, 11(12), 1304–1315. <https://doi.org/10.1111/j.1461-0248.2008.01253.x>

CRC/FoN. (2010). *Rapid Biodiversity Assessment on the Essei and Butuah Lagoons and Whin River Estuary in the Sekondi-Takoradi Metropolis of the Western Region of Ghana*. Coastal Resource Center in Partnership with Friends of the Nation on the Hen Mpoano Initiative in Ghana.

Culumber, Z. W., Anaya-Rojas, J. M., Booker, W. W., Hooks, A. P., Lange, E. C.,

Puer, B., Ramírez-Bullón, N., & Travis, J. (2019). Widespread Biases in

Ecological and Evolutionary Studies. *BioScience*, 69(8), 631–640.
<https://doi.org/10.1093/biosci/biz063>

Curry, R., Dickson, B., & Yashayaev, I. (2003). A change in the freshwater balance of the Atlantic Ocean over the past four decades. *Nature*, 426(6968), 826–829.

Dadzie-Paintsil, E., & Mensah, J. V. (2022). Effects of urbanization on coastal wetlands in the Sekondi-Takoradi Metropolis, Ghana. *Indo Pacific Journal of Ocean Life*, 6(2).

Damar, A., Ervinia, A., Kurniawan, F., & Rudianto, B. Y. (2021). Eutrophication in a tropical estuary: Is it good or bad? *IOP Conference Series: Earth and Environmental Science*, 744(1), 012010.

Darko, G., Dodd, M., Nkansah, M. A., Aduse-Poku, Y., Ansah, E., Wemegah, D. D., & Borquaye, L. S. (2017). Distribution and ecological risks of toxic metals in the topsoils in the Kumasi metropolis, Ghana. *Cogent Environmental Science*, 3(1), 1354965.

Davis, A. K., Hildebrand, M., & Palenik, B. (2006). Gene expression induced by copper stress in the diatom *Thalassiosira pseudonana*. *Eukaryotic Cell*, 5(7), 1157–1168.

Day, J. W., Yáñez-Arancibia, A., Kemp, W. M., & Crump, B. C. (2012). Introduction to Estuarine Ecology. In J. W. Day, B. C. Crump, W. M. Kemp, & A. Yáñez-Arancibia (Eds.), *Estuarine Ecology* (pp. 1–18). John Wiley & Sons, Inc. <https://doi.org/10.1002/9781118412787.ch1>

Day Jr, J. W., Yáñez-Arancibia, A., Kemp, W. M., & Crump, B. C. (2012).

Introduction to estuarine ecology. *Estuarine Ecology*, 1–18.

deGraft-Johnson, K. A. A., Blay, J., Nunoo, F. K. E., & Amankwah, C. C. (2010).

Biodiversity threats assessment of the Western Region of Ghana. *The Integrated Coastal and Fisheries Governance (ICFG) Initiative Ghana*.

Deinet, S., McRae, L., De Palma, A., Manley, R., Loh, J., & Collen, B. (2010). The

Living Planet Index for Global Estuarine Systems-Technical Report. WWF/ZSL.

Díaz, S., Purvis, A., Cornelissen, J. H. C., Mace, G. M., Donoghue, M. J., Ewers,

R. M., Jordano, P., & Pearse, W. D. (2013). Functional traits, the phylogeny of function, and ecosystem service vulnerability. *Ecology and Evolution*, 3(9), 2958–2975. <https://doi.org/10.1002/ece3.601>

Donaldson, M. R., Burnett, N. J., Braun, D. C., Suski, C. D., Hinch, S. G., Cooke,

S. J., & Kerr, J. T. (2017). Taxonomic bias and international biodiversity conservation research. *FACETS*, 1(1), 105–113.

<https://doi.org/10.1139/facets-2016-0011>

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English,

C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., & Knowlton, N. (2012). Climate change impacts on marine ecosystems. *Annual Review of Marine Science*, 4, 11–37.

Dong, H.-P., Dong, Y.-L., Cui, L., Balamurugan, S., Gao, J., Lu, S.-H., & Jiang, T.

(2016). High light stress triggers distinct proteomic responses in the marine diatom *Thalassiosira pseudonana*. *BMC Genomics*, 17, 1–14.

- D'ors, A., Bartolomé, M. C., & Sánchez-Fortún, S. (2016). Repercussions of salinity changes and osmotic stress in marine phytoplankton species. *Estuarine, Coastal and Shelf Science*, 175, 169–175.
- Dovlo, E. K. (2016). Seasonal variations in temperature and salinity in the Gulf of Guinea. *Journal of Aquaculture and Marine Biology*, 4(2).
- Duarte, C. M., Dennison, W. C., Orth, R. J., & Carruthers, T. J. (2008). The charisma of coastal ecosystems: Addressing the imbalance. *Estuaries and Coasts*, 31, 233–238.
- Duran, R., & Cravo-Laureau, C. (2016). Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. *FEMS Microbiology Reviews*, 40(6), 814–830.
- Dzakpasu, M. F. A. (2019). *Ecological assessment of some coastal lagoons and estuaries in Ghana: Abiotic and biotic approaches* [PhD Thesis]. University of Cape Coast.
- Effah, E., Aheto, D. W., Acheampong, E., & Blay, J. (2023). Ghana Case Study Two: Assessment of the Vulnerability of a Small Coastal Community to Climate Change for Adaptation Planning in Ghana. In *Challenges in Tropical Coastal Zone Management: Experiences and Lessons Learned* (pp. 227–245). Springer.
- El Gammal, M. A. M., Nageeb, M., & Al-Sabeb, S. (2017). Phytoplankton abundance in relation to the quality of the coastal water – Arabian Gulf,

Saudi Arabia. *The Egyptian Journal of Aquatic Research*, 43(4), 275–282.

<https://doi.org/10.1016/j.ejar.2017.10.004>

Elliott, M., & Whitfield, A. K. (2011). Challenging paradigms in estuarine ecology and management. *Estuarine, Coastal and Shelf Science*, 94(4), 306–314.

Emori, S., Taylor, K., Hewitson, B., Zermoglio, F., Juckes, M., Lautenschlager, M., & Stockhause, M. (2016). CMIP5 data provided at the IPCC Data Distribution Centre. *Fact Sheet of the Task Group on Data and Scenario Support for Impact and Climate Analysis (TGICA) of the Intergovernmental Panel on Climate Change (IPCC)*, 8.

EPA, M. (2011). Ghana's Second National Communication to the UNFCCC. *Environmental Protection Agency and Ministry of Environment, Science and Technology, Accra*.

Essumang, D. K. (2010). Distribution, Levels, and Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in Some Water Bodies along the Coastal Belt of Ghana. *The Scientific World JOURNAL*, 10, 972–985.
<https://doi.org/10.1100/tsw.2010.96>

Falkowski, P. (2012). Ocean Science: The power of plankton. *Nature*, 483(7387), S17–S20. <https://doi.org/10.1038/483S17a>

Falkowski, P. G. (1997). Evolution of the nitrogen cycle and its influence on the biological sequestration of CO₂ in the ocean. *Nature*, 387(6630), 272–275.

Falkowski, P. G., Laws, E. A., Barber, R. T., & Murray, J. W. (2003). Phytoplankton and Their Role in Primary, New, and Export Production. In

M. J. R. Fasham (Ed.), *Ocean Biogeochemistry* (pp. 99–121). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-55844-3_5

Falkowski, P. G., & Raven, J. A. (2013). *Aquatic photosynthesis*. Princeton University Press.

Fanesi, A., Wagner, H., & Wilhelm, C. (2017). Phytoplankton growth rate modelling: Can spectroscopic cell chemotyping be superior to physiological predictors? *Proceedings of the Royal Society B: Biological Sciences*, 284(1848), 20161956. <https://doi.org/10.1098/rspb.2016.1956>

Faseyi, C. A., Miyittah, M. K., Sowunmi, A. A., & Yafetto, L. (2022). Water quality and health risk assessments of illegal gold mining-impacted estuaries in Ghana. *Marine Pollution Bulletin*, 185, 114277.

Faseyi, C. A., Miyittah, M. K., Yafetto, L., Sowunmi, A. A., & Lutterodt, G. (2022). Pollution fingerprinting of two southwestern estuaries in Ghana. *Heliyon*, 8(8), e10337. <https://doi.org/10.1016/j.heliyon.2022.e10337>

Fatema, K., Wan Maznah, W. O., & Isa, M. M. (2015). Spatial variation of water quality parameters in a mangrove estuary. *International Journal of Environmental Science and Technology*, 12(6), 2091–2102. <https://doi.org/10.1007/s13762-014-0603-2>

Fianko, J. R., Osae, S., Adomako, D., Adotey, D. K., & Serfor-Armah, Y. (2007). Assessment of heavy metal pollution of the Iture Estuary in the central region of Ghana. *Environmental Monitoring and Assessment*, 131, 467–473.

Fidor, A., Konkel, R., & Mazur-Marzec, H. (2019). Bioactive peptides produced by cyanobacteria of the genus *Nostoc*: A review. *Marine Drugs*, 17(10), 561.

Fischer, E. M., Sedláček, J., Hawkins, E., & Knutti, R. (2014). Models agree on forced response pattern of precipitation and temperature extremes. *Geophysical Research Letters*, 41(23), 8554–8562.

Fisher, N. A. (1993). *Volunteer estuary monitoring: A methods manual*. US Environmental Protection Agency, Office of Water, Office of Wetlands

Flombaum, P., Wang, W.-L., Primeau, F. W., & Martiny, A. C. (2020). Global picophytoplankton niche partitioning predicts overall positive response to ocean warming. *Nature Geoscience*, 13(2), 116–120.

<https://doi.org/10.1038/s41561-019-0524-2>

Fryxell, G. A., & Hasle, G. R. (1979). The genus *Thalassiosira*: Species with internal extensions of the strutted processes. *Phycologia*, 18(4), 378–393. <https://doi.org/10.2216/i0031-8884-18-4-378.1>

Fu, F., Zhang, X., Zhang, X., Li, Q., & Sun, L. (2020). Comprehensive analysis and identification of heat-responsive genes in *Agarophyton vermiculophyllum* by RNA-sequencing. *Botanica Marina*, 63(5), 479–490.

Gadhia, M., Surana, R., & Ansari, E. (2013). Seasonal Variations in Physico-Chemical Characteristics of Tapi Estuary in Hazira Industrial Area. *Our Nature*, 10(1), 249–257. <https://doi.org/10.3126/on.v10i1.7811>

Gao, K., Beardall, J., Häder, D.-P., Hall-Spencer, J. M., Gao, G., & Hutchins, D. A. (2019). Effects of ocean acidification on marine photosynthetic

organisms under the concurrent influences of warming, UV radiation, and deoxygenation. *Frontiers in Marine Science*, 6, 322.

Gao, K., & Campbell, D. A. (2014). Photophysiological responses of marine diatoms to elevated CO₂ and decreased pH: a review. *Functional Plant Biology*, 41(5), 449–459.

Garcia, N., Lopez Elias, J. A., Miranda, A., Martinez Porchas, M., Huerta, N., & Garcia, A. (2012). Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases. *Latin American Journal of Aquatic Research*, 40(2), 435–440.

<https://doi.org/10.3856/vol40-issue2-fulltext-18>

Garzke, J., Ismar, S. M. H., & Sommer, U. (2015). Climate change affects low trophic level marine consumers: Warming decreases copepod size and abundance. *Oecologia*, 177(3), 849–860. <https://doi.org/10.1007/s00442-014-3130-4>

Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Pörtner, H.-O., Rogers, A. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., ... Turley, C. (2015). Contrasting futures for ocean and society from different anthropogenic CO₂ emissions scenarios. *Science*, 349(6243), aac4722.

<https://doi.org/10.1126/science.aac4722>

Gavhane, S. K., Sapkale, J. B., Susware, N. K., & Sapkale, S. J. (2021). Impact of heavy metals in riverine and estuarine environment: A review. *Res. J. Chem. Environ*, 25(5), 226–233.

Gemeda, D. O., & Sima, A. D. (2015). The impacts of climate change on African continent and the way forward. *Journal of Ecology and the Natural Environment*, 7(10), 256–262.

Gittings, J. A., Raitsos, D. E., Krokos, G., & Hoteit, I. (2018). Impacts of warming on phytoplankton abundance and phenology in a typical tropical marine ecosystem. *Scientific Reports*, 8(1), 2240.

Glamore, W. C., Rayner, D. S., & Rahman, P. F. (2016). Estuaries and Climate Change: Technical Monograph Prepared for the National Climate Change Adaptation Research Facility. *Water Research Laboratory of the School of Civil and Environmental Engineering, UNSW: Sydney, Australia*.

Griffen, B., Belgrad, B., Cannizzo, Z., Knotts, E., & Hancock, E. (2016). Rethinking our approach to multiple stressor studies in marine environments. *Marine Ecology Progress Series*, 543, 273–281. <https://doi.org/10.3354/meps11595>

Groom, J. D., Dent, L., & Madsen, L. J. (2011). Stream temperature change detection for state and private forests in the Oregon Coast Range. *Water Resources Research*, 47(1).

Guinder, V. A., Popovich, C. A., Molinero, J. C., & Marcovecchio, J. (2013). Phytoplankton summer bloom dynamics in the Bahía Blanca Estuary in

relation to changing environmental conditions. *Continental Shelf Research*, 52, 150–158. <https://doi.org/10.1016/j.csr.2012.11.010>

Haeder, D.-P., Villafane, V. E., & Helbling, E. W. (2014). Productivity of aquatic primary producers under global climate change. *Photochemical & Photobiological Sciences*, 13(10), 1370–1392.

Halpern, B. S., Frazier, M., Potapenko, J., Casey, K. S., Koenig, K., Longo, C., Lowndes, J. S., Rockwood, R. C., Selig, E. R., Selkoe, K. A., & Walbridge, S. (2015). Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nature Communications*, 6(1), 7615. <https://doi.org/10.1038/ncomms8615>

Hancock, A. M., King, C. K., Stark, J. S., McMinn, A., & Davidson, A. T. (2020). Effects of ocean acidification on Antarctic marine organisms: A meta-analysis. *Ecology and Evolution*, 10(10), 4495–4514.

Hanslow, D. J., Morris, B. D., Foulsham, E., & Kinsela, M. A. (2018). A Regional Scale Approach to Assessing Current and Potential Future Exposure to Tidal Inundation in Different Types of Estuaries. *Scientific Reports*, 8(1), 7065. <https://doi.org/10.1038/s41598-018-25410-y>

Harrison, G. I., & Morel, F. M. (1986). Response of the marine diatom *Thalassiosira weissflogii* to iron stress 1. *Limnology and Oceanography*, 31(5), 989–997.

Hasan, J., Shaha, D. C., Kundu, S. R., Yusoff, F. M., Cho, Y.-K., Haque, F., Salam, M. A., Ahmed, S., Wahab, Md. A., Ahmed, M., Hossain, Md. I., & Afrad, Md. S. I. (2022). Phytoplankton Community in Relation to

Environmental Variables in the Tidal Mangrove Creeks of the Pasur River Estuary, Bangladesh. *Conservation*, 2(4), 587–612.

<https://doi.org/10.3390/conservation2040039>

Hasle, G. R., Syvertsen, E. E., Steidinger, K. A., Tangen, K., & Tomas, C. R. (1996). *Identifying marine diatoms and dinoflagellates*. Elsevier.

[https://books.google.com/books?hl=en&lr=&id=KQxPtwnlqoC&oi=fnd&pg=PP1&dq=HASLE,+G+\(1997\).+Identifying+Marine+Phytoplankton+%7C%7C+Marine+Diatoms.,+\(\),+5%E2%80%9393385.+doi:10.1016/B978-012693018-4/50004-](https://books.google.com/books?hl=en&lr=&id=KQxPtwnlqoC&oi=fnd&pg=PP1&dq=HASLE,+G+(1997).+Identifying+Marine+Phytoplankton+%7C%7C+Marine+Diatoms.,+(),+5%E2%80%9393385.+doi:10.1016/B978-012693018-4/50004-5+&ots=3bEyTB9o5R&sig=4zuFktdZSpktFrLv6yaPsn5WDIE)

[5+&ots=3bEyTB9o5R&sig=4zuFktdZSpktFrLv6yaPsn5WDIE](https://books.google.com/books?hl=en&lr=&id=KQxPtwnlqoC&oi=fnd&pg=PP1&dq=HASLE,+G+(1997).+Identifying+Marine+Phytoplankton+%7C%7C+Marine+Diatoms.,+(),+5%E2%80%9393385.+doi:10.1016/B978-012693018-4/50004-5+&ots=3bEyTB9o5R&sig=4zuFktdZSpktFrLv6yaPsn5WDIE)

Havens, K. E., & Steinman, A. D. (2015). Ecological responses of a large shallow lake (Okeechobee, Florida) to climate change and potential future hydrologic regimes. *Environmental Management*, 55, 763–775.

Hayford, E. K., Amin, A., Osaе, E. K., & Kutu, J. (2009). Impact of gold mining on soil and some staple foods collected from selected mining communities in and around Tarkwa-Prestea area. *West African Journal of Applied Ecology*, 14(1).

Haynes, W. A., Vallania, F., Liu, C., Bongen, E., Tomczak, A., Andres-Terrè, M., Lofgren, S., Tam, A., Deisseroth, C. A., & Li, M. D. (2017). Empowering multi-cohort gene expression analysis to increase reproducibility. *PACIFIC SYMPOSIUM ON BIOCOMPUTING 2017*, 144–153.

Hernández Ruiz, L., Ekumah, B., Asiedu, D. A., Albani, G., Acheampong, E., Jónasdóttir, S. H., Koski, M., & Nielsen, T. G. (2021). Climate change and

oil pollution: A dangerous cocktail for tropical zooplankton. *Aquatic Toxicology*, 231, 105718. <https://doi.org/10.1016/j.aquatox.2020.105718>

Hernando, M., Schloss, I. R., Malanga, G., Almandoz, G. O., Ferreyra, G. A., Aguiar, M. B., & Puntarulo, S. (2015). Effects of salinity changes on coastal Antarctic phytoplankton physiology and assemblage composition. *Journal of Experimental Marine Biology and Ecology*, 466, 110–119. <https://doi.org/10.1016/j.jembe.2015.02.012>

Herr, D. (2009). *The Ocean and Climate Change: Tools and guidelines for action*. IUCN.

Heudre, D., Wetzel, C. E., Van de Vijver, B., Moreau, L., & Ector, L. (2021). Brackish diatom species (Bacillariophyta) from rivers of Rhin-Meuse basin in France. *Botany Letters*, 168(1), 56–84.

Hindarti, D., & Larasati, A. W. (2019). Copper (Cu) and Cadmium (Cd) toxicity on growth, chlorophyll-a and carotenoid content of phytoplankton *Nitzschia* sp. *IOP Conference Series: Earth and Environmental Science*, 236, 012053.

Hjorth, M., Vester, J., Henriksen, P., Forbes, V., & Dahllöf, I. (2007). Functional and structural responses of marine plankton food web to pyrene contamination. *Marine Ecology Progress Series*, 338, 21–31.

Honda, M., & Suzuki, N. (2020). Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. *International Journal of Environmental Research and Public Health*, 17(4), 1363.

Hoppenrath, M., Elbrächter, M., & Drebes, G. (2009). *Marine phytoplankton*.

- Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., & Billen, G. (2011). Coupled biogeochemical cycles: Eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Frontiers in Ecology and the Environment*, 9(1), 18–26.
- Huang, Y.-J., Jiang, Z.-B., Zeng, J.-N., Chen, Q.-Z., Zhao, Y., Liao, Y., Shou, L., & Xu, X. (2011). The chronic effects of oil pollution on marine phytoplankton in a subtropical bay, China. *Environmental Monitoring and Assessment*, 176, 517–530.
- Hülse, D., Arndt, S., Wilson, J. D., Munhoven, G., & Ridgwell, A. (2017). Understanding the causes and consequences of past marine carbon cycling variability through models. *Earth-Science Reviews*, 171, 349–382.
- Hyun, B., Kim, J.-M., Jang, P.-G., Jang, M.-C., Choi, K.-H., Lee, K., Yang, E. J., Noh, J. H., & Shin, K. (2020). The Effects of Ocean Acidification and Warming on Growth of a Natural Community of Coastal Phytoplankton. *Journal of Marine Science and Engineering*, 8(10), 821.
<https://doi.org/10.3390/jmse8100821>
- IPCC. (2021). *Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. In Press.*

IPCC. (2022). *The Ocean and Cryosphere in a Changing Climate: Special Report of the Intergovernmental Panel on Climate Change* (1st ed.). Cambridge University Press. <https://doi.org/10.1017/9781009157964>

Irwin, A. J., Finkel, Z. V., Müller-Karger, F. E., & Troccoli Ghinaglia, L. (2015). Phytoplankton adapt to changing ocean environments. *Proceedings of the National Academy of Sciences*, 112(18), 5762–5766. <https://doi.org/10.1073/pnas.1414752112>

Ishida, Y., Hiragushi, N., Kitaguchi, H., Mitsutani, A., Nagai, S., & Yoshimura, M. (2000). A highly CO₂-tolerant diatom, *Thalassiosira weissflogii* H1, enriched from coastal sea, and its fatty acid composition. *Fisheries Science*, 66(4), 655–659. <https://doi.org/10.1046/j.1444-2906.2000.00105.x>

ISSER. (2020). *Policy brief: Water Pollution in Ghana*. Institute for Social and Economic Research (ISSER), University of Ghana.

James, N. C., Van Niekerk, L., Whitfield, A. K., Potts, W. M., Götz, A., & Paterson, A. W. (2013). Effects of climate change on South African estuaries and associated fish species. *Climate Research*, 57(3), 233–248.

Jian, J., Zeng, D., Wei, W., Lin, H., Li, P., & Liu, W. (2017). The combination of RNA and protein profiling reveals the response to nitrogen depletion in *Thalassiosira pseudonana*. *Scientific Reports*, 7(1), 8989.

Jiao, K., Yang, H., Huang, X., Liu, F., & Li, S. (2023). Effects of phosphorus species and zinc stress on growth and physiology of the marine diatom *Thalassiosira weissflogii*. *Chemosphere*, 139308.

Jonah, F. E., Mensah, E. A., Edziyie, R. E., Agbo, N. W., & Adjei-Boateng, D.

(2016). Coastal Erosion in Ghana: Causes, Policies, and Management.

Coastal Management, 44(2), 116–130.

<https://doi.org/10.1080/08920753.2016.1135273>

Jones, B. L., Unsworth, R. K., Udagedara, S., & Cullen-Unsworth, L. C. (2018).

Conservation concerns of small-scale fisheries: By-catch impacts of a shrimp and finfish fishery in a Sri Lankan lagoon. *Frontiers in Marine Science*, 5, 52.

Karikari, A. Y., Asante, K. A., & Biney, C. A. (2006). Water quality characteristics at the estuary of Korle Lagoon in Ghana. *West African Journal of Applied Ecology*, 10(1).

Kennish, M. J. (2002). Environmental threats and environmental future of estuaries. *Environmental Conservation*, 29(1), 78–107.

Kennish, M. J., & Paerl, H. W. (2010). *Coastal lagoons: Critical habitats of environmental change*. CRC press.

Kent, M. L., Whyte, J. N. C., & LaTrace, C. (1995). Gill lesions and mortality in seawater pen-reared Atlantic salmon *Salmo salar* associated with a dense bloom of *Skeletonema costatum* and *Thalassiosira* species. *Diseases of Aquatic Organisms*, 22(1), 77–81.

Kirchman, D. L., Morán, X. A. G., & Ducklow, H. (2009). Microbial growth in the polar oceans—Role of temperature and potential impact of climate change. *Nature Reviews Microbiology*, 7(6), 451–459.

<https://doi.org/10.1038/nrmicro2115>

Kirst, G. O. (1977). The cell volume of the unicellular alga, *Platymonas subcordiformishazen*: Effect of the salinity of the culture media and of osmotic stresses. *Zeitschrift Für Pflanzenphysiologie*, 81(5), 386–394.

Kovalenko, K. E., & Reavie, E. D. (2022). Taxonomic bias in freshwater phytoplankton communities and its effect on environmental assessment metrics. *Phycologia*, 61(4), 436–443.

<https://doi.org/10.1080/00318884.2022.2073077>

Kwiatkowski, L., Bopp, L., Aumont, O., Ciais, P., Cox, P. M., Laufkötter, C., Li, Y., & Séférian, R. (2017). Emergent constraints on projections of declining primary production in the tropical oceans. *Nature Climate Change*, 7(5), 355–358. <https://doi.org/10.1038/nclimate3265>

Lalèyè, A. P., Villanueva, M. C., Entsua, M. M., & Moreau, J. (2007). A review of the aquatic living resources in Gulf of Guinea lagoons, with particular emphasis on fisheries management issues. *Journal of Afrotropical Zoology*, 3(Special issue), 123–136.

Lancelot, C., & Muylaert, K. (2011). 7.02-trends in estuarine phytoplankton ecology. In *Treatise on estuarine and coastal science* (pp. 5–15). Academic Press Waltham.

Laws, E. A. (2013). Evaluation of In Situ Phytoplankton Growth Rates: A Synthesis of Data from Varied Approaches. *Annual Review of Marine Science*, 5(1), 247–268. <https://doi.org/10.1146/annurev-marine-121211-172258>

Lee, J. G., Roberts, S. B., & Morel, F. M. (1995). Cadmium: A nutrient for the marine diatom *Thalassiosira weissflogii*. *Limnology and Oceanography*, 40(6), 1056–1063.

Legendre, L., & Michaud, J. (1999). Chlorophyll a to estimate the particulate organic carbon available as food to large zooplankton in the euphotic zone of oceans. *Journal of Plankton Research*, 21(11), 2067–2083.

Li, F., Jiang, L., Zhang, T., Qiu, J., Lv, D., Su, T., Li, W., Xu, J., & Wang, H. (2021). Combined effects of seawater acidification and benzo(a)pyrene on the physiological performance of the marine bloom-forming diatom *Skeletonema costatum*. *Marine Environmental Research*, 169, 105396. <https://doi.org/10.1016/j.marenvres.2021.105396>

Li, G., & Campbell, D. A. (2013a). Rising CO₂ interacts with growth light and growth rate to alter photosystem II photoinactivation of the coastal diatom *Thalassiosira pseudonana*. *Plos One*, 8(1), e55562.

Li, W. (2012). Volcano plots in analyzing differential expressions with mRNA microarrays. *Journal of Bioinformatics and Computational Biology*, 10(06), 1231003.

Li, X., Lu, C., Zhang, Y., Zhao, H., Wang, J., Liu, H., & Yin, K. (2020). Low dissolved oxygen in the Pearl River estuary in summer: Long-term spatio-temporal patterns, trends, and regulating factors. *Marine Pollution Bulletin*, 151, 110814. <https://doi.org/10.1016/j.marpolbul.2019.110814>

Litchman, E., Edwards, K. F., Klausmeier, C. A., & Thomas, M. K. (2012). Phytoplankton niches, traits and eco-evolutionary responses to global environmental change. *Marine Ecology Progress Series*, 470, 235–248.

Liu, G., He, W., & Cai, S. (2020). Seasonal Variation of Dissolved Oxygen in the Southeast of the Pearl River Estuary. *Water*, 12(9), 2475. <https://doi.org/10.3390/w12092475>

Liu, Q., Zhang, Y., Wu, H., Liu, F., Peng, W., Zhang, X., Chang, F., Xie, P., & Zhang, H. (2020). A review and perspective of eDNA application to eutrophication and HAB control in freshwater and marine ecosystems. *Microorganisms*, 8(3), 417.

Lohbeck, K. T., Riebesell, U., & Reusch, T. B. (2012). Adaptive evolution of a key phytoplankton species to ocean acidification. *Nature Geoscience*, 5(5), 346–351.

Luber, G., & Lemery, J. (2015). *Global climate change and human health: From science to practice*. Jossey-Bass.

Lukhabi, D. K., Mensah, P. K., Asare, N. K., Akwetey, M. F. A., & Faseyi, C. A. (2024). Benthic macroinvertebrates as indicators of water quality: A case study of estuarine ecosystems along the coast of Ghana. *Heliyon*, 10(7), e28018. <https://doi.org/10.1016/j.heliyon.2024.e28018>

Mackas, D. L. (2011). Does blending of chlorophyll data bias temporal trend? *Nature*, 472(7342), E4–E5. <https://doi.org/10.1038/nature09951>

Mahu, E., Danso, P., Edusei, M. O., & deGraft-Johnson, K. A. A. (2023). Impact of agricultural practices on ecosystem health of lagoons: A case study of the

Keta Lagoon Complex in Ghana, West Africa. *Environmental Monitoring and Assessment*, 195(5), 1–15.

Marshall, D., Proum, S., Hossain, M. B., Adam, A., Lim, L. H., & Santos, J. H.

(2016). Ecological responses to fluctuating and extreme marine acidification: Lessons from a tropical estuary (the Brunei Estuarine System). *Scientia Bruneiana*, 15. <https://doi.org/10.46537/scibru.v15i0.33>

Masson-Delmotte, V., Zhai, P., Pörtner, H.-O., Roberts, D., Skea, J., & Shukla, P.

R. (2022). *Global Warming of 1.5 C: IPCC special report on impacts of global warming of 1.5 C above pre-industrial levels in context of strengthening response to climate change, sustainable development, and efforts to eradicate poverty*. Cambridge University Press.

Mensah, P. K., Mgaba, N., Griffin, N., Odume, O. N., & Palmer, C. G. (2017).

Development of a procedure for determining the mixing ratios in ecotoxicological experiments and its application in binary salt mixture experiments. *Ecotoxicology*, 26(8), 1011–1017.

<https://doi.org/10.1007/s10646-017-1829-9>

Miththapala, S. (2013). *Lagoons and Estuaries. Coastal Ecosystems Series*. IUCN

Sri Lanka Country Office.

Montagna, P. A., Palmer, T. A., Beseres Pollack, J., Montagna, P. A., Palmer, T.

A., & Beseres Pollack, J. (2013). Conceptual model of estuary ecosystems. *Hydrological Changes and Estuarine Dynamics*, 5–21.

Murphy, G. E. P., Romanuk, T. N., & Worm, B. (2020). Cascading effects of climate change on plankton community structure. *Ecology and Evolution*, 10(4), 2170–2181. <https://doi.org/10.1002/ece3.6055>

Muylaert, K., Sabbe, K., & Vyverman, W. (2009). Changes in phytoplankton diversity and community composition along the salinity gradient of the Schelde estuary (Belgium/The Netherlands). *Estuarine, Coastal and Shelf Science*, 82(2), 335–340.

Nagelkerken, I., Sheaves, M., Baker, R., & Connolly, R. M. (2015). The seascape nursery: A novel spatial approach to identify and manage nurseries for coastal marine fauna. *Fish and Fisheries*, 16(2), 362–371.

Nunoo, E. K. F., Quansah, E. E., & Ofori-Danson, P. K. (2016). Preliminary studies on impacts of ocean acidification on diversity of fish species landed by artisanal and semi-industrial fisheries in Ghana. *International Journal of Marine Science*, 6.

Nyarko, E., & Klubi, B. E. (2011). Polycyclic aromatic hydrocarbons (PAHs) levels in two commercially important fish species from the coastal waters of Ghana and their carcinogenic health risks. *West African Journal of Applied Ecology*, 19(1).

Okyere, I. (2019). Implications of the deteriorating environmental conditions of River Pra estuary (Ghana) for marine fish stocks. *Journal of Fisheries and Coastal Management*, 1(1), 15.

<https://doi.org/10.5455/jfcom.20190315062201>

- Okyere, I., & Nortey, D. D. N. (2018). Assessment of the ecological health status of river pra estuary (Ghana) and adjoining wetland using physico-chemical conditions and macroinvertebrate bioindicators. *West African Journal of Applied Ecology*, 26(2), 44–55.
- Osei, K., Chuku, E. O., Effah, E., Kent, K., & Crawford, B. (2021). *Participatory Assessment of Shellfisheries in the Estuarine and Mangrove Ecosystems of Ghana*. Centre for Coastal Management (Africa Centre of Excellence in Coastal Resilience) University of Cape Coast, Ghana and Coastal Resources Center, Graduate School of Oceanography, University of Rhode Island.
- Othman, H. B., Lanouguère, É., Got, P., Hlaili, A. S., & Leboulanger, C. (2018). Structural and functional responses of coastal marine phytoplankton communities to PAH mixtures. *Chemosphere*, 209, 908–919.
- Page, T. S., Almeda, R., Koski, M., Bournaka, E., & Nielsen, T. G. (2022). Toxicity of tyre wear particle leachates to marine phytoplankton. *Aquatic Toxicology*, 252, 106299. <https://doi.org/10.1016/j.aquatox.2022.106299>
- Palm, L. M., Carboo, D., Yeboah, P. O., Quasie, W. J., Gorleku, M. A., & Darko, A. (2011). Characterization of polycyclic aromatic hydrocarbons (PAHs) present in smoked fish from Ghana. *Advance Journal of Food Science and Technology*, 3(5), 332–338.
- Parmar, T. K., Rawtani, D., & Agrawal, Y. K. (2016). Bioindicators: The natural indicator of environmental pollution. *Frontiers in Life Science*, 9(2), 110–118. <https://doi.org/10.1080/21553769.2016.1162753>

- Pliński, M., & Józwiak, T. (1999). Temperature and N:P ratio as factors causing blooms of blue-green algae in the Gulf of Gdańsk. *Oceanologia*, 73–80.
- Polechová, J., & Storch, D. (2008). Ecological niche. *Encyclopedia of Ecology*, 2, 1088–1097.
- Pontes, L. P., Vafeiadou, A.-M., de França, F. J. L., Cavalcante, R. A., de Araújo França, D. A., Brito, C. M., Alves, R. N., de Carvalho, P. S. M., & dos Santos, G. A. P. (2021). Toxic effects of phenanthrene intensify with an increase of temperature for the populations of a free-living nematode. *Ecological Indicators*, 120, 106868.
<https://doi.org/10.1016/j.ecolind.2020.106868>
- Potapova, M. (2011). Patterns of Diatom Distribution In Relation to Salinity. In *The Diatom World* (pp. 313–332). <https://doi.org/10.1007/978-94-007-1327-7>
- Preisendorfer, R. W. (1986). Secchi disk science: Visual optics of natural waters 1. *Limnology and Oceanography*, 31(5), 909–926.
- Pritchard, D. W. (1967). *What is an estuary: Physical viewpoint*.
- Radchenko, I. G., & Il'Yash, L. V. (2006). Growth and photosynthetic activity of diatom *Thalassiosira weissflogii* at decreasing salinity. *Biology Bulletin*, 33, 242–247.
- Rahmstorf, S. (2007). A Semi-Empirical Approach to Projecting Future Sea-Level Rise. *Science*, 315(5810), 368–370.
<https://doi.org/10.1126/science.1135456>
- Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J.

- P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94(3), 849–873. <https://doi.org/10.1111/brv.12480>
- Robins, P. E., Skov, M. W., Lewis, M. J., Giménez, L., Davies, A. G., Malham, S. K., Neill, S. P., McDonald, J. E., Whitton, T. A., & Jackson, S. E. (2016). Impact of climate change on UK estuaries: A review of past trends and potential projections. *Estuarine, Coastal and Shelf Science*, 169, 119–135.
- Rose, R. M. (2015). The impact of climate change on human security in the Sahel Region of Africa. *Donnish Journal of African Studies and Development*, 1(2), 9–14.
- Rowbury, R. J. (2003). Temperature effects on biological systems: Introduction. *Science Progress*, 86(1–2), 1–8.
- S Zaher, S., & M Helal, A. (2020). How culture medium pH range influence phytoplankton growth performance and biochemical content. *Egyptian Journal of Aquatic Biology and Fisheries*, 24(6), 103–116.
- Saarinen, T. S., & Kløve, B. (2012). Past and future seasonal variation in pH and metal concentrations in runoff from river basins on acid sulphate soils in Western Finland. *Journal of Environmental Science and Health, Part A*, 47(11), 1614–1625. <https://doi.org/10.1080/10934529.2012.680783>
- Salgado-Hernanz, P. M., Regaudie de Gioux, A., Antoine, D., & Basterretxea, G. (2021). *Pelagic primary production in the coastal Mediterranean Sea:*

Variability, trends and contribution to basin scale budgets [Preprint].

Biogeochemistry: Coastal Ocean. <https://doi.org/10.5194/bg-2020-457>

- Sampaio, E., & Rosa, R. (2020). Climate Change, Multiple Stressors, and Responses of Marine Biota. In W. Leal Filho, A. M. Azul, L. Brandli, P. G. Özuyar, & T. Wall (Eds.), *Climate Action* (pp. 264–275). Springer International Publishing. https://doi.org/10.1007/978-3-319-95885-9_90
- Santos, J. W., Correia, R. A., Malhado, A. C. M., Campos-Silva, J. V., Teles, D., Jepson, P., & Ladle, R. J. (2020). Drivers of taxonomic bias in conservation research: A global analysis of terrestrial mammals. *Animal Conservation*, 23(6), 679–688. <https://doi.org/10.1111/acv.12586>
- Sathicq, M. B., Gómez, N., Bauer, D. E., & Donadelli, J. (2017). Use of phytoplankton assemblages to assess the quality of coastal waters of a transitional ecosystem: Río de la Plata estuary. *Continental Shelf Research*, 150, 10–17. <https://doi.org/10.1016/j.csr.2016.08.009>
- Schaum, C.-E., Buckling, A., Smirnoff, N., Studholme, D. J., & Yvon-Durocher, G. (2018). Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom. *Nature Communications*, 9(1), 1719. <https://doi.org/10.1038/s41467-018-03906-5>
- Schaum, C.-E., Rost, B., & Collins, S. (2016). Environmental stability affects phenotypic evolution in a globally distributed marine picoplankton. *The ISME Journal*, 10(1), 75–84.

- Scheren, P. A., Ibe, A. C., Janssen, F. J., & Lemmens, A. M. (2002). Environmental pollution in the Gulf of Guinea – a regional approach. *Marine Pollution Bulletin*, 44(7), 633–641. [https://doi.org/10.1016/S0025-326X\(01\)00305-8](https://doi.org/10.1016/S0025-326X(01)00305-8)
- Segner, H., Schmitt-Jansen, M., & Sabater, S. (2014). Assessing the Impact of Multiple Stressors on Aquatic Biota: The Receptor's Side Matters. *Environmental Science & Technology*, 48(14), 7690–7696. <https://doi.org/10.1021/es405082t>
- Sew, G., & Todd, P. (2020). Effects of Salinity and Suspended Solids on Tropical Phytoplankton Mesocosm Communities. *Tropical Conservation Science*, 13, 194008292093976. <https://doi.org/10.1177/1940082920939760>
- Sheaves, M., Baker, R., Nagelkerken, I., & Connolly, R. M. (2015). True Value of Estuarine and Coastal Nurseries for Fish: Incorporating Complexity and Dynamics. *Estuaries and Coasts*, 38(2), 401–414. <https://doi.org/10.1007/s12237-014-9846-x>
- Simier, M., Osse, O. J. F., Sadio, O., & Ecoutin, J. (2021). Biology and ecology of sea catfish (Ariidae) of estuarine, lagoon and coastal ecosystems in West Africa. *Journal of Fish Biology*, 99(2), 629–643. <https://doi.org/10.1111/jfb.14751>
- Solomon, S. (2007). *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC* (Vol. 4). Cambridge university press.

Sournia, A., Chrdtiennot-Dinet, M.-J., & Ricard, M. (1991). Marine phytoplankton: How many species in the world ocean? *Journal of Plankton Research*, 13(5), 1093–1099.

Stocker, T. (2014). *Climate change 2013: The physical science basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge university press.

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, 183, 85–92. <https://doi.org/10.1016/j.biocon.2014.11.038>

Su, Y., Qi, H., Hou, Y., Gao, M., Li, J., Cai, M., Zhu, X., Chen, M., Ge, C., Fu, D., Wang, Z., & Peng, L. (2022). Combined Effects of Microplastics and Benzo[a]pyrene on the Marine Diatom *Chaetoceros muelleri*. *Frontiers in Marine Science*, 8. <https://www.frontiersin.org/articles/10.3389/fmars.2021.779321>

Sun, J., Hutchins, D. A., Feng, Y., Seubert, E. L., Caron, D. A., & Fu, F.-X. (2011). Effects of changing pCO₂ and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom *Pseudo-nitzschia multiseries*. *Limnology and Oceanography*, 56(3), 829–840.

Sylla, M. B., Elguindi, N., Giorgi, F., & Wisser, D. (2016). Projected robust shift of climate zones over West Africa in response to anthropogenic climate change for the late 21st century. *Climatic Change*, 134(1–2), 241–253.

- Taffs, K. H., Saunders, K. M., & Logan, B. (2017). Diatoms as Indicators of Environmental Change in Estuaries. In K. Weckström, K. M. Saunders, P. A. Gell, & C. G. Skilbeck (Eds.), *Applications of Paleoenvironmental Techniques in Estuarine Studies* (Vol. 20, pp. 277–294). Springer Netherlands. https://doi.org/10.1007/978-94-024-0990-1_11
- Tallam, K., & White Jr, E. (2023). The role of diseases in unifying the health of global estuaries. *Frontiers in Marine Science*, 10, 1185662.
- Talley, L. D. (2002). Salinity patterns in the ocean. *The Earth System: Physical and Chemical Dimensions of Global Environmental Change*, 1, 629–640.
- Tanaka, Y., Minggat, E., & Roseli, W. (2021). The impact of tropical land-use change on downstream riverine and estuarine water properties and biogeochemical cycles: A review. *Ecological Processes*, 10(1), 1–21.
- Teoh, M.-L., Phang, S.-M., & Chu, W.-L. (2013). Response of Antarctic, temperate, and tropical microalgae to temperature stress. *Journal of Applied Phycology*, 25(1), 285–297. <https://doi.org/10.1007/s10811-012-9863-8>
- Thomas, M. K., Aranguren-Gassis, M., Kremer, C. T., Gould, M. R., Anderson, K., Klausmeier, C. A., & Litchman, E. (2017). Temperature–nutrient interactions exacerbate sensitivity to warming in phytoplankton. *Global Change Biology*, 23(8), 3269–3280.
- Thomas, M. K., Kremer, C. T., Klausmeier, C. A., & Litchman, E. (2012). A Global Pattern of Thermal Adaptation in Marine Phytoplankton. *Science*, 338(6110), 1085–1088. <https://doi.org/10.1126/science.1224836>

- Thrush, S. F., Townsend, M., Hewitt, J. E., Davies, K., Lohrer, A. M., Lundquist, C., Cartner, K., & Dymond, J. (2013). The many uses and values of estuarine ecosystems. *Ecosystem Services in New Zealand—Conditions and Trends*. Manaaki Whenua Press, Lincoln, New Zealand, 226–237.
- Tolosa, I., de Mora, S., Sheikholeslami, M. R., Villeneuve, J.-P., Bartocci, J., & Cattini, C. (2004). Aliphatic and aromatic hydrocarbons in coastal Caspian Sea sediments. *Marine Pollution Bulletin*, 48(1–2), 44–60.
- Torres Palenzuela, J. M., González Vilas, L., Bellas, F. M., Garet, E., González-Fernández, Á., & Spyarakos, E. (2019). *Pseudo-nitzschia* blooms in a coastal upwelling system: Remote sensing detection, toxicity and environmental variables. *Water*, 11(9), 1954.
- Torstensson, A., Chierici, M., & Wulff, A. (2012). The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom *Navicula directa*. *Polar Biology*, 35(2), 205–214.
- Tsikoti, C., & Genitsaris, S. (2021). Review of harmful algal blooms in the coastal Mediterranean Sea, with a focus on Greek waters. *Diversity*, 13(8), 396.
- Tyree, M. A., Bishop, I. W., Hawkins, C. P., Mitchell, R., & Spaulding, S. A. (2020). Reduction of taxonomic bias in diatom species data. *Limnology and Oceanography: Methods*, 18(6), 271–279. <https://doi.org/10.1002/lom3.10350>
- Ukwe, C. N., Ibe, C. A., Alo, B. I., & Yumkella, K. K. (2003). Achieving a paradigm shift in environmental and living resources management in the

Gulf of Guinea: The large marine ecosystem approach. *Marine Pollution Bulletin*, 47(1–6), 219–225.

Urban, M. C., De Meester, L., Vellend, M., Stoks, R., & Vanoverbeke, J. (2012).

A crucial step toward realism: Responses to climate change from an evolving metacommunity perspective. *Evolutionary Applications*, 5(2), 154–167.

US EPA, O. (2015, December 23). *Overview of Greenhouse Gases* [Overviews and Factsheets]. <https://www.epa.gov/ghgemissions/overview-greenhouse-gases>

Utermöhl, H. (1958). Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. *Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: Mitteilungen*, 9(1), 1–38.

Van De Waal, D. B., & Litchman, E. (2020). Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375(1798), 20190706. <https://doi.org/10.1098/rstb.2019.0706>

Varela, M., & Prego, R. (2003). Hydrography and phytoplankton in an isolated and non-pristine ria area: The A Coruña Harbour (NW Spain). *Acta Oecologica*, 24(2), 113–124. [https://doi.org/10.1016/S1146-609X\(03\)00048-1](https://doi.org/10.1016/S1146-609X(03)00048-1)

Vincent, F., & Bowler, C. (2020). Diatoms Are Selective Segregators in Global Ocean Planktonic Communities. *mSystems*, 5(1), e00444-19. <https://doi.org/10.1128/mSystems.00444-19>

- Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116(5), 882–892. <https://doi.org/10.1111/j.0030-1299.2007.15559.x>
- Vos, P. C., & de Wolf, H. (1993). Diatoms as a tool for reconstructing sedimentary environments in coastal wetlands; methodological aspects. *Hydrobiologia*, 269–270(1), 285–296. <https://doi.org/10.1007/BF00028027>
- Vrieling, E. G., Sun, Q., Tian, M., Kooyman, P. J., Gieskes, W. W. C., Van Santen, R. A., & Sommerdijk, N. A. J. M. (2007). Salinity-dependent diatom biosilicification implies an important role of external ionic strength. *Proceedings of the National Academy of Sciences*, 104(25), 10441–10446. <https://doi.org/10.1073/pnas.0608980104>
- Vu, H. L. (2018). *Anthropogenic influences on ecosystem processes in a tropical estuary: Feedback connections and environmental management targets-a case study of Dong Ho Estuary, Kien Giang, Vietnam*.
- Wahyu, D., Hindarti, D., & Permana, R. (2020). Cadmium toxicity towards marine diatom *Thalassiosira* sp. And its alteration on chlorophyll-a and carotenoid content. *World News of Natural Sciences*, 31, 48–57.
- Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, 148, 1–13.
- Walter, B., Peters, J., van Beusekom, J. E. E., & St. John, M. A. (2015). Interactive effects of temperature and light during deep convection: A case study on growth and condition of the diatom *Thalassiosira weissflogii*. *ICES Journal*

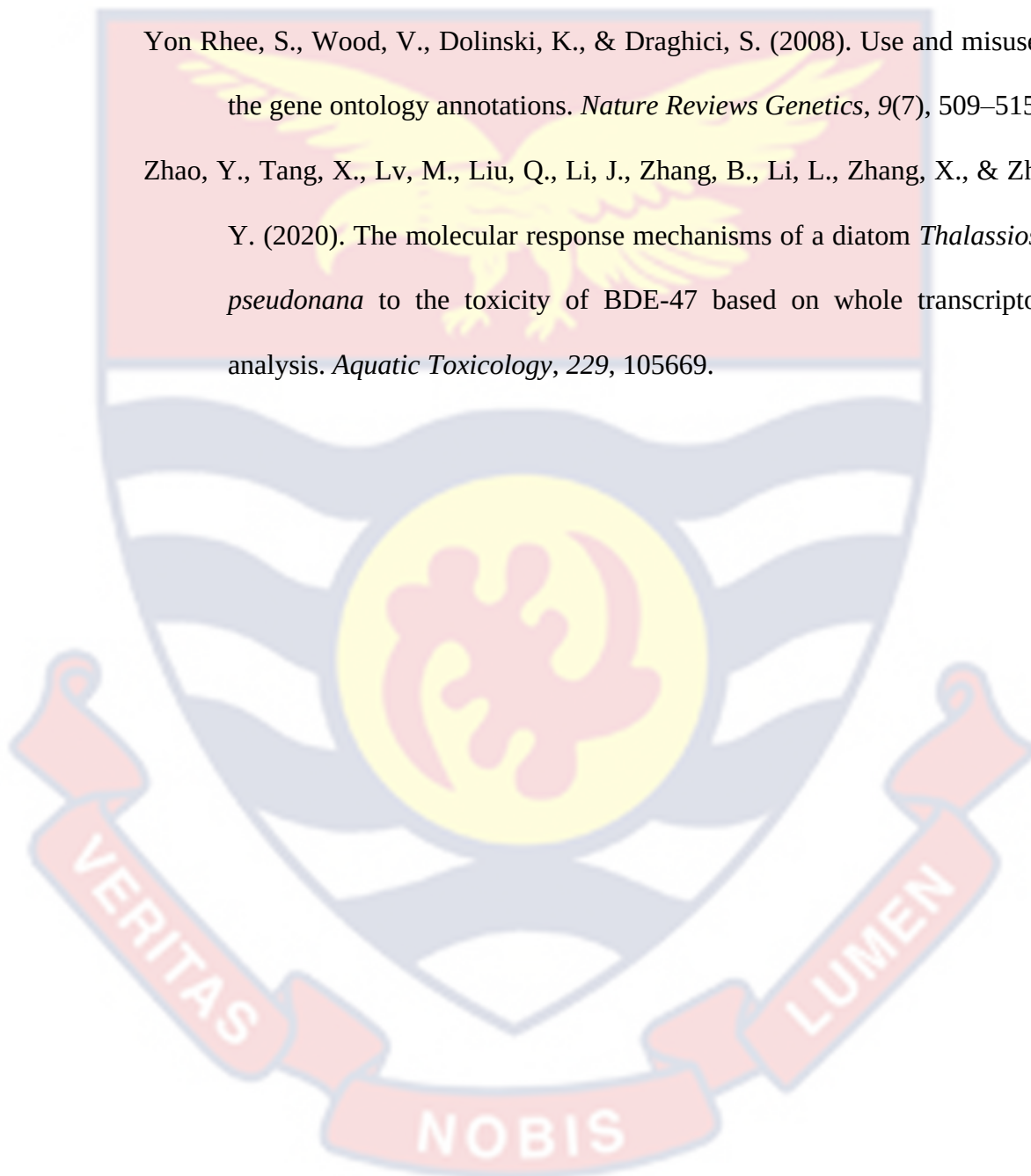
of Marine Science, 72(6), 2061–2071. <https://doi.org/10.1093/icesjms/fsu218>

- Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., Calladine, A., Fourqurean, J. W., Heck, K. L., Hughes, A. R., Kendrick, G. A., Kenworthy, W. J., Short, F. T., & Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences*, 106(30), 12377–12381. <https://doi.org/10.1073/pnas.0905620106>
- Weinstock, G. M. (2007). ENCODE: more genomic empowerment. *Genome Research*, 17(6), 667–668.
- Wentzky, V. C., Tittel, J., Jäger, C. G., Bruggeman, J., & Rinke, K. (2020). Seasonal succession of functional traits in phytoplankton communities and their interaction with trophic state. *Journal of Ecology*, 108(4), 1649–1663.
- Wu, Y., Gao, K., & Riebesell, U. (2010). CO₂-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*. *Biogeosciences*, 7(9), 2915–2923.
- Xu, Y., & Morel, F. M. (2013). Cadmium in marine phytoplankton. *Cadmium: From Toxicity to Essentiality*, 509–528.
- Yadav, S., Anam, G. B., & Ahn, Y.-H. (2022). *Comparative growth characteristics and interspecific competitive interaction of two cyanobacteria, Phormidium autumnale and Nostoc sp.* Wiley Online Library.

Yankson, K., & Obodai, E. (2007). An update of the number, types and distribution of coastal lagoons in Ghana. *Journal of the Ghana Science Association*, 2(2). <https://doi.org/10.4314/jgsa.v2i2.17967>

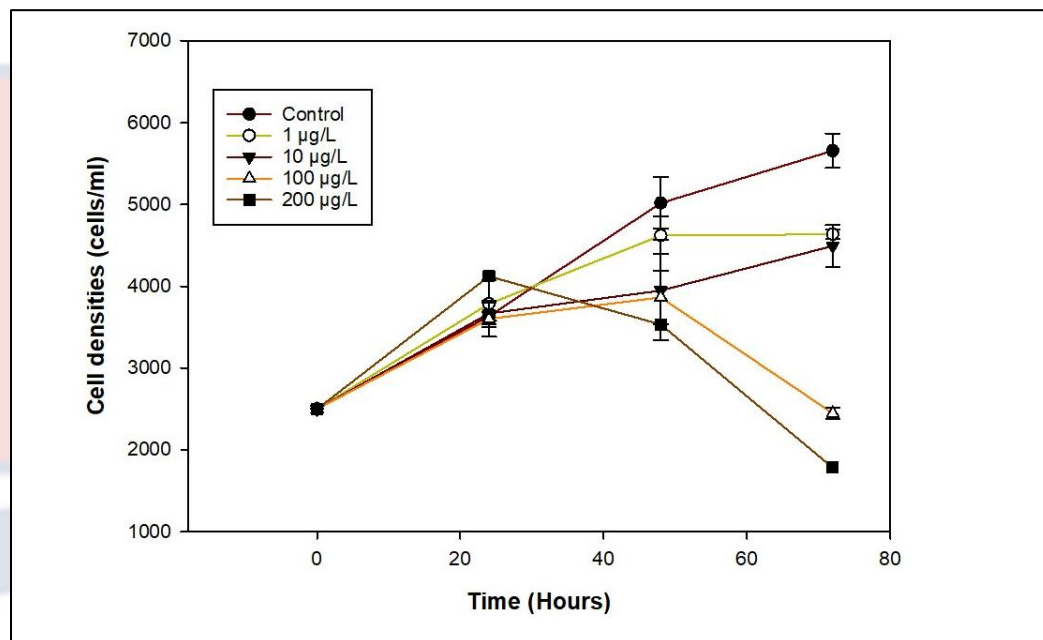
Yon Rhee, S., Wood, V., Dolinski, K., & Draghici, S. (2008). Use and misuse of the gene ontology annotations. *Nature Reviews Genetics*, 9(7), 509–515.

Zhao, Y., Tang, X., Lv, M., Liu, Q., Li, J., Zhang, B., Li, L., Zhang, X., & Zhao, Y. (2020). The molecular response mechanisms of a diatom *Thalassiosira pseudonana* to the toxicity of BDE-47 based on whole transcriptome analysis. *Aquatic Toxicology*, 229, 105669.



APPENDICES

Appendix A: Range finding experiment for cadmium exposure experiments



Appendix B: Calculations for the pyrene and cadmium concentrations in the combination experiments

EC50 Values

1. Cadmium: 2.131 µg/L
2. Pyrene: 0.8708nM = 174.45 µg/L

Percentage Contribution

% Pyrene Contribution

$$= ((\text{Pyrene conc.}) / ((\text{Pyrene conc.}) + (\text{Cadmium conc.}))) \times 100$$

% Cadmium contribution

$$= (\text{cadmium conc.}) / ((\text{Pyrene conc.}) + (\text{Cadmium conc.})) \times 100$$

$$\text{Pyrene contribution} = 174.45 / (174.45 + 2.131) \times 100 = 0.99\%$$

$$\text{Cadmium contribution} = 2.131 / (171.45 + 2.131) \times 100 = 0.01\%$$

No.	Total Cadmium and Pyrene Concentration	Pyrene	Cadmium
1.	10 µg/L	9.9 µg/L	0.1 µg/L
2.	100 µg/L	99 µg/L	1 µg/L
3.	1000 µg/L	990 µg/L	10 µg/L

Appendix C: Percentage growth rate, chlorophyll-*a*, and dry biomass concentrations in comparison to the control for the individual experiments

NB: values are for the different days and cumulatively.

The (-) signify the control values in the experiments

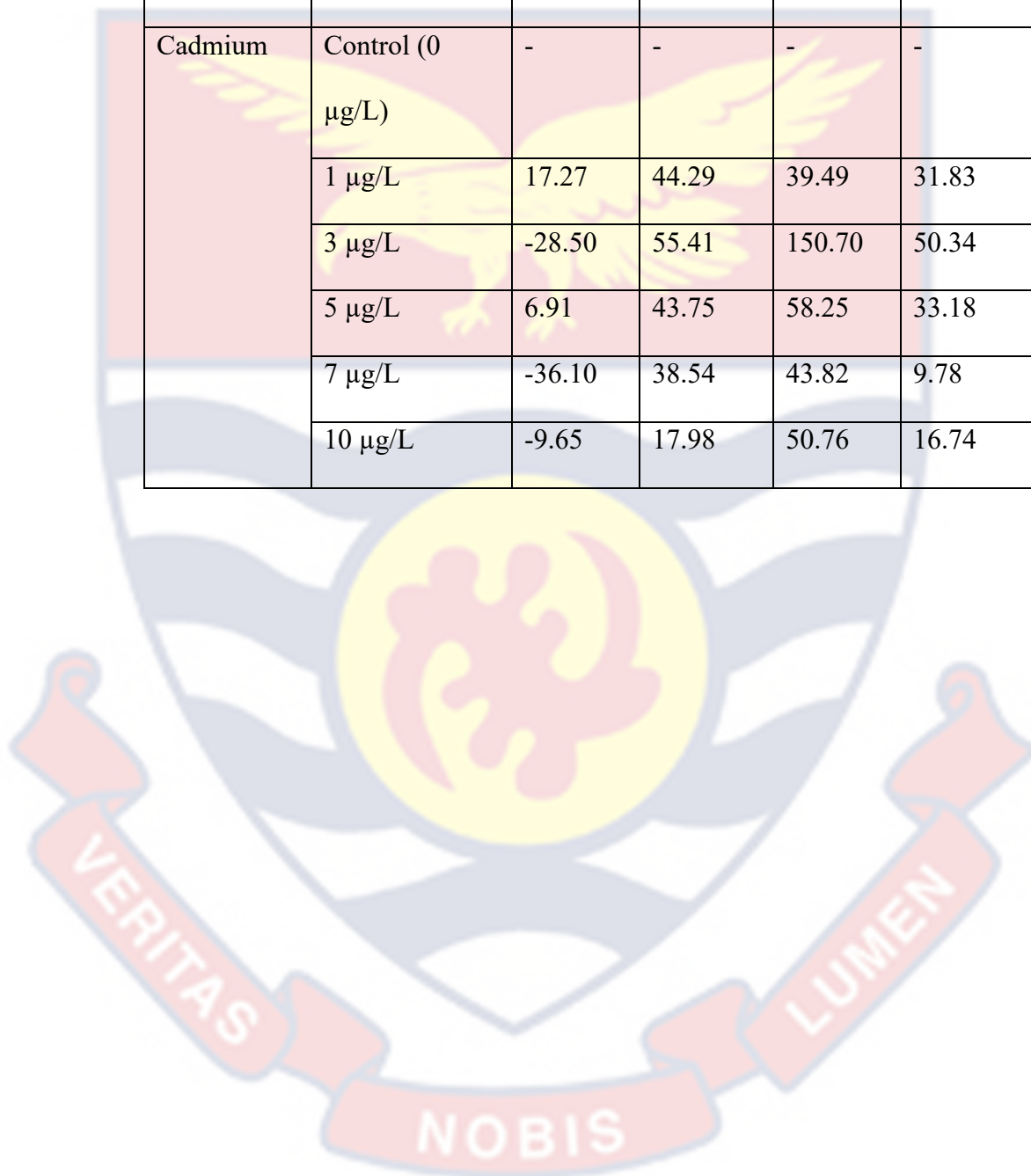
1. Cell Densities (Cells/mL) (%) in comparison to the control					
Stressor	Stressor Level	Day 1	Day 2	Day 3	Cumulative
Temperature	24	-25.69	10.09	4.61	-2.15
	26	-19.38	-10.01	2.49	-8.02
	28*	-	-	-	-
	30	10.22	27.67	-8.67	9.14
	32	-5.40	25.05	2.79	8.06
Salinity	1	-51.68	-90.27	-95.32	-81.34
	5	-51.39	-83.00	-92.88	-77.99
	15	-0.50	9.07	-15.86	-3.63
	25	0.00	0.00	0.00	0.00
	35	-8.96	13.48	10.32	5.84
pH	5	-2.42	-24.50	-47.68	-26.09
	6	-19.71	-12.06	-20.38	-17.29
	7	0.00	0.00	0.00	0.00
	8	-21.92	-25.98	-21.64	-23.23
	9	-16.66	-22.84	-13.08	-17.54
Pyrene	Control (no pyrene)	-	-	-	-

	Control (Acetone)	20.38	-1.40	9.49	8.35
	1 nM	11.70	-25.48	-17.69	-12.83
	10 nM	10.20	-27.49	-21.49	-15.37
	100 nM	-4.94	-29.30	-25.44	-21.47
	100+ nM	3.78	-24.55	-40.30	-22.92
Cadmium	Control (0 µg/L)	-	-	-	-
	1 µg/L	-3.10	-4.16	-13.77	-7.91
	3 µg/L	0.24	7.36	-18.59	-5.53
	5 µg/L	-20.16	-7.78	-9.38	-11.88
	7 µg/L	-16.07	-14.14	-23.18	-18.48
	10 µg/L	-19.35	-9.39	-27.55	-19.79
2. Chlorophyll-a concentration (µg/L) (%) in comparison to the control					
Stressor	Stressor Level	Day 1	Day 2	Day 3	Cumulative
Temperature	24	-38.05	3.97	-6.22	-15.53
	26	-42.87	6.96	-2.88	-15.62
	28*	0.00	0.00	0.00	0.00
	30	-6.38	46.41	16.41	17.71
	32	-24.40	1.74	-7.37	-11.07
Salinity	1	-55.16	-63.94	-65.90	-61.45

	5	-43.87	-67.48	-72.71	-60.78
	15	-3.54	-24.58	-37.65	-21.28
	25	0.00	0.00	0.00	0.00
	35	14.29	-21.05	-13.50	-6.14
pH	5	-12.48	-25.12	-40.14	-26.56
	6	0.33	-5.08	-16.49	-7.43
	7	0.00	0.00	0.00	0.00
	8	-10.35	-35.14	-23.57	-23.70
	9	17.49	9.57	-22.05	0.95
Pyrene	Control (no pyrene)	0.00	0.00	0.00	0.00
	Control (Acetone)	8.12	-14.81	-24.57	-7.22
	1 nM	6.74	-11.90	2.44	-0.49
	10 nM	14.88	-7.47	-17.11	-0.10
	100 nM	34.48	-14.77	9.31	12.13
	100+ nM	23.28	-11.90	-0.77	5.89
Cadmium	Control (0 $\mu\text{g/L}$)	-	-	-	-
	1 $\mu\text{g/L}$	14.01	183.93	46.17	47.31
	3 $\mu\text{g/L}$	-50.49	130.36	5.22	-5.79
	5 $\mu\text{g/L}$	-20.52	33.33	58.58	19.72
	7 $\mu\text{g/L}$	-13.26	102.38	3.64	7.25

	10 µg/L	-30.49	135.71	82.37	37.16
3. Dry Biomass (µg/L) (%) in comparison to the control					
Stressor	Stressor Level	Day 1	Day 2	Day 3	Cumulative
Temperature	24	2.18	15.06	-28.46	-0.93
	26	12.95	-9.80	3.43	1.26
	28*	-	-	-	-
	30	5.75	-5.37	-46.99	-12.94
	32	1.08	-4.76	-21.92	-7.47
Salinity	1	87.80	21.86	-56.25	17.35
	5	47.92	-0.94	-35.85	4.23
	15	11.31	-22.67	-20.09	-9.13
	25	-	-	-	-
	35	37.95	-15.99	51.93	29.15
pH	5	0.27	16.69	-19.83	-1.57
	6	17.66	8.70	-5.34	6.64
	7	-	-	-	-
	8	55.99	24.33	-13.16	21.34
	9	12.54	4.96	6.01	7.80
Pyrene	Control (no pyrene)	0.00	0.00	0.00	0.00
	Control (Acetone)	-2.18	-20.06	8.20	-4.95
	1 nM	-13.87	-6.96	-8.48	-9.76

	10 nM	-8.70	-2.43	-25.08	-11.85
	100 nM	-9.42	-0.20	2.56	-2.39
	100+ nM	-10.77	-4.94	1.81	-4.71
Cadmium	Control (0 µg/L)	-	-	-	-
	1 µg/L	17.27	44.29	39.49	31.83
	3 µg/L	-28.50	55.41	150.70	50.34
	5 µg/L	6.91	43.75	58.25	33.18
	7 µg/L	-36.10	38.54	43.82	9.78
	10 µg/L	-9.65	17.98	50.76	16.74



**Appendix D: Percentage growth rate, chlorophyll-a, and dry biomass
concentrations in comparison to the control
for the combination experiments**

NB: values are for the different days and cumulatively.

The (-) signify the control values in the experiments

1. Cell densities (cells/mL) (%) in comparison to the control						
Climate Stressor	Climate stressor level	Cadmium and pyrene (µg/L)	Day 1	Day 2	Day 3	Cumulative
Control	-	0	-	-	-	-
Temperature (°C)	24	10	-11.48	-0.28	0.28	-3.09
		100	-0.96	-5.43	-8.63	-5.59
		1000	5.17	-38.27	-39.56	-27.02
	28	10	-16.30	-26.00	-27.85	-24.16
		100	-21.99	-8.60	-43.46	-27.13
		1000	-9.72	-19.68	-47.08	-28.68
	32	10	-17.61	-25.61	-9.75	-16.66
		100	2.11	10.79	-8.63	0.13
		1000	-14.55	-8.20	-47.08	-26.54
Salinity (‰)	15	10	-33.82	-18.09	-45.96	-34.28
		100	-33.82	-37.82	-49.31	-41.64
		1000	-40.84	-56.07	-52.64	-50.47
	25	10	-7.53	-8.60	-18.11	-12.37

pH		100	-11.04	-12.16	-49.02	-27.61
		1000	-30.33	-7.41	-48.47	-31.19
	35	10	-17.61	8.42	-16.99	-9.51
		100	32.78	3.67	-23.95	-0.23
		1000	-20.25	-13.34	-44.85	-28.68
	5	10	-26.90	-29.97	-56.13	-40.32
		100	-24.51	-36.68	-42.62	-35.91
		1000	-35.14	-32.73	-57.66	-44.04
	7	10	-19.80	-12.16	-20.62	-17.85
		100	-13.88	-17.31	-44.01	-27.79
		1000	-11.04	-27.19	-52.09	-33.45
	9	10	-17.18	-4.63	-29.81	-18.80
		100	-20.69	-15.72	-46.80	-30.36
		1000	-4.02	-23.20	-49.02	-29.03

i. Chlorophyll-*a* concentration (($\mu\text{g/L}$) (%)) in comparison to the control

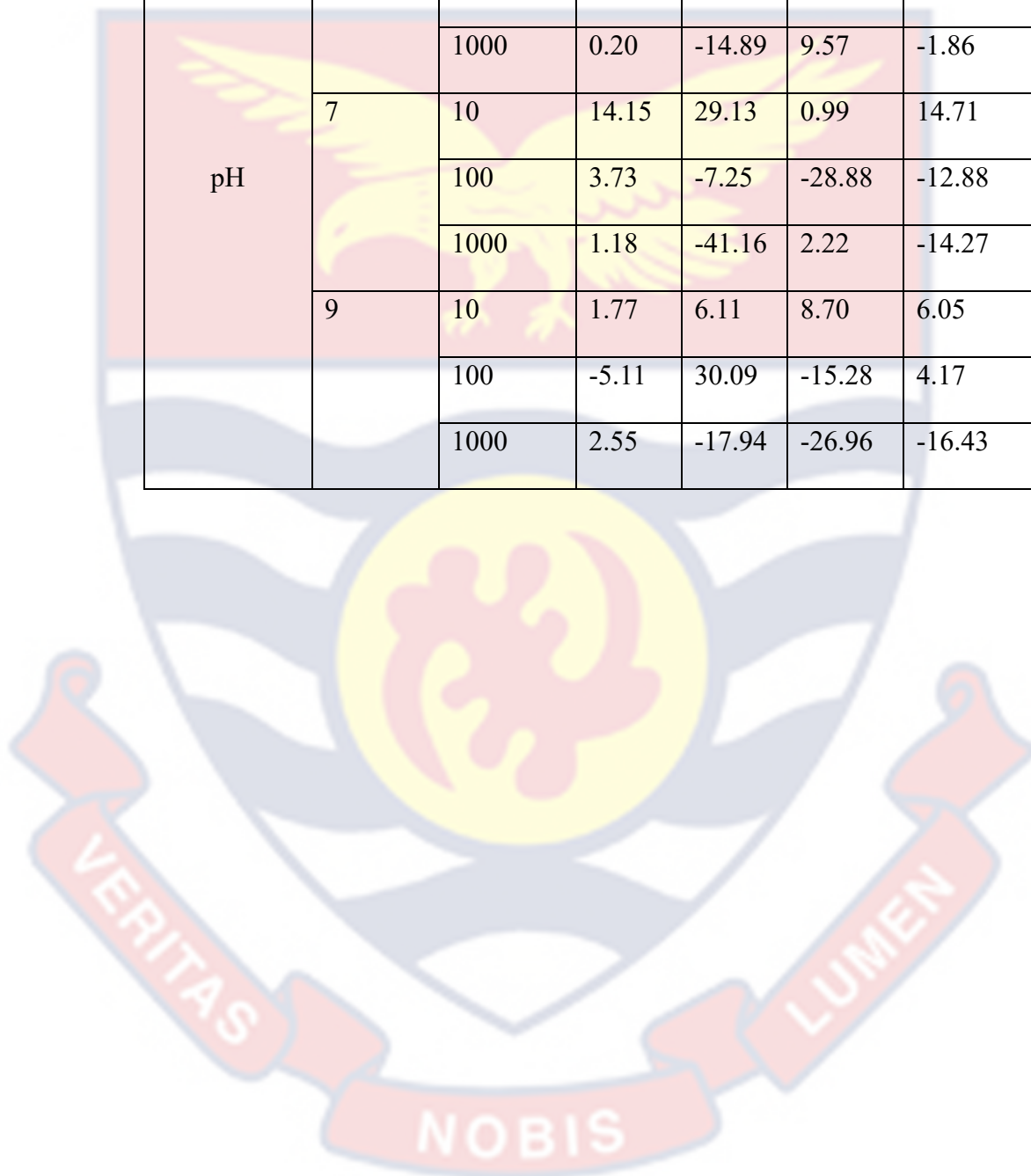
Climate Stressor	Climate stressor level	Cadmium and pyrene ($\mu\text{g/L}$)	Day 1	Day 2	Day 3	Cumulative
Control	0	0	-	-	-	-
Temperature	24	10	2.04	8.08	4.78	4.53

(°C)		100	-2.36	-2.41	5.91	0.89
		1000	- 10.80	12.90	-5.13	-3.03
28	10	10	- 24.78	-3.97	-16.21	-16.54
		100	- 18.84	-1.43	-19.54	-15.05
		1000	- 20.67	-0.04	-19.31	-15.32
32	10	10	- 15.02	-27.44	-7.15	-14.81
		100	5.20	-15.26	2.85	-0.50
		1000	- 10.85	-14.97	6.71	-4.89
Salinity (‰)	15	10	- 45.57	-51.25	-32.52	-41.75
		100	- 53.49	-36.45	-43.43	-45.54
		1000	- 54.56	-46.68	-48.59	-50.37
	25	10	- 24.14	-9.93	-27.17	-22.02

pH	35	100	- 34.96	-22.15	-30.44	-30.19
		1000	- 44.38	-26.26	-39.15	-38.09
		10	- 10.54	-2.19	-4.38	-6.16
		100	13.15	21.91	-4.05	8.41
		1000	- 15.12	-2.96	-19.54	-14.02
	5	10	- 84.52	-80.08	-85.81	-84.00
		100	- 83.53	-70.76	-86.99	-81.91
		1000	- 86.66	-44.94	-87.19	-77.13
		10	2.65	-5.75	-9.08	-3.94
		100	7.19	-6.51	-9.48	-2.58
	7	1000	-2.79	-14.21	-12.18	-9.16
		10	-8.73	2.08	-21.11	-11.09
		100	5.70	8.97	-10.03	0.26
	9	1000	34.16	20.01	-19.56	9.66

Dry biomass ($\mu\text{g/L}$)						
Climate Stressor	Climate stressor level	Cadmium and pyrene ($\mu\text{g/L}$)	Day 1	Day 2	Day 3	Cumulative
Control	0	0	-	-	-	-
Temperature ($^{\circ}\text{C}$)	24	10	-10.41	-58.65	-10.43	-28.48
		100	-5.89	-36.90	-22.83	-23.99
		1000	-4.72	-43.00	-4.60	-19.00
	28	10	1.77	-25.89	11.18	-4.98
		100	-0.98	-13.99	-11.93	-10.05
		1000	-4.13	5.22	3.73	2.38
	32	10	-3.34	-6.36	-9.57	-6.86
		100	2.95	-24.43	-6.96	-11.10
		1000	-4.52	-11.70	5.84	-3.24
Salinity (‰)	15	10	0.79	2.54	2.36	2.05
		100	15.52	-3.69	-10.56	-1.67
		1000	-7.66	-11.96	0.62	-6.10
	25	10	4.52	-2.35	-0.87	-0.12
		100	15.72	8.65	2.36	7.95
		1000	6.68	10.94	-1.74	5.05
	35	10	10.61	-2.29	-1.74	1.05
		100	0.00	-0.03	-1.74	-0.68

		1000	20.63	-13.49	-0.12	-0.10
pH	5	10	-11.59	-4.20	16.09	1.79
		100	-4.32	-66.60	-10.25	-29.90
		1000	0.20	-14.89	9.57	-1.86
	7	10	14.15	29.13	0.99	14.71
		100	3.73	-7.25	-28.88	-12.88
		1000	1.18	-41.16	2.22	-14.27
	9	10	1.77	6.11	8.70	6.05
		100	-5.11	30.09	-15.28	4.17
		1000	2.55	-17.94	-26.96	-16.43



Appendix E: Ethical clearance from the Institutional Review Board,
University of Cape Coast

UNIVERSITY OF CAPE COAST
INSTITUTIONAL REVIEW BOARD SECRETARIAT

TEL: 0558093143 / 0508878309
E-MAIL: irb@ucc.edu.gh
OUR REF: UCC/IRB/A/2016/1265
YOUR REF:
OMB NO: 0990-0279
IORG #: IORG0009096



22TH FEBRUARY, 2022

Mrs. Rael Teresa Adhiambo
Department of Fisheries and Aquatic Sciences
University of Cape Coast

Dear Mrs. Adhiambo,

ETHICAL CLEARANCE – ID (UCCIRB/CANS/2022/01)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted Provisional Approval for the implementation of your research titled **Ecotoxicological Assessment of Climate Change Related Stressors on Selected Plankton within Tropical Estuarine Systems**. This approval is valid from 22th February, 2022 to 21th February, 2023. You may apply for a renewal subject to submission of all the required documents that will be prescribed by the UCCIRB.

Please note that any modification to the project must be submitted to the UCCIRB for review and approval before its implementation. You are required to submit periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithfully,

Samuel Asiedu Owusu, PhD
UCCIRB Administrator

ADMINISTRATOR
INSTITUTIONAL REVIEW BOARD
UNIVERSITY OF CAPE COAST

Appendix F: Recipe for Marine Water Enrichment Solutions

	*Ingredient		Quantity Needed
	Formula	Name	to Prepare 1 L of Solution
Solution 1	Na ₂ SiO ₃ ·9H ₂ O	Sodium metasilicate nonahydrate	30 mg
Solution 2	Na ₂ EDTA	Disodium ethylenediaminetetraacetate dehydrate (alias EDTA disodium salt)	45 g
	NaNO ₃	Sodium nitrate	100 g
	H ₃ BO ₃	Boric acid	33.6 g
	NaH ₂ PO ₄	Sodium dihydrogen phosphate	20 g
	MnCl ₂ ·4H ₂ O	Manganese(II) chloride tetrahydrate (alias Manganous chloride tetrahydrate)	0.36 g
	FeCl ₃ ·6H ₂ O	Iron(III) chloride hexahydrate (Alias Ferric chloride hexahydrate)	1.3 g
Solution 3	ZnCl ₂	Zinc chloride	2.1 g
	CoCl ₂ ·6H ₂ O	Cobalt (II) chloride hexahydrate (alias Cobaltous chloride)	2.0 g

		hexahydrate)	
	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	Ammonium heptamolybdate tetrahydrate (alias Ammonium molybdate; Molybdic acid ammonium salt tetrahydrate)	0.9 g
	CuSO ₄ ·5H ₂ O	Copper(II) Sulfate Pentahydrate (alias Blue vitriol; Cupric sulfate pentahydrate)	2.0 g
	Add drops of 0.1 N HCl until clear solution		
Vitamins	Thiamin		200 mg
	Biotin		1 mg
	Cyanocobalamin		1 mg

(modified from Hansen, 1989)

*Available for purchase from Sigma-Aldrich at

www.sigmaaldrich.com/germany.html

Preparation of the Solutions

- Prepare all solutions using demineralized water in a separate bottle
- Prepare solutions 1-3 in screw-capped polystyrene flask/bottles (DURAN[®] bottles may be used
- To avoid precipitation, prepare solution 2 following the sequence (starting with Na₂.EDTA) given in the table above

iv. Autoclave the separate solutions after precipitation at 15 psi for 15 minutes

v. Store the solutions 1-3 in $\approx 4^{\circ}\text{C}$; store Vitamin solutions at -20°C

vi. All solutions must be utilised within a period of 1 year.

vii. Prepare B1 growth media by adding 1000 ml of solution 2 and 1 ml of solution 3.

Usage:

i. For growing diatoms

- Add to each liter of water: 1 ml of solution 1, 1.1 ml of the B1 media and 1 ml of the vitamin solution

ii. For growing non-diatoms

- Add to each liter of sea water: 1.1 ml of B1 media and 1ml of the vitamin stock solution

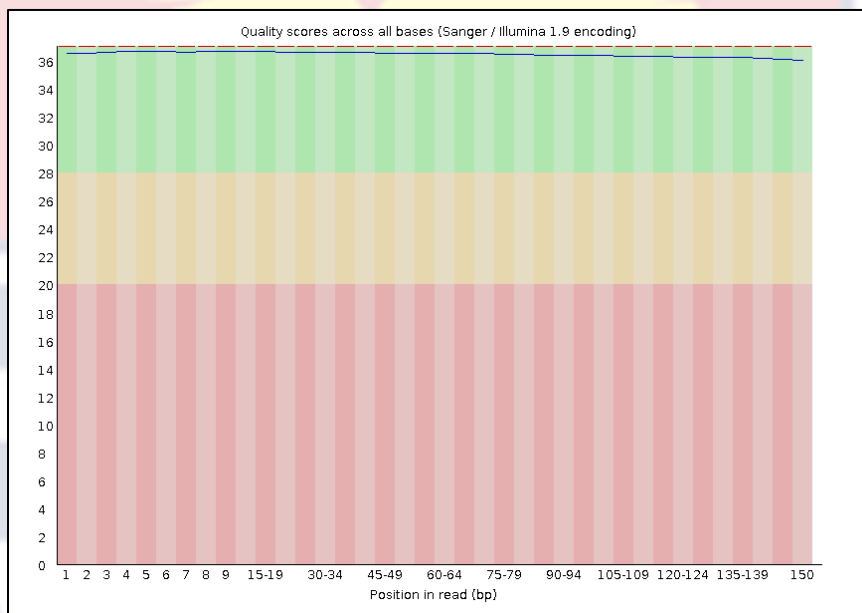
Guide References

Hansen, P. J. (1989). The red tide dinoflagellate *Alexandrium tamarense*: Effects on behavior and growth of a tintinnid ciliate. *Marine ecology progress series*. 53(2), 105-116.

Appendix G: Preliminary results from the RNA-sequencing

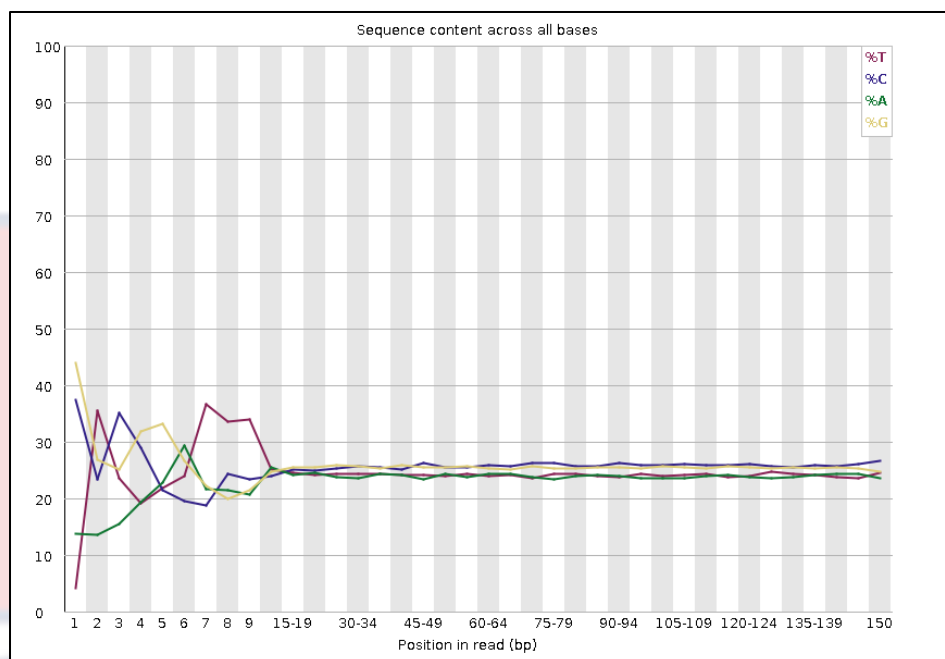
1. Data quality control

Sequencing quality distribution for sample Control. NB: The horizontal axis represents the base position while the vertical axis represents the single base quality.



2. GC Content

The distribution of adenine, thymine, guanine and cytosine (A/T/G/C) for sample control analysis. The base position is represented on the horizontal axis while the single base percentage is represented on the vertical axis. For the current research, a majority of the GC content was distributed between 20-30 across different base pair reads up to 150 bp



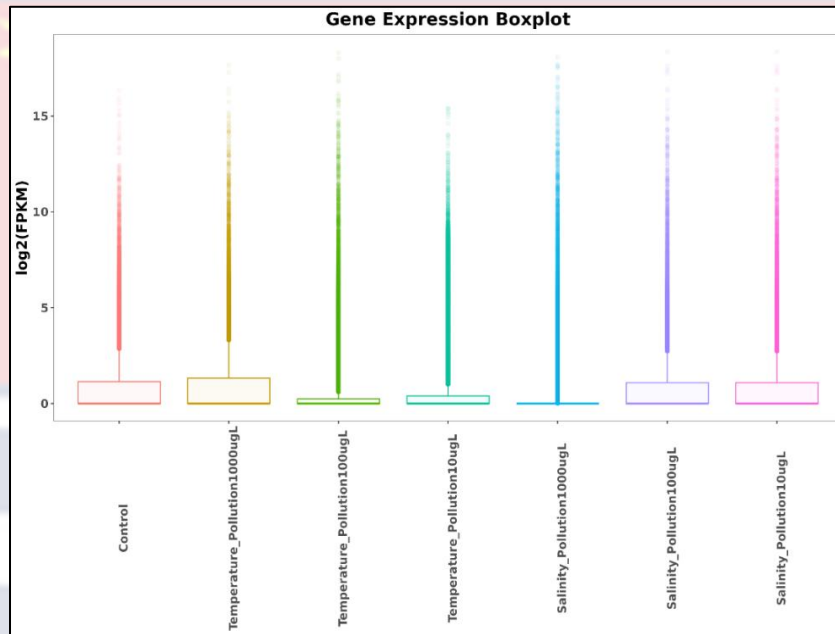
3. Quality Control Summary

Statistics of data quality

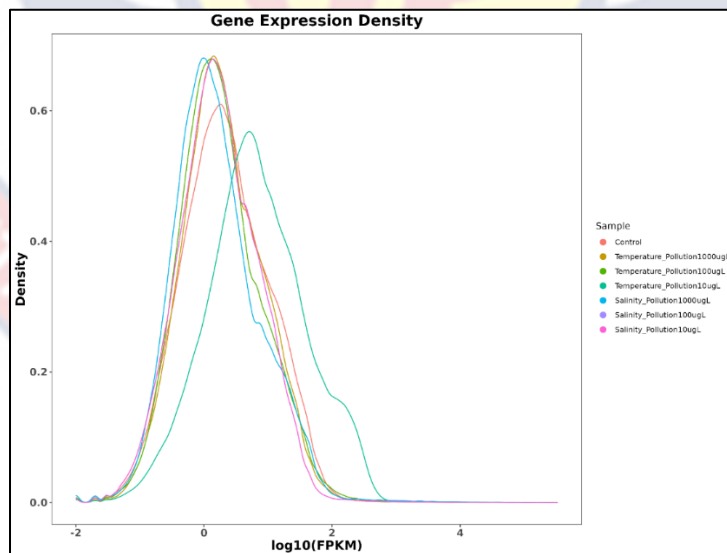
Sample ID	Clean reads	Clean bases	%≥Q30	GC Content
Control	40534580	5931229750	95.66	51.64
Salinity_Pollution1000ugL	42254454	6139850509	95.96	50.08
Salinity_Pollution100ugL	51570494	7545375568	95.67	51.3
Salinity_Pollution10ugL	55335074	8021617680	95.91	49.78
Temperature_Pollution1000ugL	48547002	7075702325	95.64	48.6
Temperature_Pollution100ugL	46586456	6703031812	95.26	49.3
Temperature_Pollution10ugL	28802296	3979091813	93.99	49.49

3. Gene Expression Distribution

- a. Boxplot of FPKM for each sample. The sample names are indicated in the x axis while the $\log_{10}(\text{FPKM})$ is indicated in the y axis.

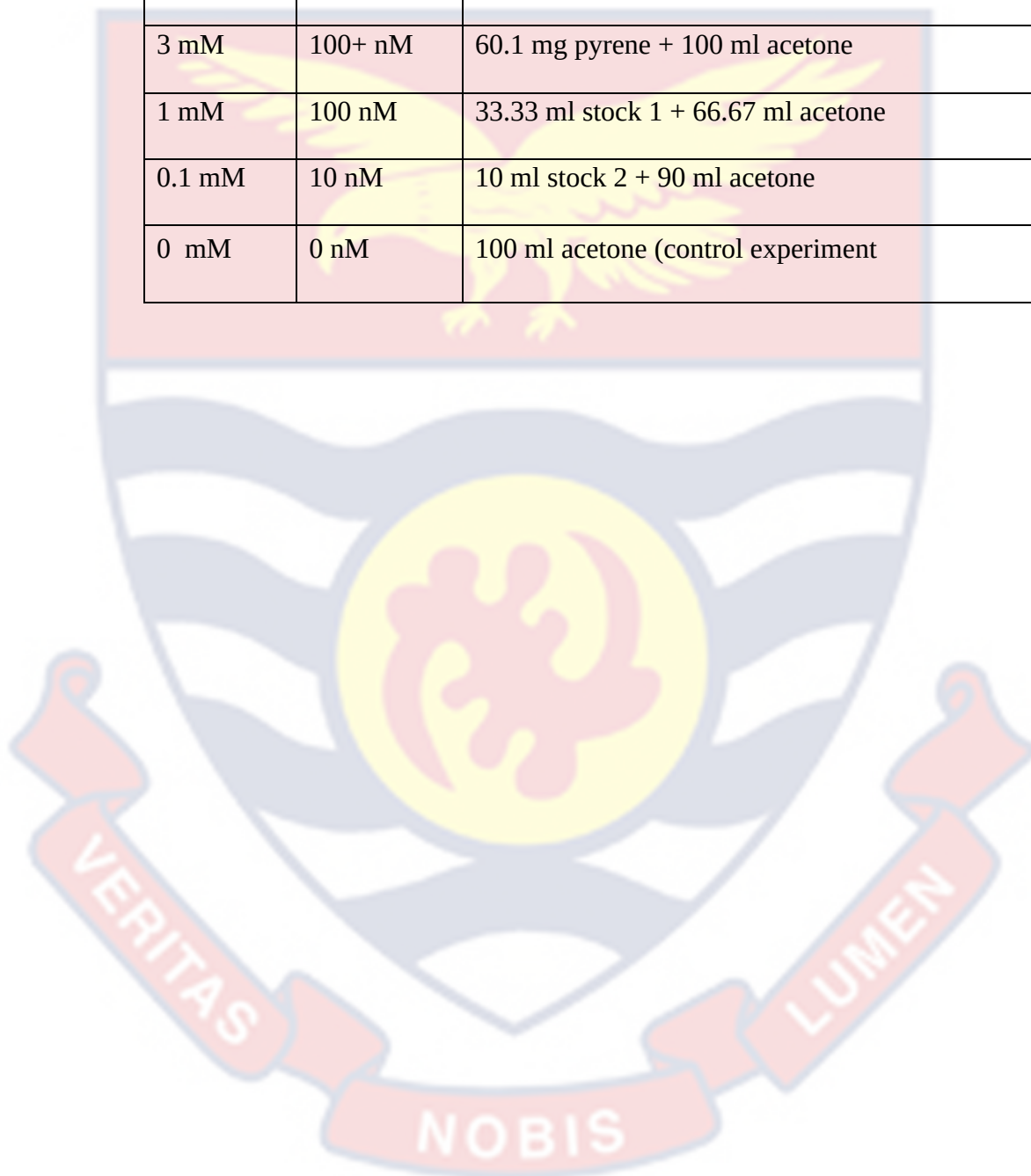


- b. FPKM density distribution. The $\log_{10}(\text{FPKM})$ is indicated in the x axis while the y-axis shows the gene density.



Appendix H: Pyrene stock and exposure solutions used in the experiments

Stock	Exposure Solution	Content
3 mM	100+ nM	60.1 mg pyrene + 100 ml acetone
1 mM	100 nM	33.33 ml stock 1 + 66.67 ml acetone
0.1 mM	10 nM	10 ml stock 2 + 90 ml acetone
0 mM	0 nM	100 ml acetone (control experiment)



Appendix I: Review Article



Review

Widespread Geographical Disparities in Phytoplankton Ecology Research in the Face of Climate Change: A Review

Rael Adhiambo ^{1,2,3,*} , Paul Kojo Mensah ^{2,4} and Emmanuel Acheampong ^{1,2}

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² Department of Fisheries and Aquatic Sciences, School of Biological Sciences, University of Cape Coast, Cape Coast 00223, Ghana; pkmensah@ucc.edu.gh

³ National Commission for Science, Technology, and Innovation, Nairobi 30623-00100, Kenya

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Abstract: Climate change is a global threat that is better understood through global research spanning many regions, countries, and life-supporting ecosystems. This review focused on the bibliometric analysis of the distribution of a global research compendium on phytoplankton, an aquatic community that accounts for half of the carbon fixation in the global carbon cycle. A total of 716 primary research papers published in the immediate past decade (2012–2022) were reviewed. The articles were sampled from Web of Science and described field and laboratory experiments quantifying the impact of global climate change on phytoplankton from different climate zones (tropical, subtropical, temperate, subpolar, and polar) and ecosystems (freshwater, brackish, and marine). Analyses of these studies suggest that the bulk of the global research effort (80%) focused on high-latitude areas, and only a few (17%) were carried out in the tropics, largely led by four countries (Australia, Brazil, India, and Saudi Arabia), while Africa's contribution was minuscule (<1%). In terms of ecosystems, the majority (76%) of the research was in marine waters, irrespective of the climate zone. Analyses of these studies also highlighted widespread disparities in phytoplankton research in tropical aquatic ecosystems, particularly in Africa. Strategic investment in terms of targeted funding, institutional networks, and partnerships between the global north and global south are necessary to increase phytoplankton research across different ecosystems in the tropics.



Citation: Adhiambo, R.; Mensah, P.K.; Acheampong, E. Widespread Geographical Disparities in Phytoplankton Ecology Research in the Face of Climate Change: A Review. *Water* **2023**, *15*, 4288. <https://doi.org/10.3390/w15040288>

Keywords: Africa; aquatic productivity; climate change; phytoplankton research; research biases; tropical ecosystems

Appendix J: Research Article

Aquatic Sciences (2024) 86:70
<https://doi.org/10.1007/s00027-024-01085-8>

Aquatic Sciences

RESEARCH ARTICLE



Plankton assemblages in a tropical West African estuary

Rael Adhiambo^{1,2,3} · Paul Kojo Mensah^{1,4} · Emmanuel Acheampong^{1,2} · Eric Appiah Krampah^{1,2}

Received: 15 November 2023 / Accepted: 4 May 2024
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Abstract

The taxonomic composition, abundance, diversity and spatial distribution of phytoplankton and zooplankton, as well as the prevailing hydrographic conditions, were examined in the Whin Estuary, which is considered one of the most pristine and productive coastal ecosystems of Ghana, which is located on the Gulf of Guinea. Hydrographic and plankton variables were determined along the gradient of the estuary between March and July 2022 across two seasons and tidal periods. A total of 50 genera of phytoplankton and 27 genera of zooplankton were recorded across the two seasons and sampling periods. Diatoms and copepods dominated the plankton communities across the estuary. Plankton genera such as *Navicula*, *Nitzschia*, *Thalassiosira*, *Cyclops*, *Paracalanus* and *Temora* were recorded across all the seasons and tidal periods, despite the varying hydrographic conditions. The environmental variables varied across the seasons, tidal periods and sampling stations in the estuary. For instance, temperature, DO, transparency, and chlorophyll *a* varied significantly ($p < 0.05$) across both the seasons and tidal periods. pH values were significantly ($p < 0.05$) lower in the rainy season compared to the dry season. There was a strong salinity gradient across the estuary, with the upper reaches dominated by freshwater organisms, such as cladocerans and cyanobacteria, and the lower reaches by marine organisms, primarily diatoms and copepods. In sum, the results reported here are important as they highlight the distribution of plankton communities in a tropical estuarine ecosystem and can serve as a reference for the management of tropical brackish ecosystems.

Keywords Brackish ecosystems · Environmental variables · Phytoplankton · Zooplankton · Gulf of guinea

