UNIVERSITY OF GHANA COLLEGE OF BASIC AND APPLIED SCIENCES

SCHOOL OF BIOLOGICAL SCIENCES

EXPLORING THE USE OF Mg/Ca RATIOS OYSTER SHELLS AS PROXY FOR WATER TEMPERATURE IN THE ANYANUI CREEK.

BY

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11004572

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN

MARINE SCIENCE

DEPARTMENT OF MARINE AND FISHERIES SCIENCES

DECLARATION

This dissertation represents the research conducted by Dorcas Antwi in the Department of Marine and Fisheries Sciences at the University of Ghana, under the supervision of Prof. Edem Mahu and Dr. Benjamin Botwe. I declare that this work is entirely my own and has not been previously published or submitted for any degree or diploma at any other institution.

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ABSTRACT

The research focuses on exploring the potential of Mg/Ca ratios in the shells of *Crassostrea tulipa*, an oyster species found in the Anyanui Creek, as a proxy for water temperature. In the field of paleoclimatology, foraminifera and oyster shells have been widely used as proxies to infer historical water temperatures. Given the complex environmental conditions of estuaries, which are influenced by seasonal variations in freshwater influx and salinity, this research aimed to determine whether Mg/Ca ratios could reliably reflect temperature in a setting where additional factors, such as salinity and pH, may interfere. Monthly water and oyster shell samples were collected from three stations in the Creek between March 2023 and April 2024. Temperature, salinity, and pH were measured in situ, and the shells underwent hot plate digestion with hydrofluoric acid and aqua regia to release trace metals for analysis. Mg/Ca ratios in the shells were analysed and correlated with temperature using regression models, including Linear, Ridge, Lasso, and Random Forest regressions, to assess the strength of the Mg-temperature relationship across variable salinity conditions. Results showed moderate correlations between Mg/Ca ratios and temperature at the upstream station, suggesting some potential for using Mg/Ca ratios as temperature indicators in more stable environments. However, correlations were weaker in the midstream and ocean-fed stations, where salinity variability due to freshwater input disrupted the Mg-temperature relationship. The pH also influenced Mg incorporation, particularly in midstream zones, where fluctuating conditions complicated Mg/Ca ratios as standalone proxies for temperature. The findings indicate that while Mg/Ca ratios can offer insights into temperature in estuarine environments, their accuracy is limited in variable salinity and pH settings. A multi-proxy approach incorporating additional indicators is recommended for future studies to improve estuary temperature reconstruction. This research contributes to understanding the environmental applications of biomineralization and highlights the need for multi-faceted approaches in complex estuarine ecosystems as the variability in temperature in estuarine systems like Anyanui Creek can

make the correlation between temperature and Mg/Ca ratios insignificant, especially when other environmental factors, such as salinity, pH, and biological regulation, exert stronger influences.

DEDICATION

I dedicate this work to my family, loved ones, and friends. Their unwavering love and support made this accomplishment possible. Without them, I could not have completed this journey.

ACKNOWLEDGEMENT

I am deeply thankful to God for His grace and strength throughout my studies. I sincerely thank Prof. Edem Mahu for her indispensable supervision and the resources provided through the ARISE Project that made this work a success. I also thank Dr. Benjamin Botwe for his expertise and invaluable assistance. I am grateful for the financial support from ARISE, which enabled all field campaigns and laboratory work. My heartfelt thanks go to Samuel Tawiah and Maurice Oti-Edusei for their assistance during my fieldwork and data analysis.

I appreciate the University of Ghana for providing the research facilities and space. I am profoundly indebted to my family, whose encouragement and prayers have been my pillar of support. Finally, I thank my course mates and friends in the Department of Marine & Fisheries Sciences, University of Ghana.

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CHAPTER 1
INTRODUCTION

1.0 Background

An estuary is a partially enclosed coastal water body that maintains either permanent or periodic connectivity with the sea, receives at least occasional freshwater input from rivers, and exhibits spatial and temporal salinity variation, typically lower than seawater but potentially hypersaline under conditions of high evaporation and minimal tidal or fluvial exchange (Potter et al., 2010). Physically, estuaries are shaped by tidal regimes, wave energy, and river discharge, which influence water circulation, sediment transport, and the formation of stratified or well-mixed zones (Kennish, 2002). Chemically, estuarine waters exhibit variable concentrations of nutrients, dissolved oxygen, and pH, reflecting the continuous interaction between marine and terrestrial inputs as well as biological processes such as photosynthesis, respiration, and decomposition ((Borum et al., 2012; Cloern et al., 2017). As a result, fish populations in estuaries often experience significant shifts in their numbers and makeup due to alterations in several factors, such as river flow, the state of the estuary's entrance, the availability of habitats, temperature, salinity, and water clarity (Chilton et al., 2021). All these factors are susceptible to significant influence from climate change. Although the term "creek" typically refers to a small, narrow stream, the Anyanui Creek functions ecologically and hydrologically as part of an estuarine system. It is a tributary of the Volta estuary and connects upstream with the Keta Lagoon and discharges into the Gulf of Guinea downstream. Its tidal exchange, brackish water conditions, and sediment deposition patterns align it more closely with the functional definition of an estuary than with a purely fluvial creek (Elliott & Whitfield, 2011).

Estuaries play a pivotal role in supporting critical ecological functions such as nutrient recycling, carbon sequestration, energy transfer through food webs, and the creation of diverse habitats that sustain high biodiversity (Barbier et al., 2011; Elliott & Whitfield, 2011). In addition to these ecological roles, estuaries also offer significant economic benefits, including support for aquaculture, commercial and subsistence fisheries, tourism, and coastal protection services (Costanza et al., 1997; Barbier et al.,

2011). Moreover, estuaries serve as crucial breeding grounds for economically valuable fish species (Nagelkerken et al., 2013). Although these estuaries provide indispensable ecosystem services in their specific locales, research conducted in various geographic regions has revealed that they all encounter varying stress levels attributable to climate change and its fluctuations. Estuarine water temperatures influence a wide range of biological processes, including metabolism, reproduction, and species distributions. In paleoclimatology, where long-term instrumental records are often lacking, proxy indicators are indispensable. These are indirect measures used to infer past environmental conditions like temperature, salinity, and pH (Tripati et al., 2003). An ideal temperature proxy would accurately and immediately capture unbiased temperature shifts while remaining unaffected by changes that occur after deposition. While no temperature proxy perfectly fulfils these criteria, several techniques are resilient enough to yield quantitative findings (Ortiz, 2013). Foraminifera, corals, and bivalve shells have been widely used as proxies to infer historical water temperatures. Foraminiferal Mg/Ca ratios have been established as reliable paleotemperature indicators in marine sediments (Elderfield & Ganssen, 2000). Similarly, corals incorporate temperature-dependent trace elements and isotopes in their skeletons, making them valuable for reconstructing past sea surface temperatures (Gagan et al., 2000). Bivalve shells, such as those of oysters and clams, have also been used to infer seasonal and interannual variability in estuarine and coastal water temperatures (Surge & Lohmann, 2008). Bivalve shells grow incrementally, allowing for fine-grained sampling that captures detailed intra-annual records (Stenzel, 1964, Kirby et al., 1998). Moreover, bivalves thrive in diverse climate conditions and habitats. Their typically robust and thick calcitic shells also contribute to the exceptional preservation of fossils. Oysters have been around for over 200 million years. Their calcite shells are durable, often remaining well-preserved with minimal alterations over time (Bougeois et al., 2015). Moreover, their large shell size allows for detailed sampling, enabling the study of short-term variations across multiple years of growth, such as seasonal or annual changes (Mouchi et al., 2017). Shell development of oysters also captures information about the water temperature. Traditionally, stable oxygen isotopes (e.g., $\delta 1^{8}O$) have been used to reconstruct temperature histories, however, $\delta^{18}O$ is influenced by both

temperature and the isotopic composition of water, which is itself dependent on salinity (Freitas et al., 2005). In estuarine environments where salinity varies significantly due to freshwater influx and tidal mixing, the reliability of $\delta^{18}O$ as a temperature proxy is therefore reduced (Dissard et al., 2009; Goodwin et al., 2013). In contrast, elemental ratios like magnesium-to-calcium (Mg/Ca) offer an alternative approach that may be less sensitive to salinity variations and more directly influenced by temperature (Surge & Lohmann, 2008; Freitas et al., 2011). The Mg/Ca ratio in biogenic carbonate is based on the principle that magnesium is incorporated into calcite or aragonite shells during the organism's growth, with the amount influenced by temperature (Dodd & Crisp, 1982). Studies have established the positive correlation between temperature and Mg incorporation in a wide range of marine calcifiers, including foraminifera, ostracods, and corals (Elderfield & Ganssen, 2000., Lear et al., 2000). However, recent studies have extended this principle to bivalves, including oysters (Surge & Lohmann, 2008; Tynan et al., 2016. In tropical estuarine systems, bivalves such as the West African mangrove oyster, Crassostrea tulipa (Lamarck, 1819), serve as valuable proxies for environmental reconstruction due to their ecological sensitivity and shell growth patterns. This species inhabits intertidal mangrove zones, where it firmly attaches to submerged substrates and mangrove roots exhibits continuous shell accretion (Osei et al., 2022). Structurally, the shell comprises two asymmetrical valves one deeply cupped and cemented to the substrate, and the other flatter and free-moving characteristic of oysters in the family Ostreidae (Ronny, 2024). Internally, the shell is composed primarily of aragonite, with growth rings that reflect seasonal environmental changes, making it a valuable archive for reconstructing estuarine conditions (Atindana, 2021). Recent studies have demonstrated the value of elemental proxies like Mg/Ca and Sr/Ca in bivalves and ostracods to infer past environmental conditions. For instance, Surge and Lohmann (2008) validated Mg/Ca-temperature relationships in *Crassostrea virginica*, while Schleinkofer et al. (2021) showed how multiple elemental ratios vary with environmental drivers in deep-sea bivalves. Cao et al. (2023) further supported the use of these proxies in high-pressure, low-temperature cold seep environments.

This research investigates whether Mg/Ca ratios in C. tulipa shells can be used to reconstruct water temperature across Anyanui Creek over a 12-month period.

1.1 Hypothesis

Null Hypothesis: The incorporation of Mg/Ca ratios in the oyster shells at Anyanui is influenced by temperature.

Alternative Hypothesis: The incorporation of Mg/Ca ratios in the oyster shells at Anyanui is not influenced by temperature.

1.2 Aim of the study

This study aims to explore Mg/Ca ratios in oyster shells (*Crassostrea tulipa*) as a potential proxy for water temperature in the Anyanui creek.

1.2.1 Specific Objectives

1. To determine Mg/Ca ratios in both water and oyster shells (C. tulipa) across spatial and temporal gradients in the creek.
2. To evaluate the relationships between Mg/Ca ratios in shells and environmental variables (temperature, salinity, and pH).
3. To develop a predictive model using Mg/Ca ratios in oyster shells as a proxy for water temperature.

1.3 Justification

Mangrove trees along the bank along the Anyanui Creek act as a natural barrier against erosion and offer a habitat for a diverse range of fish and marine life. Local communities rely on the creek for both sustenance and income. It's also a picturesque and unique destination, popular among tourists and locals, serving as a hub for activities like swimming, fishing, boating, irrigation, and transportation. Temperature fluctuations, particularly warmer temperatures, can adversely affect aquatic ecosystems. They influence the distribution and abundance of aquatic species and water quality within the creek.

Temperature changes can limit the creek's utility in various ways. Monitoring temperature is crucial for tracking ecosystem changes and assessing the creek's overall health, yet historical temperature is totally missing (Ubaldini et al., 2024). This hinders our understanding of the Creek and its ecosystem response to climate limits our ability to predict how it may respond to future environmental shifts. Long-term temperature data monitoring is essential for sustainable creek management and contributes valuable information for restoration and conservation initiatives. Numerous techniques exist for reconstructing historical water temperatures, including stable oxygen isotopes ($\delta^{18}O$) in carbonate shells, sediment core analyses, and instrumental historical records (Pearson, 2012; Kämpf et al., 2019; Meister et al., 2024). While stable isotopes, for example, $\delta^{18}O$ values are well established proxies, they are strongly influenced by salinity, making them less reliable in environments like the estuarine part of the Anyanui Creek where salinity varies with both tidal inflow and freshwater discharge (Dissard et al., 2009). Sediment-based proxies are often limited by poor chronological resolution and the absence of continuous biogenic indicators in shallow or disturbed environments (Goodwin et al., 2013). In contrast, elemental ratios such as Mg/Ca in bivalve shells provide a cost effective and biologically integrated method for assessing past water temperatures. Unlike $\delta^{18}O$, Mg incorporation into shells is more directly temperature-dependent and less affected by salinity, especially when applied cautiously in multi-proxy contexts (Freitas et al., 2011). *C. tulipa* is considered a useful proxy for water temperature reconstruction primarily because of its ecological and biological characteristics that allow it to record environmental variability in its shell (Atindana, 2021). Like many oyster species, *C. tulipa* produces growth bands in its shell that reflect seasonal and environmental changes, including temperature fluctuations and these growth increments in the shell calcium carbonate can be analysed to infer past water temperatures, as the oyster's biomineralization processes are influenced by surrounding conditions such as temperature and salinity (Azzoug et al., 2012). *C. tulipa* is sedentary and long-lived, often remaining attached to mangrove roots or hard substrates for years. Its limited mobility ensures that shell records reflect local environmental conditions rather than mixed signals from multiple habitats. Despite the ecological significance of tropical estuaries in West Africa, there is

a paucity of studies that explore elemental tracers in bivalves from this region. Most prior research in the Keta Lagoon Complex and its adjoining water bodies, including Anyanui Creek, has primarily focused on water quality assessments, fish population dynamics, and mangrove degradation (Lamptey et al., 2013, Armah and Amlalo, 2020, Mahu et al., 2023). While these studies have advanced our understanding of ecological pressures in the region, there remains a notable absence of research employing high resolution geochemical proxies for reconstructing past environmental and climate conditions in this tropical estuarine system. This study therefore seeks to address a critical knowledge gap by evaluating whether Mg/Ca ratios in *C. tulipa* shells can be used as a proxy for water temperature under the dynamic salinity conditions of a tropical tidal creek system. Moreover, the findings from this study could contribute to the development of proxy-based environmental monitoring methods that can be applied to other tropical estuarine systems. The findings will have important implications for climate change research, environmental monitoring, and the conservation of tropical estuarine ecosystems.

CHAPTER TWO
LITERATURE REVIEW

2.0 Introduction

Knowledge on past water temperature is crucial for climate and environmental research, providing insights into historical climate variations. Proxies are indispensable in paleoclimate research, allowing scientists to reconstruct historical environmental conditions like temperature, salinity, and pH from natural records (Mouchi et al., 2017). Common examples of these proxies are ice cores, tree rings, sediment deposits, and biogenic carbonates. Using biogenic carbonates, such as bivalve shells, as environmental proxies have become valuable in reconstructing past marine and estuarine conditions (Richardson, 2001). Oysters incorporate biogenic carbonates into their shells as they grow, leaving behind a geochemical signature of the surrounding water conditions. Analysing Mg/Ca ratios in oyster shells has become a valuable method for reconstructing past environmental conditions, especially water temperature (Freitas et al., 2011). This approach is based on the principle that the incorporation of magnesium (Mg) into calcium carbonate ($CaCO_3$) shells is affected by various environmental factors, with temperature being a key influence (Surge et al., 2003). By analysing the Mg/Ca ratio of oyster shells, researchers can infer the prevailing water temperatures during their growth.

2.1 Importance of Water Temperature Reconstruction

The role of water temperature in shaping aquatic ecosystems and influencing marine life underscores the significance of reconstructing historical temperatures (Petersen et al., 2021). In terms of biological growth, temperature directly affects metabolic rates, enzyme activities, and cell function, thereby controlling the growth and development of organisms. For example, Osei et al. (2021) demonstrated seasonal growth oscillations in *C. tulipa* in Ghana's Densu Delta, with faster growth during warmer periods using the seasonally oscillating von Bertalanffy Growth Function (soVBGF). Temperature also influences survival, as organisms often have thermal tolerance limits beyond which physiological stress or death occurs. Atindana (2021) found that *C. tulipa* condition indices and catch rates varied significantly with seasonal temperature changes in the Densu Estuary, affecting survival and

productivity. Temperature further affects water mixing through its influence on water density. Water temperature and density are inversely related, with maximum density at 4°C in freshwater, causing convection currents that promote mixing of water layers which is important for distributing oxygen and nutrients throughout aquatic ecosystems (Bonacina et al., 2022). Assessing changes in water temperature can be challenging at times due to limited historical observations (Bougeois et al., 2015). Photosynthesis, the foundation of primary production, is temperature sensitive, as enzyme activities involved in photosynthesis have optimal temperature ranges; too low or too high temperatures reduce photosynthetic efficiency. For instance, extreme temperatures can reduce the photosynthetic rate in plants and algae, impacting ecosystem productivity (Verfaillie et al., 2009). Shifts in the thermal regime of estuaries can profoundly affect biological communities and the overall health of these ecosystems. Most aquatic organisms thrive within specific temperature ranges, and changes in water temperature directly influence their growth rates, behaviour, and distribution (Caissie, 2006). In the context of a warming climate, estuarine water temperature rises due to increased heat exchange with the atmosphere. There is strong evidence that global water temperatures are increasing due to climate change, further exacerbated by rising water extractions to meet human needs (Huang et al., 2022). In this era of climate change, datasets tracking estuarine water temperature are particularly valuable, as they reflect the direct impacts of climate change on river ecosystems (Kędra & Wiejaczka, 2017). Understanding these dynamics particularly in terms of continuity, completeness, and time horizon is crucial (Chen et al., 2015). Although estuarine water temperature has been consistently observed for an extended period, this fundamental dataset is not readily available in many parts of the world.

2.2 Paleotemperature Proxies

The Earth's climate has undergone significant changes over time, prompting scientists to contextualize recent climate observations within a broader historical framework (Felis et al., 2004). To extend climate records beyond the reach of modern instruments, researchers rely on proxies as indirect measures of past climate conditions preserved in natural archives like marine and terrestrial sediments,

trees, and ice cores (Tynan et al., 2016). Paleotemperature proxies are particularly important for reconstructing historical climate conditions, offering critical insights into past temperature variations. These proxies include physical, chemical, and biological materials preserved in the geologic record, enabling their comparison with modern climate parameters (Mauri et al., 2015). Among the most widely used paleotemperature proxies are the shells of foraminiferans and diatoms, which contain stable oxygen isotopes, allowing researchers to infer past water temperatures. These organisms, found in both aquatic and marine environments, provide valuable information about historical environmental conditions (Felis et al., 2004; Barker et al., 2004). Pollen is another widely used proxy for reconstructing ancient climates. Certain types of fossilised pollen found in rocks or ocean sediments can indicate past climate conditions. By analysing the distribution of pollen types, researchers can infer historical climate belts and temperature variations (Mauri et al., 2015). Ice cores are essential proxies for reconstructing past atmospheric gas levels. These cores contain trapped air bubbles that provide information on past atmospheric composition. By analysing the isotopes of hydrogen and oxygen in ice cores, scientists can reconstruct past climatic conditions (Abram et al., 2013). Sediments collected from oceans and lakes are commonly used as proxy records for reconstructing climate over various timescales. These sediments contain organic matter, minerals, and microfossils that offer insights into past environmental conditions (Leng & Marshall, 2004). Proxy data is converted into paleotemperature estimates through statistical methods or transfer functions that rely on biogeochemical properties or taxonomic assemblages. These estimates are derived using calibration-in-time and calibration-in-space approaches, each of which carries its own degree of uncertainty. The database encompasses a diverse array of proxy types, including pollen assemblages, dinocysts, chlorophyll abundance, and isotopic compositions (Kaufman et al., 2020). Research emphasises the importance of addressing uncertainties related to calibration, proxy biases, and spatiotemporal coverage when using paleotemperature proxies, as these limitations can significantly affect the accuracy and reliability of temperature reconstructions (Yan et al., 2014).

2.3 Mg/Ca Ratios in Bivalve Shells

For a long time, paleo ecologists have been trying to use the chemical properties of fossils to understand past environmental conditions (Bougeois et al., 2015). A key area of research has been the development of techniques to quantitatively assess past temperatures and salinities. This involves analysing trace and minor element chemistry, as well as the oxygen and carbon isotopic composition of fossilised shells (Dodd & Crisp, 1982). Due to their sequential shell deposition, marine bivalves play a significant role as paleoenvironmental archives, enabling the reconstruction of environmental conditions with high temporal resolutions across a broad spectrum of time, spanning from decades to centuries (Richardson, 2001). Additionally, these bivalves have been prevalent throughout the fossil record since the Cretaceous period and are found in various contemporary maritime environments, ranging from coastal seas to hydrothermal zones (Stenzel, 1964). The full potential of most geochemical proxies in bivalves remains unrealised due to a scarcity of completed calibration and validation studies, despite the notable significance of these proxies (Richardson, 2001). It is crucial to conduct further research to explore the applicability of elemental geochemical proxies to other bivalve species, as there is still a possibility that species-specific effects may come to light. Bivalves, including oysters, precipitate calcium carbonate ($CaCO_3$) to form their shells, using ions from the surrounding seawater. The process is biologically mediated, involving the secretion of shell matrix proteins that regulate nucleation and crystal growth (Huang & Zhang, 2022). The mineralogy of the shell varies with life stage: larval shells are primarily aragonitic, while adult shells contain more calcite (Andersson et al. 2008). The incorporation of trace elements like magnesium (Mg) and strontium (Sr) occurs during shell formation, and their concentrations can reflect environmental conditions at the time of deposition (Freitas et al., 2011). Magnesium can replace calcium in the carbonate lattice, with the substitution influenced by environmental factors like temperature, salinity, and pH (Murphy et al., 2024). Elderfield et al. (1996) found that the incorporation of magnesium into calcium carbonate shells is mainly determined by the Mg/Ca ratio in seawater and the surrounding water temperature. However, the exact amount of magnesium that gets integrated into the shell depends on the organism's physiology and environmental

conditions (Carré et al., 2006). Since Mg/Ca ratio is relatively unaffected by salinity, it is viewed as an effective temperature indicator (Dodd & Crisp, 1982). Studies have shown that trace element ratios in biogenic carbonates, such as Mg/Ca in biogenic calcite and Sr/Ca in aragonite, to serve as potential temperature proxies (e.g. Lear et al., 2000, Rosenthal, Bova, & Zhou, 2022.). These ratios are often considered alongside or in place of $\delta^{18}O$ carbonate. Calibration curves for Mg/Ca temperature relationships have been successfully developed for various organisms, including foraminifera (Elderfield & Ganssen, 2000), gastropods, and ostracods (e.g., Elmore et al., 2012; Cobo et al., 2017). It is widely recognised that the Mg/Ca ratio in seawater remains stable unless salinity drops below 10 PSU (Dodd & Crisp, 1982). Nonetheless, using fossil trace chemistry to determine paleotemperatures has encountered difficulties, chiefly due to uncertainties in the relationship between temperature and trace element concentrations (Freitas et al., 2006). Some species show a positive correlation between certain trace elements and temperature, while others correlate negatively with the same elements. The biomineralization process in oysters is particularly noteworthy for their significant economic value and because they frequently appear as fossils in sedimentary records (Bougeois et al., 2015). Numerous studies have delved into various aspects of oyster biomineralization, examining the roles of organic molecules, the composition of bulk shells and amino acids, and the elemental concentrations in foliate and prismatic layers (Ullmann et al., 2013, Surge et al., 2003, Mouchi et al., 2013, Bougeois et al., 2015). Oyster shells are predominantly made of low-magnesium calcite, known for being the most resistant form of calcium carbonate to alteration, significantly contributing to the exceptional preservation of their fossils (Surge & Lohmann, 2008). Additionally, oysters thrive in diverse ecosystems, demonstrating their adaptability to fluctuations in salinity and showcasing extensive stratigraphic, geographic, and latitudinal distributions, which makes them excellent fossils (Stenzel, 1971).

2.4 Factors Influencing Mg Incorporation into $CaCO_3$ Shells

Temperature and salinity are the two most significant factors influencing Mg incorporation into $CaCO_3$ shells. As Foster et al. (2010) discussed, higher temperatures tend to increase the amount of Mg

incorporated into shell carbonate. This is because higher temperatures can increase the solubility of Mg in seawater, leading to greater Mg incorporation into the shell. Hence, magnesium ions are more readily substituted for calcium at higher temperatures in the aragonite lattice (Elderfield et al. 1996). However, salinity also plays a critical role. Changes in salinity, resulting from freshwater input and tidal variations, affect the Mg concentration available for incorporation (Allison and Finch 2004). In estuarine environments, where salinity fluctuates due to freshwater inputs, the availability of Mg^{2+} in the water changes, thereby affecting how much is incorporated into the shell. This suggests that both temperature and salinity must be considered when using Mg/Ca ratios as proxies for environmental conditions (Surge & Lohmann, 2008).

Variations in pH can alter the solubility of Mg and Ca, impacting the Mg/Ca ratio in shells (Schöne et al., 2005). Foster et al. (2010) found that while pH has a minor impact on Mg incorporation, it can influence the overall carbonate chemistry of the water, which in turn affects shell formation. In highly alkaline environments, lower levels of available magnesium may reduce Mg incorporation into the shell. However, in most natural environments, pH is relatively stable compared to temperature and salinity, making its influence on Mg/Ca ratios less pronounced. Similarly, Elderfield et al. (1996) observed that nutrient levels and dissolved oxygen also affect the incorporation of trace elements in marine carbonates, but these effects are generally secondary to the primary controls of temperature and salinity. Calcifying organisms, which are vital for paleoclimate reconstructions, display intricate biological mechanisms affecting the incorporation of magnesium (Mg) and calcium (Ca) into their carbonate structures (Carré et al., 2006). These processes, in turn, impact the Mg/Ca ratios in their shells or skeletons. These mechanisms represent a dynamic interplay between biological and environmental factors. The metabolic activity of calcifying organisms plays a role in Mg/Ca ratios. Metabolic variations, such as differences in energy expenditure and pathways, can influence the selective uptake of Mg and Ca during calcification (Elmore et al., 2012). The site and mode of calcification contribute to Mg/Ca ratios. For instance, the outer versus inner shell layers in molluscs exhibit distinct Mg/Ca signatures, as observed in studies on bivalves (Freitas et al., 2009). Planktonic

and benthic foraminifera, with different calcification modes, also display variations in Mg/Ca ratios (Spero et al., 1997). Organisms exert significant control over biomineralization processes, which impacts the Mg/Ca ratios in their carbonate structures. The regulation of biomineralization involves the active management of mineral precipitation, thus influencing how magnesium (Mg) and calcium (Ca) are incorporated (Kirby et al., 1998). Physiological responses to environmental changes also play a crucial role. Factors such as temperature, salinity, and pH can alter these processes, subsequently affecting the Mg/Ca ratios within the organisms' carbonate skeletons (Schöne et al., 2005). Different species may exhibit unique biochemical pathways and genetic controls governing the uptake and incorporation of Mg and Ca, leading to species-specific Mg/Ca signatures (Gillikin et al., 2005). The metabolic processes of oysters can influence the Mg/Ca ratio in their shells, potentially through selective ion uptake or internal regulation. The selective uptake of ions during calcification influences Mg/Ca ratios. Organisms may selectively incorporate Mg or Ca ions based on availability and physiological requirements, resulting in variations in Mg/Ca ratios within their carbonate structures (P. S. Freitas et al., 2009). Understanding these biological mechanisms is paramount for interpreting Mg/Ca ratios as environmental proxies. Human activities in estuarine environments, such as urbanization and pollution, introduce additional factors influencing Mg/Ca ratios. Anthropogenic inputs can alter water chemistry, impacting Mg availability and the biomineralization processes of calcifying organisms (Gillikin et al., 2005).

2.5 Relationship Between Mg/Ca Ratios and Water Temperature in Marine Organisms

The relationship between Mg/Ca ratios and water temperature has been extensively investigated in marine organisms. For example, Lea et al. (1999) demonstrated that foraminifera, a type of marine microorganism, incorporate higher levels of magnesium into their shells as water temperature rises. This principle has facilitated the widespread use of Mg/Ca ratios in marine systems for reconstructing past sea surface temperatures. In bivalves like oysters, a similar trend has been observed. Surge et al. (2001) found that Mg/Ca ratios in *Crassostrea virginica* shells from Chesapeake Bay correlated with

seasonal water temperature changes, suggesting that the Mg/Ca ratio could serve as a proxy for temperature in estuarine environments. However, the correlation between temperature and Mg/Ca ratios in estuaries is often complicated by other factors, particularly salinity. The incorporation of Mg into the $CaCO_3$ structure is temperature-dependent due to kinetic factors (Mucci, 1987). However, this process is also influenced by the specific species, as different organisms have varying sensitivities to temperature and other environmental factors (Freitas et al., 2006).

2.6 Relationship Between Mg/Ca Ratios and Salinity in Marine Organisms

Salinity plays a crucial role in regulating Mg/Ca ratios in estuarine environments. Several studies have documented a negative correlation between salinity and Mg/Ca ratios, where higher salinity reduces the incorporation of magnesium into shell carbonate. This is particularly important in estuarine environments where freshwater influx from rivers or rain can significantly alter the salinity levels.

Ullmann et al. (2013) found that in Crassostrea gigas, Mg/Ca ratios decreased as salinity increased, complicating their use as straightforward temperature proxies in estuarine environments. This finding supports the notion that salinity has a significant effect on Mg/Ca ratios, which can obscure the temperature signal in environments where salinity fluctuates. Freitas et al. (2008) conducted controlled laboratory experiments on Mytilus edulis and Pecten maximus and found a similar negative correlation between salinity and Mg/Ca ratios. Their findings suggest that in estuarine settings, Mg/Ca ratios are more sensitive to salinity than temperature, emphasizing the need to account for salinity changes when using these ratios as environmental proxies.

2.7 Method of Mg/Ca Ratio Analysis

Efficiently and accurately identifying these elements in small samples with varying compositions is essential for optimizing the use of Mg/Ca and Sr/Ca ratios in marine carbonates as proxies for palaeoceanographic conditions. Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICPAES) is an analytical technique that allows for the simultaneous determination of multiple elements in a sample. This method is particularly useful for analysing trace elements with high

precision and accuracy, making it invaluable in paleoclimate research. The sample, which can be a liquid or a dissolved solid, is typically prepared by digesting it in a suitable acid to break down the matrix and make the elements available for analysis. The prepared sample is then introduced into a high temperature argon plasma. The plasma is generated by passing argon gas through a radiofrequency

(RF) coil, inducing a high-energy discharge that ionizes the argon atoms, creating a high-temperature (around 10,000 K) and high-energy environment. As the sample enters the plasma, it is quickly vaporized and atomized. The high temperatures of the plasma excite the atoms or ionize them, depending on the specific conditions. When these excited atoms or ions return to their ground state, they emit light at specific wavelengths unique to each element. This characteristic light allows for the identification and quantification of elements based on their emission spectra, which is fundamental for techniques like Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). The emitted light then passes through an optical system that may include a diffraction grating or prism, dispersing the light into its component wavelengths and creating a spectrum. The dispersed light is directed onto a detector, usually a photomultiplier tube or a charge- coupled device (CCD), which measures the intensity of the emitted light at different wavelengths. The resulting data, displayed as a spectrum, is analysed to identify peaks corresponding to the emission lines of elements in the sample. The intensity of these peaks is directly proportional to the concentration of the respective elements. To convert the intensity of the emission lines into quantitative concentrations, a calibration curve is created using standard reference materials with known concentrations of the target elements. This curve helps establish the relationship between the intensity of the emission lines and the element concentrations in the sample. This process ensures accurate identification and quantification of elements, making it invaluable in analysing environmental samples for paleoclimate research.

ICP-AES is recognized for its high sensitivity, wide dynamic range, and capacity to analyse a diverse array of elements simultaneously. It is extensively used in fields such as environmental analysis, geochemistry, and materials science due to its ability to deliver accurate and precise multi-element

analysis. According to De Villiers et al. (1995), a significant challenge in conducting ICP-AES analysis on marine carbonates is the interference caused by the calcium (Ca) matrix and the self-matrix effect. Even with attempts to match the calcium concentration in sample solutions, the resulting inaccuracies can often equal or exceed the variations observed among different samples. However, a non-traditional calibration approach that utilizes reference solutions with identical calcium concentrations, but differing Mg/Ca and Sr/Ca values can effectively mitigate this matrix effect.

2.8 Existing Studies on Mg/Ca Ratios in Oyster Shells

The relationship between Mg/Ca ratios and water temperature in oyster shells has been thoroughly investigated in temperate regions as shown in Table 1. Surge and Lohmann (2008) examined the potential of using Mg/Ca ratios in the shells of the estuarine oyster *Crassostrea virginica* as a temperature proxy. Their research addressed the complexities of oxygen isotope ratios ($\delta^{18}O$) in estuarine environments, where both temperature and salinity can vary simultaneously. Unlike $\delta^{18}O$, Mg/Ca ratios in water were assumed to remain constant above 10 practical salinity units (PSU). The study examined whether the water salinity in estuarine settings affects the chemical composition of shells. They found a small amount of mixing between low-salinity and normal marine water conditions, indicating that this assumption might not always hold true in estuaries. By analysing powdered shell samples for oxygen and carbon isotopes, as well as magnesium-to-calcium ratios, researchers gained insights into the environmental factors that influenced these organisms. By comparing the measured $\delta^{18}O$ and $\delta^{13}C$ values in the shells to their predicted values, researchers were able to assign dates to the shell samples. These dates, once aligned, allowed for the determination of corresponding temperatures and salinities, which facilitated comparisons between the Mg/Ca ratios in the shells and temperature. The study found a weak but statistically significant link between the Mg/Ca_{shell} and temperature ($r^2 = 0.05$, $p < 0.01$); however, when analysing the last year of growth, a stronger positive relationship emerged between Mg/Ca_{shell} and the partition coefficient (DMg). While still moderate, these correlations were statistically significant, suggesting a potential influence of DMg on shell chemistry, especially during periods of rapid growth. This improved correlation could be influenced by

ontogenetic effects impacting magnesium incorporation into the younger parts of the shell or potential inaccuracies in date assignments. In another study, Tynan et al. (2016) investigated the potential of using Mg/Ca ratios in the shells of Saccostrea glomerata, also known as the Sydney rock oyster, as a temperature record. The findings demonstrated that these oyster shells' Mg/Ca ratios could be reliably used to estimate historical sea surface temperatures. Factors such as salinity, pH, and the availability of calcium and magnesium ions in the water influenced these ratios, providing valuable information about past climate conditions. Additionally, Bougeois et al. (2015) investigated Mg/Ca ratios in fossilised oyster shells as proxies for paleotemperatures, concentrating on the Palaeogene period in Central Asia. Their study showed that Mg/Ca ratios in specific oyster species and specimens could be used to reconstruct historical variations in seawater temperatures.

Table 1. Comparing Mg/Ca seawater temperature relationships for calcitic bivalves.

Species	**Environment**	**Temperature range**	**Mg/Ca = f(T)**	**Reference**
Crassostrea gigas	Marine	5–25 °C	$Mg/Ca = -0.50 + 0.27 * T$	Mouchi et al., 2013
Crassostrea virginica	Estuarine	18–32 °C	$Mg/Ca = -0.23 + 0.72 * T$	Surge and Lohmann, 2008
Mytilus trossolus	Marine	6–23 °C	$Mg/Ca = 2.25 (\pm 0.63) + 0.30 (\pm 0.04) * T$	Klein et al., 1996
Mytilus edulis	Brackish water	7–19 °C	$Mg/Ca = 5.44 (\pm 0.31) + 0.77 (\pm 0.22) * T$	Wanamaker et al., 2008
	Culturing	10–20 °C	$Mg/Ca = 1.50 (\pm 0.57) + 0.27 (\pm 0.04) * T$	Freitas et al., 2008
	Estuarine	5–20 °C	$Mg/Ca = -0.63 (\pm 0.29) + 0.70 (\pm 0.02) * T$	Putten et al. (2000)

Table adopted from Bougeois et al. (2015)

Ullmann et al. (2013) utilized the Giant Pacific Oyster, *Crassostrea gigas*, as a contemporary model for fossilised oysters. Their research involved detailed sampling of two shell structures, the chalky

substance, and the foliate layers, to analyse trace element distributions and stable isotope variability. They observed seasonal patterns in the oxygen isotopes, with consistent carbon isotope values across different oysters. The researchers suggested that calcium isotope ratios in oyster shells could be used as a proxy for paleo-seawater calcium isotope ratios. Notably, they found that Mg/Ca ratios in the chalky substance negatively correlated with $\delta^{18}O$ values, indicating temperature dependence, a pattern not observed in the foliate layers. Seasonal Sr/Ca variations were linked to metabolic processes, with Mg/Ca ratios also showing additional environmental control.

The methodologies used in these studies generally involve collecting oyster shells from various environmental settings, measuring the Mg/Ca ratios using techniques like inductively coupled plasma atomic emission spectrometry (ICP-AES), and correlating these ratios with environmental data such as temperature, salinity, and pH.

2.9 Identification of Gaps in Current Knowledge

Although using Mg/Ca ratios as temperature proxies is well-documented in marine environments, there are still knowledge gaps regarding how this relationship operates in tropical estuarine systems. Further research is necessary to understand the combined influences of temperature, salinity, and other environmental factors on Mg/Ca ratios in these settings. Additionally, while much of the research has focused on temperate species like *Crassostrea virginica* and *Crassostrea gigas*, there is limited research on tropical species such as *Crassostrea tulipa*, which is the focus of this study. Many existing studies have been conducted in temperate estuaries such as Chesapeake Bay or the Danish Wadden Sea, where seasonal temperature variations are pronounced. In contrast, Anyanui Creek is in a tropical region with less pronounced temperature variability but significant salinity fluctuations due to seasonal rainfall and freshwater input. This makes it an ideal location to study the combined effects of temperature and salinity on Mg/Ca ratios. Also, this study addresses a critical knowledge gap in the application of elemental proxies for environmental reconstruction in tropical coastal ecosystems.

CHAPTER THREE
METHODOLOGY

3.0 Sample Area

The geological characteristics of Ghana's coast contribute significantly to its dynamic nature. The coastline is characterized by sedimentary formations, including sandy beaches, coastal plains, and rocky outcrops. Erosional and depositional processes are driven by factors such as wave action, tides, and riverine inputs. The coast of Ghana spans approximately 550 km along the Gulf of Guinea, ranging from 06º.06; 01º.12 E to 05º.05; 03º.06 W. This coastal stretch, which spans from the eastern border with the Republic of Togo to the western border with Cote d'Ivoire, is crucial to the country's socio-economic landscape. The coastal region has been classified into three major geomorphic zones: Eastern, Central, and Western beaches. Each zone exhibits distinct characteristics, influenced by factors such as coastal processes, sedimentation, and human activities. The Eastern coast, where the study area is located, is notably influenced by the Volta River, a major watercourse that significantly impacts the region's geomorphology. This section of the coast experiences unique features shaped by the interaction between the river and coastal processes.

3.1 Study Site

Anyanui Creek is a tributary of the Volta Estuary located in the Anloga District of Ghana's Volta Region and forms part of a dynamic hydrological network that connects the Volta River, the Keta Lagoon, and the Atlantic Ocean (Mahu et al., 2024). The creek flows through dense mangrove forests, creating a biologically rich and ecologically sensitive corridor that supports species like *Crassostrea tulipa* (West African mangrove oyster), tilapia, and various crustaceans. It receives water from several rivers, including the Volta, Todzie, and Belikpa Rivers, which flow through the Avu Lagoon on its northwest border into the lagoon. It is subject to tidal influence and salinity fluctuations due to the mixture of seawater and freshwater from inland sources. Farmers engage in manure-intensive horticulture, cultivating crops such as shallots, peppers, okra, tomatoes, and carrots year-round, exerting considerable pressure on the lagoon's resources (Mahu et al. 2023). Following a preliminary survey

conducted one month prior to the main study in February 2023, three sampling stations were strategically established along Anyanui Creek to capture spatial variability in physicochemical conditions. The upstream station (Lagoon-fed), located at latitude 5.7767°N and longitude 0.8081°E, is influenced by inflow from the Keta Lagoon and is characterized by high turbidity and low salinity. The midstream station (latitude 5.8025°N, longitude 0.7473°E) represents a transitional zone with moderate salinity and enhanced tidal mixing. The downstream station (Ocean-fed), situated closest to the estuarine mouth at latitude 5.7786°N and longitude 0.6998°E, exhibits elevated salinity levels and strong tidal influence. Both climate change and anthropogenic activities have impacted the Creek. For instance, in March 2023, a sandbar blocked Anyanui Creek's connection to the sea by tidal wave action, denoted as downstream, gradually reducing salinity in this area. During September 2023, a controlled spill from the Akosombo Dam may alter the water hydrology in Anyanui Creek, affecting conditions across the sampling stations.

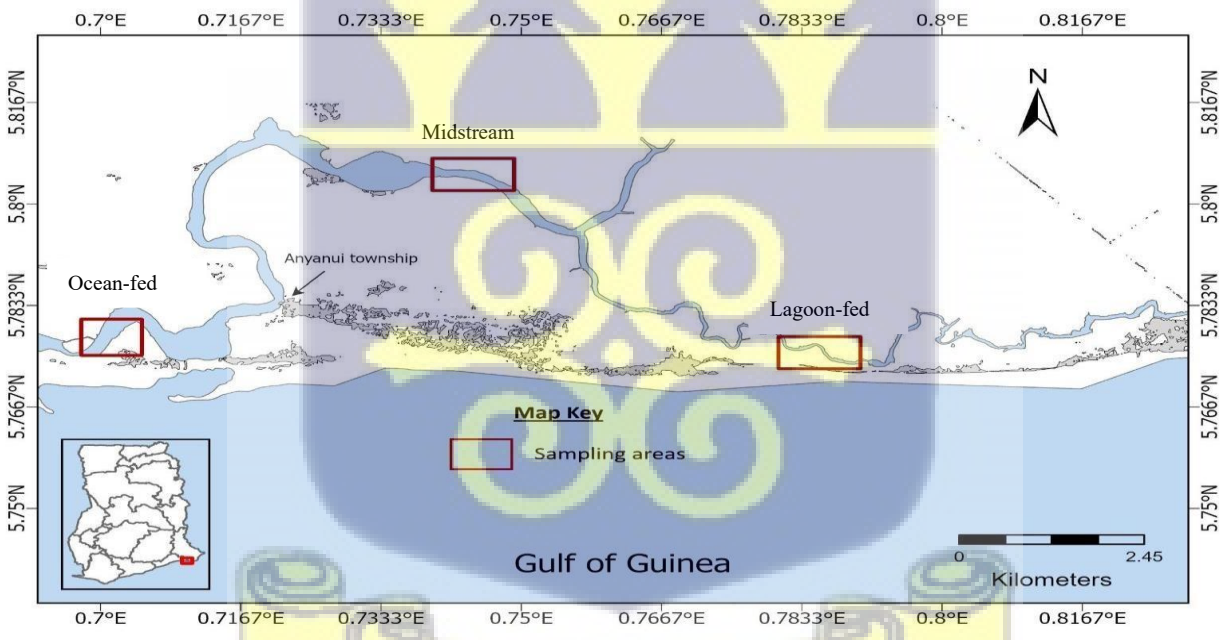


Figure 1: A map of the three sampling stations across the Anyanui Creek: Lagoon-fed (Upstream), Midstream (transitional zone), and Ocean-fed (Downstream).

3.2 Field Sampling

Oyster and water samples were collected monthly from March 2023 to April 2024 at each station. At each of the three sampling stations, three water samples and three live oysters were collected monthly

during low tide, yielding a total of 108 water samples and 108 oyster specimens over the 12-month study period (3 samples × 3 stations × 12 months). Oyster specimens were handpicked from permanent mangrove root attachments, individuals used were between the ranges of 4 cm to 6 cm in shell length selected for analysis. Water samples were manually collected by submerging clean 500 ml polyethylene bottles approximately 20 cm below the surface while holding the bottle by hand to ensure direct contact with the ambient water column, immediately stored on ice to preserve integrity prior to laboratory analysis. In situ measurements of temperature, pH, and salinity were conducted using a handheld Horiba U-50 multiparameter water quality meter. Hourly data was also collected from a specific oyster reef using a fixed data logger at the three stations. The precision of the temperature and salinity probes for both the HOBO data logger and the Horiba probe was ±0.15°C and ±0.1 ppt, respectively. Weekly data reduction was performed to accommodate time averaging, which is necessary for the geochemical analysis of oyster shells. Using a multi-probe device allowed accurate real-time monitoring, reflecting the dynamic water chemistry of the creek.

Figure 2: Harvesting of oysters from study site.

3.3 Laboratory Analysis

3.3.1 Water Sample Preparation

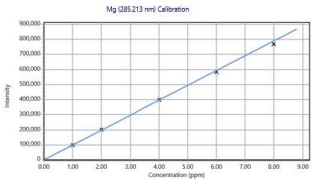
Sampling activities included collecting 108 water samples and live oysters from an oyster reef in Anyanui's creek. Shell preparation for trace metal analysis followed established protocols adapted from Surge & Lohmann (2008) and Schöne et al. (2010). Water samples were gathered at three sites:

Lagoon-fed, midstream, and ocean-fed to determine Mg and Ca levels. The interaction between salinity and Mg/Ca levels in the water was evaluated along the gradient from freshwater to saltwater. Water samples were filtered to remove particles and then treated with nitric acid to maintain a pH of 2 (Surge & Lohmann, 2008). The acidified samples were stored in specially cleaned plastic bottles to prevent contamination. This acidification process helps preserve the Mg and Ca ions, preventing precipitation and maintaining sample integrity for geochemical analysis. To analyse the magnesium and calcium content, a small amount of water. (150 microliters) was mixed with ultrapure water to dilute it. After an initial measurement to determine the concentrations, the samples were further diluted to a specific level (100 parts per million) for consistent analysis. The Mg/Ca ratios in the water samples were analysed using an Agilent 4210 MP- AES at the University of Ghana. This atomic emission spectrometer, known for its high sensitivity, is an alternative to traditional flame Atomic Absorption Spectroscopy (AAS). It operates on air instead of combustible gases through microwave plasma, making it a safer and more cost-effective option. Figure 4 shows the calibration curve which was achieved using ICP multi-element calibration standard solutions and a Certified Reference Material of seawater (CRM-SW, Greyhound, High Purity Standards).

Figure 3: Acidification, filtering, and storing of water samples for laboratory analysis.

Calibration Curves:
Mg (285.213 nm)
Intensity = 98891.16824053 * Concentration + 81.87637060
Correlation coefficient: 0.99980

Standards	Intensity	Method Concentration	Calculated Concentration	% Error
Blank	0.21	0.00	0.00	N/A
Standard 1	100317.45	1.00	1.01	1.36
Standard 2	201637.62	2.00	2.04	1.91
Standard 3	401114.34	4.00	4.06	1.38
Standard 4	586390.78	6.00	5.93	1.19
Standard 5	771075.30	8.00	7.80	2.55



Ca (393.366 nm) Calibration
Intensity = (129547.68265934 * Concentration + 648.23984060) / (1 - 0.07746032 * Concentration)
Correlation coefficient: 0.99994

Standards	Intensity	Method Concentration	Calculated Concentration	% Error
Blank	548.02	0.00	0.00	N/A
Standard 1	144718.80	1.00	1.02	2.35
Standard 2	297035.00	2.00	1.94	2.86
Standard 3	762112.80	4.00	4.04	0.95
Standard 4	1466619.99	6.00	6.03	0.48
Standard 5	2721691.61	8.00	7.99	0.07

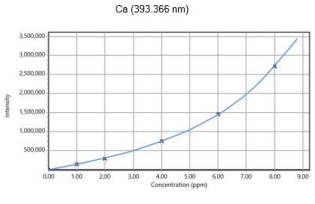


Figure 4: Calibration curve established using certified multielement standards (Agilent #5188-6525)

3.3.2 Shell Sample Preparation

After collection, the oysters were immediately opened, and their soft tissues removed. The shells underwent a cleaning process involving a 6-hour soak in hydrogen peroxide, a 20-minute acid wash, and a thorough rinse with demineralized water to disinfect the shell. To prepare them for analysis, the hinge areas were embedded in epoxy resin to avoid breaking during sectioning, and the shells were cut lengthwise from the hinge to the outer edge, following the direction of maximum growth. A dental drill was used to extract about 50-100 µg of carbonate powder for trace elemental analyses. The left valve was used for analysis, specifically targeting the growth rings at the umbo. This region primarily consists of low-Mg calcite, which forms the dominant mineral phase in adult *C. tulipa* shells. Although aragonite is present in early ontogenetic stages, adult oyster shells are overwhelmingly calcitic, which enhances their preservation and geochemical stability (Lee et al., 2011; Schöne et al., 2010; Surge & Lohmann, 2008). The oyster shell samples were digested to release trace metals by treating them in Teflon vessels with a mix of hydrofluoric acid (HF) and aqua regia. About 0.2 g of the dry sample was combined with 1 ml of aqua regia and 6 ml of HF, then heated to 120 °C for 2.5 hours. After cooling, the samples were transferred to polypropylene tubes containing a boric acid solution, rinsed with Milli-Q water and placed in an ultrasonic bath at 60 °C to ensure complete dissolution. Finally, the solution

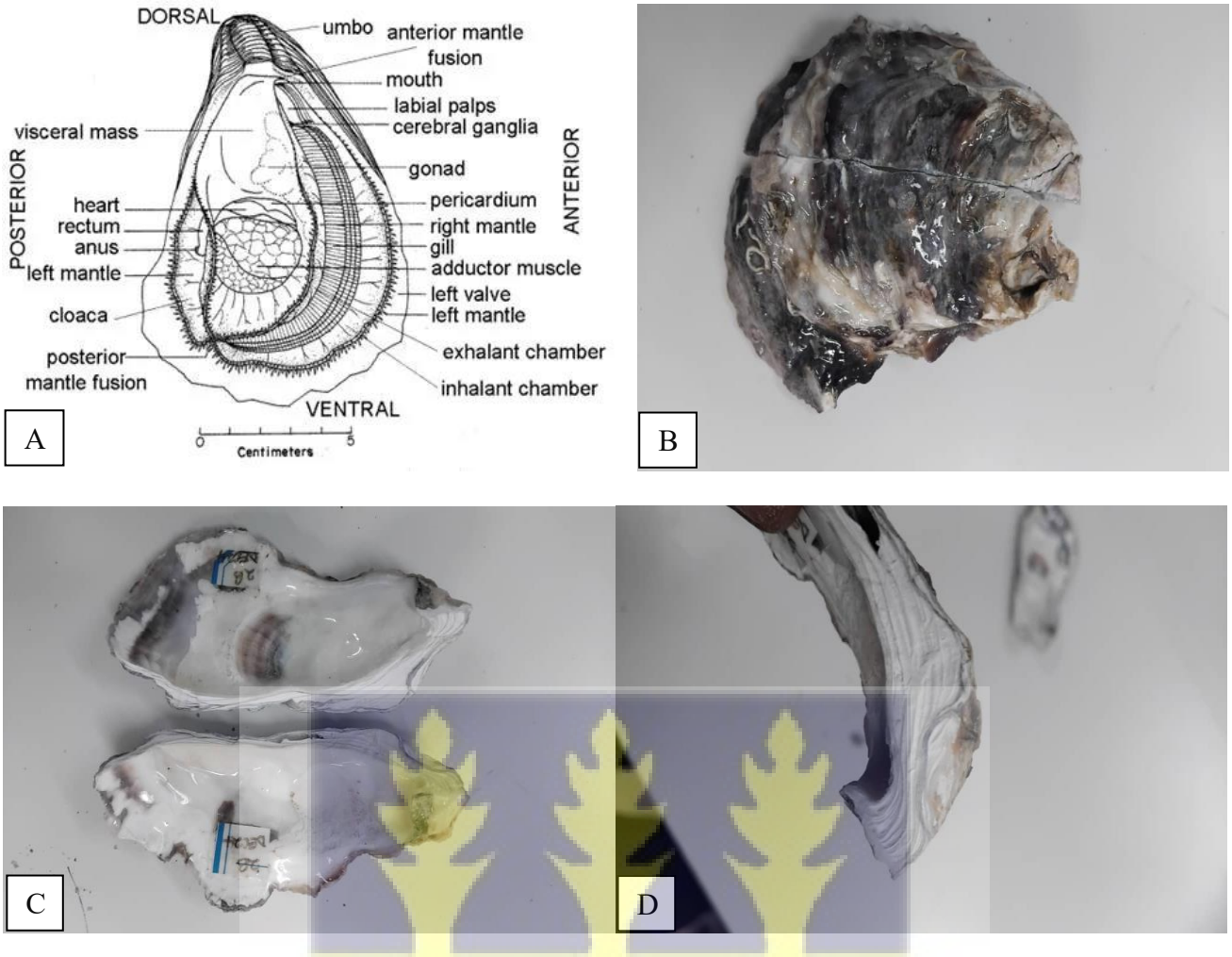


Figure 5: A diagram of Crassostrea tulipa indicating (A)A labelled diagram of the shell (B)the dorsal view of the shell (C) the shell was cut lengthwise from the hinge to the outer edge (D) the umbo of the left valve and sampling region

was diluted to 50 ml for analysis. Quality control measures included preparing reagent blanks and certified reference materials to validate accuracy and detect potential contamination. Samples were analysed for Mg/Ca ratios using a microwave plasma atomic emission spectrometer with an Agilent 4210 MP-AES instrument.

Figure 6: Shucking, cleaning, drying, sectioning, and drilling of oyster shells for laboratory analysis.

3.4 Geochemical Analysis

Powdered shell samples were analyzed for magnesium (Mg) and calcium (Ca) concentrations using the Agilent 4210 Microwave Plasma–Atomic Emission Spectrometer (MP-AES). Prior to analysis, samples were diluted to a uniform concentration of 100 ppm based on preliminary testing to ensure measurement consistency across samples. Calibration was performed using multi-element standard solutions and certified seawater reference standards, following protocols validated in previous shell geochemistry studies (e.g., Freitas et al., 2008; Surge & Lohmann, 2008). The Mg/Ca ratio was subsequently calculated from the measured concentrations, with care taken to ensure consistent treatment of all samples. The laboratory procedures followed standard geochemical protocols for trace metal analysis in biogenic carbonates (Freitas et al., 2011; Schleinkofer et al., 2021). The selection of the hinge region is consistent with methods employed in previous oyster paleoproxy studies (Surge &

Lohmann, 2008; Ubaldini et al., 2024). The instrument specifications and digestion protocols align with the recommendations from Agilent Technologies for marine biomineral analysis.

Figure 7. Summary of using the Agilent 4210 MP-AES to analyse for Mg and Ca ions in samples.

3.5 Data Processing and Statistical Analysis

Environmental data (temperature, salinity, and pH) collected at each station were first processed into weekly time-averaged values to account for short-term variability. These values were then aligned temporally with the oyster shell samples for integrated analysis. For data that followed a normal distribution, One-way Analysis of Variance (ANOVA) were applied to examine relationships and test for significant differences between groups. Correlation analyses were conducted to explore the relationships between Mg/Ca ratios in oyster shells and environmental variables (temperature, salinity, pH) at each of the three stations. Pearson correlation coefficients (r) were calculated to evaluate the strength and direction of these relationships, helping to assess the potential of Mg/Ca as a temperature proxy and to explore the influence of other factors. All statistical summaries, including mean, standard deviation, and correlation matrices, were generated using Microsoft Excel 2016. To further examine the predictive power of Mg/Ca ratios, four regression models were applied to compare different modelling approaches to understand the strengths and limitations of each. The linear models were included as baseline, while Ridge and Lasso addressed overfitting and variable selection. Random Forest captured non-linearity.

a. Linear Regression was used to determine the basic strength of linear relationships between

Mg/Ca and temperature.

b. Ridge Regression (a regularized linear model) was employed to address potential multicollinearity among environmental variables by penalizing large coefficients, leading to more robust predictions.

c. Lasso Regression was applied to perform variable selection by reducing less relevant coefficients to zero, identifying which environmental factors most significantly influence Mg/Ca ratios.

d. Random Forest Regression, a non-linear ensemble method, was used to capture complex, nonlinear interactions among variables, which are common in estuarine environments.

The dataset was split into a training set (70%) and a testing set (30%). Each model was trained on the training data and evaluated on the testing data using standard performance metrics: the correlation coefficient (R), root mean square error (RMSE) and mean absolute error (MAE). These metrics were used to compare model performance and determine the most suitable model for predicting temperature from Mg/Ca ratios in oyster shells. model's ability to capture the relationship between Mg/Ca ratios and temperature across the three stations.

CHAPTER FOUR
RESULTS

4.0 Results

Based on the objectives of this study, this chapter provides findings on the following:

1. The Mg/Ca ratios in the water and oyster shells (*C. tulipa*) across the three stations in the creek.
2. The relationship between the Mg/Ca ratio in the oyster shells and environmental conditions (temperature, salinity and pH).
3. Develop a predictive model using Mg/Ca ratios in oyster shells as a proxy for water temperature

4.1 Physicochemical conditions across the stations.

4.1.1 Variability in Temperature and Salinity across the three stations

Temperature variabilities across the Creek are shown in Figure 6. Lagoon-fed, located upstream, is primarily influenced by the inflow from the Keta Lagoon. This section is closer to the freshwater source, making its salinity, temperature, and pH quite distinct from the other stations. The temperature at Lagoon-fed ranged from 28.75 °C to 33.60 °C and generally followed the seasonal climatic patterns of the region. The mean temperature was 30.5 °C ± 0.15. However, it is important to note that readings were not taken at a fixed time of day. At Midstream, temperature was relatively stable, ranging from 28.87 °C to 33.00 °C. Ocean-fed recorded values between 29.12 °C and 32.88 °C. Changes in salinity across the study sites are shown in Figure 6. Salinity at Lagon-fed ranged from 3.64 to 18.04 PSU, with the highest value in March 2023 and a progressive decline through April 2024. Midstream exhibited moderate salinity levels, ranging from 4.45 to 14.19 PSU. Ocean-fed recorded the lowest and most variable salinity values, ranging from 2.95 PSU in September 2023 to 14.91 PSU in March 2023.

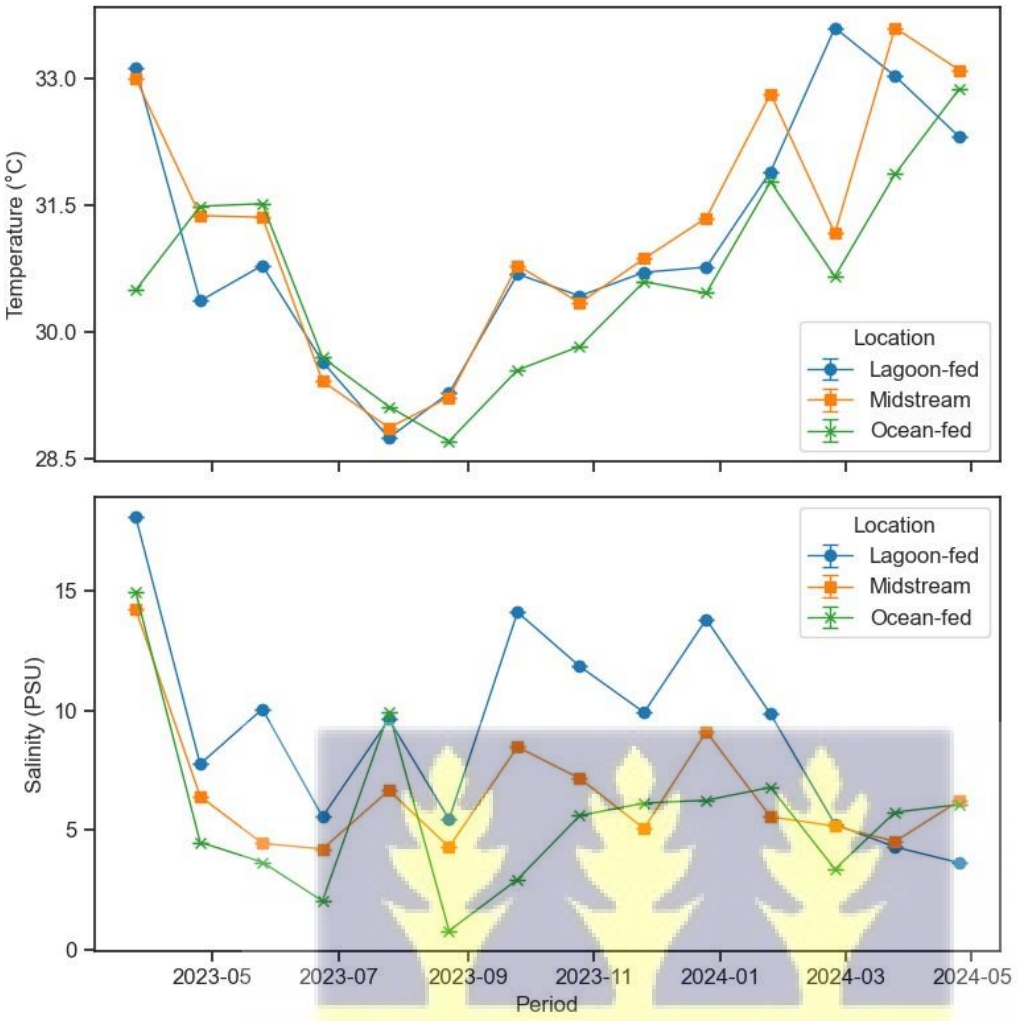


Figure 8. Monthly Variation in Surface Water Temperature and Salinity Across Lagoon-fed, Midstream, and Ocean-fed Stations in Anyanui Creek (March 2023 – April 2024)

4.1.2. Variability in pH conditions the three stations

Changes in pH across the study sites are shown in Figure 7. Lagoon-fed, pH values ranged from 7.12 to 8.57 and remained relatively stable throughout the study period. Midstream exhibited similar stability, with pH values ranging from 7.07 to 8.36. As a transitional zone, this station experienced fewer extreme fluctuations than the upstream or downstream sites. Ocean-fed, pH ranged from 7.20 to 8.44. Across all stations, pH values remained within the expected range for estuarine surface waters.

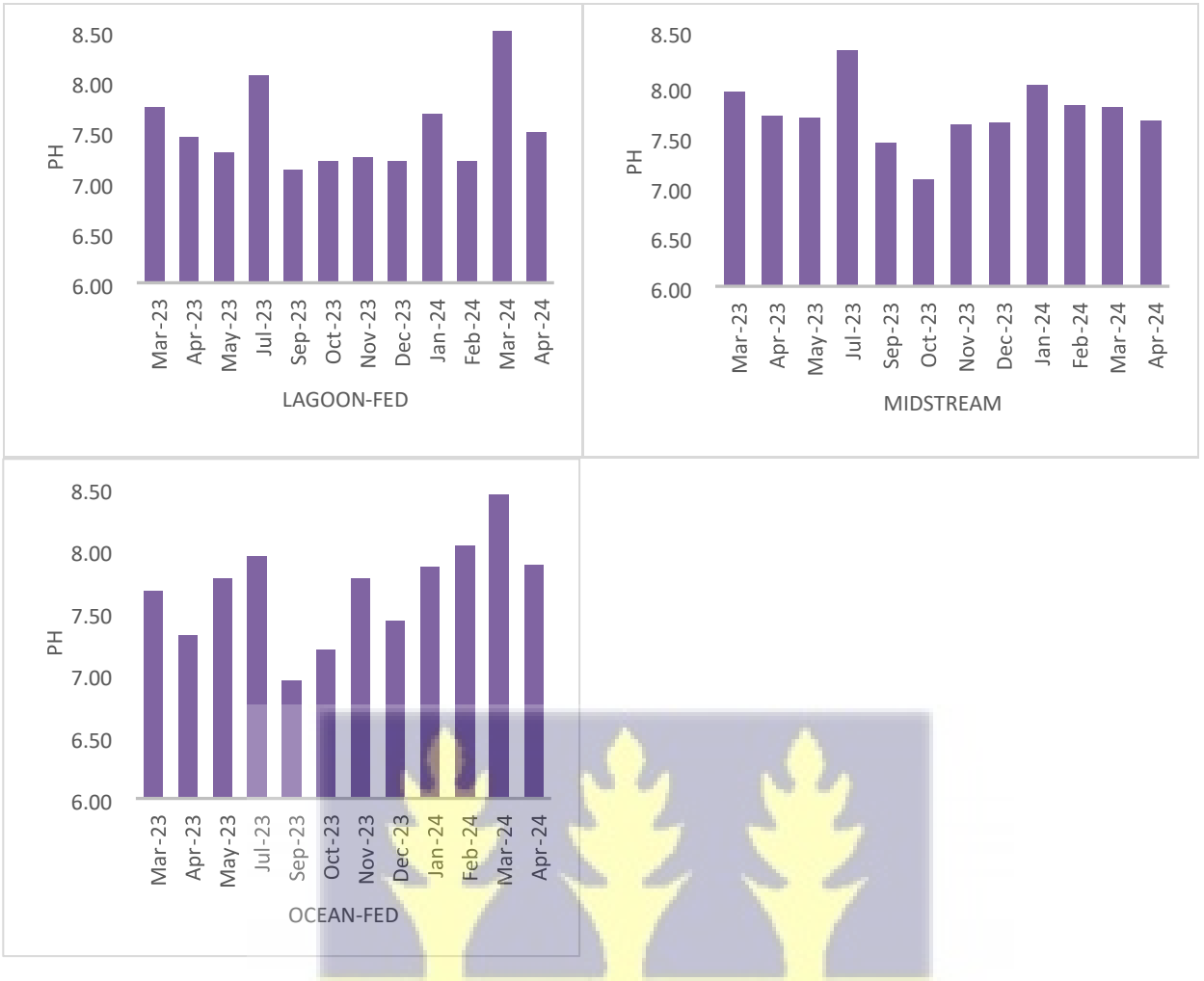


Figure 9. Monthly records of surface water pH at Lagoon-fed, Midstream, Ocean-fed along Anyanui Creek, Ghana, from March 2023 to April 2024.

4.2 Mg/Ca Ratios in Water and Shell Samples

4.2.1 Mg/Ca ratios in water and oyster shells across Stations

The Mg/Ca ratios in water samples from the three stations (Lagoon-fed, Midstream, and Ocean-fed at Anyanui Creek, shown in Figure 9, varied throughout the sampling period from March 2023 to April 2024. Mean Mg/Ca values were 2.12 ± 0.35 mmol/mol at Lagoon-fed, 2.07 ± 0.39 mmol/mol at Midstream and 2.11 ± 0.33 mmol/mol at Ocean-fed.

Figure 10. Monthly Mg/Ca ratios in surface water at three stations (Lagoon-fed: Lagoon-fed, Midstream: Midstream, Ocean-fed: Ocean-fed) along Anyanui Creek, Ghana, from March 2023 to April 2024

Table 2 shows that the means of the Mg/Ca ratios are quite similar across the three zones, indicating that, on average, the Mg/Ca ratios are consistent across the locations, with only minor differences. These values, as reflected in the ANOVA test (P-value: 0.98087), showed no significant differences between the stations, suggesting that Mg/Ca levels in the water were relatively consistent across the creek despite environmental fluctuations. This lack of statistical significance suggests that the Mg/Ca ratios are relatively consistent across the three zones when considering the natural variability within each zone.

Table 2: Anova of Mg:Ca in water from March 2023 through April 2024 at the three stations

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Lagoon-fed	36	76.21	2.12	1.39

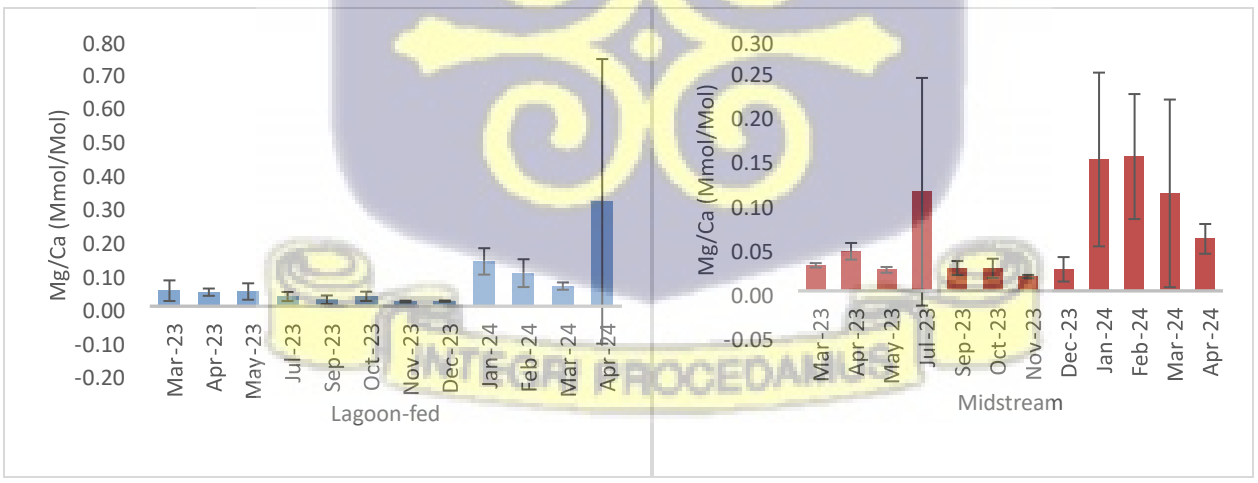
Midstream	36	74.35	2.07	1.74
Ocean-fed	36	75.99	2.11	1.31

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.06	2	0.03	0.02	0.98	3.08
Within Groups	155.41	105	1.48			
Total	155.47	107				

4.2.2 Mg/Ca ratios in oyster shells across the stations

Figure 10 illustrates the monthly Mg/Ca ratios measured in oyster shells over a 12-month period across the three stations at Anyanui Creek. At Lagoon-fed), Mg/Ca ratios ranged from 0.01 to 0.31 mmol/mol. The trend was generally low and consistent during the early months, with a gradual rise from January 2024 and a pronounced peak in April 2024. Midstream exhibited the highest values overall, ranging from 0.02 to 0.15 mmol/mol. Peaks were observed in July 2023 and sustained through early 2024, particularly in January and February. Ocean-fed maintained consistently low ratios throughout the sampling period, ranging from 0.01 to 0.25 mmol/mol. A noticeable spike occurred in September 2023, followed by a return to lower values.



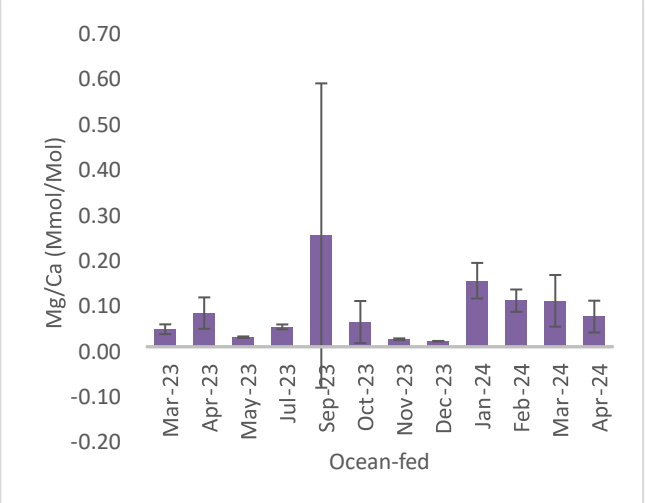


Figure 11. Monthly Mg/Ca ratios in oyster shells collected from three stations Lagoon-fed, Midstream, and Ocean-fed at Anyanui Creek, Ghana, from March 2023 to April 2024

From Table 3, the mean Mg/Ca ratios in oyster shells from the three stations (Lagoon-fed: 0.07 ± 0.02 mmol/mol, Midstream: 0.06 ± 0.01 mmol/mol, Ocean-fed: 0.08 ± 0.02 mmol/mol) were relatively low compared to the water samples. One-way ANOVA results indicated no statistically significant differences in shell Mg/Ca ratios among the stations ($p = 0.887$). Although mean values differed slightly, the overlapping standard deviations suggest that most of the variability in shell Mg/Ca occurred within stations rather than between them.

Table 3: ANOVA of Mg/Ca in water samples from March 2023 through April 2024 at the three stations

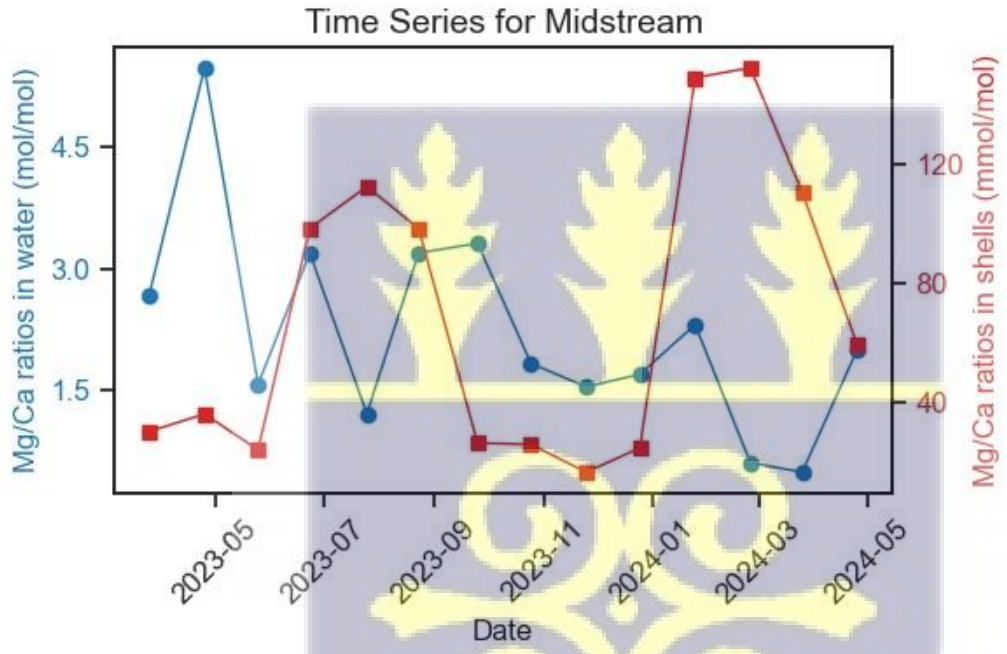
Groups	*Count*	*Sum*	*Average*	*Variance*
S 1	36	2.53	0.07	0.02
S 2	36	2.32	0.06	0.01
S 3	36	2.76	0.08	0.01

ANOVA

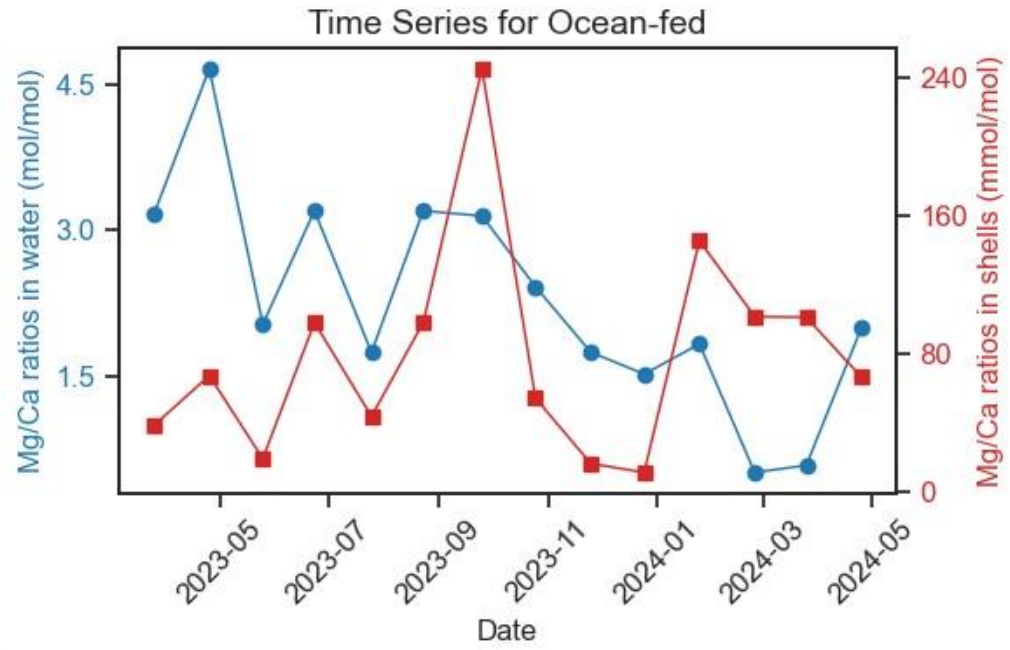
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.003	2	0.001	0.12	0.89	3.08
Within Groups	1.17	105	0.011			
Total	1.17	107				

4.2.3 Temporal Trends in Mg/Ca Ratios

Figure 11a shows the monthly variation in average Mg/Ca ratios in both water and oyster shells across all stations from March 2023 to April 2024. Mg/Ca ratios in water fluctuated more widely compared to shell values, which remained relatively stable over time. Figures 11b–d present the same trends for individual stations. In the lagoon-fed site (Figure 11b), water Mg/Ca values showed sharp temporal fluctuations, whereas shell values remained relatively constant. In the midstream zone (Figure 11c), both water and shell Mg/Ca ratios exhibited seasonal trends, with some overlap in peak periods. In the ocean-fed site (Figure 11d), Mg/Ca ratios in water and shell showed similar temporal patterns with



(c)



(d)

Figure 12. Timeseries trends in Mg/Ca ratios in shells and water. (a) Mean for the whole system, (b) Mean for Lagoon fed area, (c) Mean for Midstream, (d) Mean for Ocean fed area.

4.3. Shell Mg/Ca Ratios vs Temperature

Figure 12 illustrates the linear relationships between Mg/Ca ratios in Crassostrea tulipa shells and measured water temperatures across salinity regime. In Figure 5a, there was no statistically significant relationship between the Mg/Ca ratios in shells and water temperature ($R^2 = 0.0711$, $p > 0.05$).

The regression line $y = 0.041x - 1.1869$ indicates a negligible slope, suggesting that temperature accounted for only 1% of the variability in shell Mg/Ca ratios.

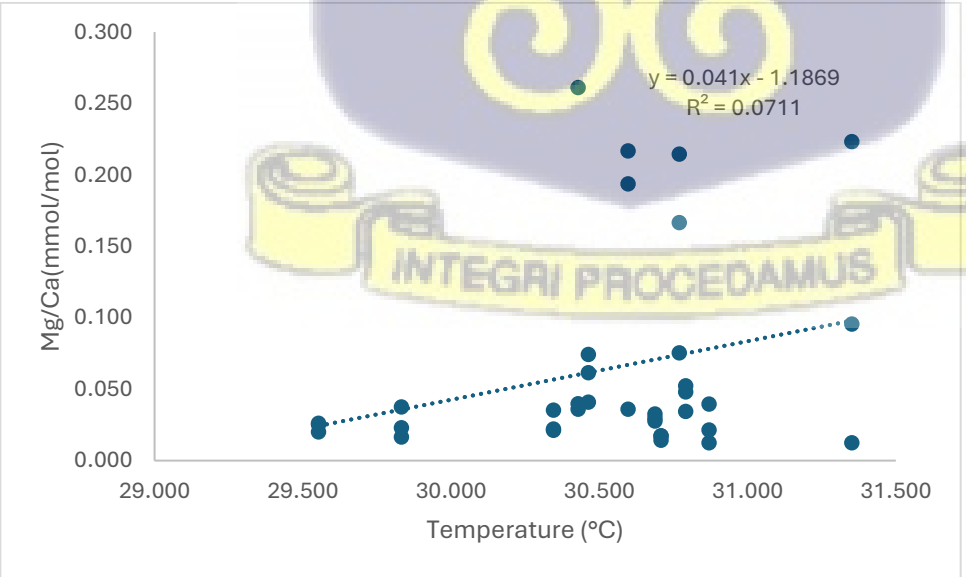


Figure. 13. Relationship between Mg/Ca ratios in shells and temperature.

4.4 Predictive model using Mg/Ca ratios in oyster shells at Lagoon-fed as proxy for water temperature

Different types of regression models using Mg/Ca ratios in oyster shells as proxy for water temperature are shown in Figure 11. The actual temperature data fluctuates throughout the period, with notable drops around July 2023 and January 2024. It shows a peak at certain intervals (April 2023 and April 2024). Linear Regression, Ridge Regression, and Lasso Regression models show consistent, flat predictions across the timeline, averaging around 31°C, irrespective of the observed fluctuations in actual temperature. These models show nearly identical predictions that are quite stable and flat across the timeline, hovering close to 31°C. Random Forest displays more variance in its predictions and attempts to follow the trend of the actual temperature more closely, although it does not fully capture extreme fluctuations seen in July 2023 and April 2024. Random Forest offers a more responsive prediction that partially captures the peaks and troughs in actual temperature data, though it still falls short in accurately mirroring sharp temperature declines, particularly around July 2023.

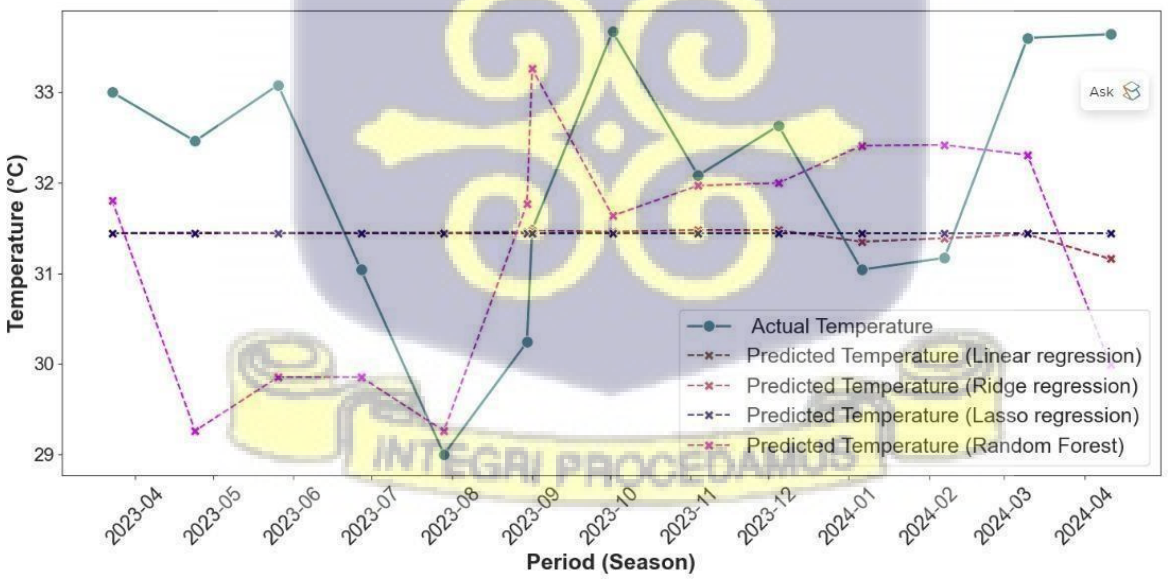


Figure 14. Observed and Predicted Water Temperatures (April 2023–April 2024): Comparison of actual temperature measurements with predictions generated by four machine learning models, Linear Regression, Ridge Regression, Lasso Regression, and Random Forest.

CHAPTER FIVE
DISCUSSIONS

5.0 Discussion

This study investigated the potential of Mg/Ca ratios in Crassostrea tulipa shells as a proxy for water temperature in the Anyanui Creek environment. Over a 12-month sampling period, Mg/Ca ratios were analysed in both oyster shells and water samples from three stations representing lagoon-fed, midstream, and ocean-fed zones. Environmental parameters including temperature, salinity, and pH were monitored concurrently. The relationship between environmental variables temperature, salinity, and pH and Mg/Ca ratios in both water and oyster shells were explored to assess if the shells could serve as reliable indicators of historical water temperature changes. Here, we discuss the findings in relation to the research objectives, while also comparing with existing literature on similar studies.

5.1 Temperature, salinity and pH Influence on Mg/Ca Ratios in Crassostrea tulipa across the stations

Temperature, salinity, and pH varied both spatially and temporally across the three sampling stations, reflecting the dynamic nature of the Anyanui Creek system. These parameters were influenced by seasonal rainfall patterns, freshwater inflows, tidal exchange, and anthropogenic disturbances, such as the Volta River dam spillage and the formation of a sandbar in March 2023. Temperature ranged from approximately 28.7 °C to 33.6 °C across all stations, with peaks typically observed during the dry season (March 2023 – April 2024) and dips during periods of increased rainfall. While temperature variation was modest, it still exhibited a clear seasonal pattern consistent with tropical coastal climate regimes. Upstream experienced slightly higher fluctuations, possibly due to reduced water volume and buffering capacity compared to the midstream and downstream zones. However, temperature was not consistently measured at the same time of day, which introduces some diurnal variability into the dataset.

Salinity showed the most pronounced spatial and temporal variation, ranging from as low as 2.95 PSU at the ocean-fed station to as high as 18.04 PSU at the lagoon-fed. The closure of the tidal inlet by a sandbar in March 2023 significantly altered the dynamics of the system, especially downstream. Prior to this event, downstream was strongly marine influenced, but afterward, its salinity levels declined

and began resembling those at the midstream station, indicating reduced seawater intrusion and enhanced freshwater dominance. This hydrological shift likely influenced other physicochemical parameters and the geochemical signatures in oyster shells.

pH values across all stations were generally within the neutral to slightly alkaline range (6.8 to 8.6), with minor spatial variability. Slightly lower pH readings were observed upstream, likely due to increased organic matter and reduced tidal flushing, which can promote acidification through microbial respiration and decomposition. After the sandbar formation, pH downstream stabilized, consistent with reduced tidal exchange and a shift toward more lagoon-like conditions.

Overall, the observed environmental gradients across the creek are consistent with what is expected in tropical aquatic systems, where mixing zones between freshwater and marine inputs generate steep physicochemical gradients (Elliott & Whitfield, 2011; Cloern et al., 2017). These gradients not only affect water chemistry but also influence biological processes such as calcification, elemental uptake, and shell growth in oysters (Dissard et al., 2009; Goodwin et al., 2013). Understanding these spatial and temporal patterns is essential when interpreting geochemical proxy signals like Mg/Ca in biogenic carbonates.

5.2 Mg/Ca Ratios in Water vs Shells

At upstream, the mean Mg/Ca ratio in water at this station was 2.12 ± 0.35 mmol/mol, with relatively stable values throughout the study period, although there were some fluctuations. The mean Mg/Ca ratio at midstream was 2.07 ± 0.39 mmol/mol, which was slightly lower than that of upstream. This part of the creek acts as a transitional zone, balancing inputs from both upstream freshwater sources and downstream ocean influences. The Mg/Ca ratio in water downstream was recorded at 2.11 ± 0.33 mmol/mol. Across the three stations, the ANOVA analysis revealed no statistically significant difference in Mg/Ca ratios in water between stations (p-value: 0.98087). This finding suggests that despite environmental variability, the Mg/Ca ratios across the stations remained relatively consistent throughout the study period. The mean Mg/Ca ratios in oyster shells from the three stations

(downstream 0.08 mmol/mol, midstream 0.06 mmol/mol, upstream 0.07 mmol/mol) were relatively low compared to the water samples, with no statistically significant differences across the stations (ANOVA P-value: 0.886595).

Water samples collected across the three stations recorded higher Mg/Ca ratios (mean ≈ 2.10 mmol/mol) compared to oyster shells, which exhibited much lower ratios (mean ≈ 0.07 mmol/mol). This pattern is consistent with previous studies that have reported a reduction in Mg content during biological calcification relative to ambient water chemistry (Freitas et al., 2011; Cao et al., 2023). The Mg/Ca ratio in seawater is typically stable on short timescales; however, biological calcifiers such as oysters do not passively incorporate magnesium in proportion to its availability in the surrounding water. The observed discrepancy between water and shell Mg/Ca ratios may be attributed to a combination of abiotic and biotic processes. Firstly, oysters precipitate low-Mg calcite as their primary shell mineral, and the thermodynamics of calcite formation favour lower Mg incorporation compared to aragonite or high-Mg calcite phases (Chave, 1954; Elderfield & Ganssen, 2000). Secondly, the process of biomineralization in mollusks is biologically mediated, with oysters exerting physiological control over the ionic composition of the extrapallial fluid where calcification occurs. This compartment is chemically distinct from ambient seawater and is regulated via active ion transport mechanisms, carbonic anhydrase activity, and shell matrix proteins (Addadi & Weiner, 1985; Lee et al., 2011; Huang & Zhang, 2022). These controls allow oysters to buffer against external variability and selectively incorporate elements into their shell. As such, Mg incorporation is not solely a reflection of environmental concentrations, but also of species-specific biological factors including growth rate, metabolic activity, and ontogenetic stage. The lower Mg/Ca values in *C. tulipa* shells observed in this study likely reflect a combination of mineralogical bias toward calcite and active biological discrimination against Mg uptake. The minor variability seen in shell Mg/Ca ratios, despite much higher values in water, underscores the importance of understanding calcification physiology when interpreting elemental proxy records. This finding aligns with results from other estuarine bivalve studies that reported decoupling between water chemistry and shell composition (Schleinkofer

et al., 2021; Surge & Lohmann, 2008). Freitas et al. (2011) observed similarly low shell Mg/Ca ratios in *Acesta excavata*, despite elevated ambient Mg levels, attributing the difference to strong physiological filtering. Thus, the relatively low and stable Mg/Ca values in *C. tulipa* shells from Anyanui Creek provide further evidence that this species may tightly regulate shell chemistry, complicating the direct use of Mg/Ca as a simple temperature proxy without accounting for these internal controls.

5.3 Evaluation of Predictive Regression Models Using Shell Mg/Ca Ratios

Figure 13 illustrates the performance of four regression models Linear Regression, Ridge Regression, Lasso Regression, and Random Forest in predicting water temperature based on Mg/Ca ratios in *Crassostrea tulipa* shells from April 2023 to April 2024. The primary objective of this study was to evaluate whether Mg/Ca ratios in *Crassostrea tulipa* shells could serve as reliable proxies for reconstructing estuarine water temperature. To explore this potential, Mg/Ca ratio was considered the dependent variable and temperature the independent (predictor) variable in the regression analysis. However, because no species-specific or regional calibration equation exists for *C. tulipa* in West African estuaries, this analysis is exploratory in nature. It seeks to identify whether a statistically significant relationship exists that could justify future calibration efforts (Rosenthal et al., 2022). These models were trained on Mg/Ca ratios as a primary proxy for temperature, aiming to capture the influence of environmental variables on shell chemistry. The actual observed temperature fluctuates markedly across the sampling period, with notable drops in July 2023 and January 2024, and peaks around April 2023 and April 2024.

The linear models provide stable, flat predictions, hovering around 31°C, regardless of fluctuations in actual temperature data. This flat response indicates that these models likely failed to capture the non-linear relationship between Mg/Ca ratios and temperature, a common issue with linear regression when applied to complex environmental data (Xiao et al., 2019). In contrast, the Linear, Ridge, and Lasso regression models produce almost identical flat predictions averaging around 31°C throughout the period. These models fail to capture the temporal dynamics and respond poorly to seasonal variability.

This is not surprising given the earlier correlation and regression analysis which showed that Mg/Ca ratios in oyster shells have a weak and statistically insignificant linear relationship with water temperature. The poor model responsiveness suggests that Mg/Ca in *C. tulipa* may not be a strong predictor of temperature in this estuarine context—likely due to vital effects, salinity-mediated incorporation, or shell diagenesis (Gillikin et al., 2006; Surge & Lohmann, 2008).

Notably, the Random Forest model shows relatively better performance, with predictions that more closely follow the general trend of actual temperature changes. It partially captures the declining trend around July 2023 and the rising trend towards April 2024, albeit not perfectly. This improvement is likely due to Random Forest's non-linear learning capacity, allowing it to detect subtle interactions and non-linearities between shell geochemistry and environmental conditions. However, even the Random Forest model falls short of fully replicating extreme fluctuations seen in the observed temperature series, particularly the sharp trough in July 2023 and the rise in early 2024. This reinforces the earlier statistical finding that while shell Mg/Ca contains some signal of environmental temperature, it may be confounded by biological regulation and other environmental factors such as salinity and pH, which were not accounted for in the univariate models. The overall poor performance of the linear models and the limited success of Random Forest suggest that shell Mg/Ca alone may not be a robust or reliable proxy for reconstructing water temperature in tropical estuarine systems. It is possible that multi-variable models, incorporating salinity, pH, and possibly Sr/Ca or stable isotopes (e.g., δ18O), would yield more reliable reconstructions (Schöne et al., 2010).

5.4 Implications for Using Mg/Ca Ratios as a Proxy for Water Temperature

The findings of this study have important implications for the use of Mg/Ca ratios in tropical bivalves as proxies for paleoenvironmental reconstructions. The weak correlation between temperature and Mg/Ca in this study raises questions about the reliability of using this ratio as a standalone temperature proxy in tropical estuarine environments. This contrasts with its more established use in temperate marine systems, where stronger corelation has been established temperature-driven Mg incorporation. While Mg/Ca ratios in temperate bivalves have proven effective for reconstructing past temperatures,

the weak and variable relationships observed in *C. tulipa* suggest that this proxy may be less reliable in tropical systems. Instead, Mg/Ca ratios in tropical bivalves may reflect a combination of temperature, salinity, and local environmental conditions, making them more suitable for reconstructing estuarine dynamics rather than specific temperature records.

These results align with observations by Gillikin et al. (2005), who suggested that tropical and estuarine bivalves may exhibit a more complex relationship between Mg/Ca and environmental conditions, making them less suitable for simple temperature reconstructions. Future research in tropical systems like Anyanui Creek, multiple environmental factors including salinity, pH, and biological processes should be considered when interpreting Mg/Ca data or explore other alternative geochemical proxies, such as Sr/Ca or oxygen isotopes, which have shown more consistent temperature dependencies in both temperate and tropical molluscs (Schöne & Fiebig, 2008).

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.0 Conclusion

The study explored the potential of using Mg/Ca ratios in the shells of *Crassostrea tulipa*, the West African mangrove oyster, as proxies for water temperature in Anyanui Creek's estuarine environment. Conducted across three stations, Lagoon-fed, Midstream, and Ocean-fed, each site featured distinct environmental conditions influenced by freshwater inputs from the Keta Lagoon and marine influences from the Gulf of Guinea. Key environmental variables such as temperature, salinity, and pH were monitored to examine their influence on Mg/Ca ratios in both water and oyster shells through comprehensive fieldwork, laboratory analysis, and statistical evaluation. Findings suggest that while Mg/Ca ratios provide insights into environmental conditions, their application as temperature proxies in tropical estuarine systems like Anyanui is complicated by additional environmental influences. Mg/Ca ratios in the water were relatively consistent across the three stations, with mean values around 2.1 ± 0.33 mmol/mol. However, there were no statistically significant differences between the stations, indicating similar water chemistry despite hydrological disruptions, such as the Akosombo Dam spillage and sandbar formation. This consistency suggests that large-scale hydrological factors influence the entire system rather than localized processes. In contrast, Mg/Ca ratios in *Crassostrea tulipa* oyster shells exhibited more variability, with mean values ranging from 0.06 to 0.08 mmol/mol across the three stations. While lower than in the water, these ratios followed a similar trend of relative consistency between stations, suggesting that the oysters' physiological processes may buffer against environmental fluctuations, regulating Mg incorporation into their shells despite variability in external water conditions. The findings suggest that Mg/Ca ratios in *Crassostrea tulipa* shells may serve as a useful, albeit limited, proxy for water temperature in estuarine environments like Anyanui Creek. However, several factors need to be considered when interpreting Mg/Ca ratios in relation to temperature, including the influences of salinity, pH, and other environmental conditions. Furthermore, while this study focused on present-day relationships between shell geochemistry and environmental

parameters, the findings offer cautious implications for paleoenvironmental reconstruction. The generally weak and variable correlation between Mg/Ca ratios and temperature especially in field collected samples from a dynamic tropical estuary suggests that Mg/Ca alone may not serve as a robust paleotemperature proxy in such systems. The study does not claim to have generated a fully validated temperature calibration model for *C. tulipa* in tropical estuaries. Rather, this research serves as a foundational assessment of the potential of Mg/Ca ratios as a paleoenvironmental indicator, specifically, examining whether such ratios correlate with in-situ temperature and salinity data over an annual cycle. This distinction is crucial. In temperate regions, several studies (e.g., Surge & Lohmann, 2008; Schöne et al., 2010) have demonstrated strong Mg-temperature relationships in bivalve shells. In contrast, this study highlights the limitations of directly transferring these assumptions to tropical estuarine settings particularly where salinity fluctuations, ontogenetic shell growth, and shell mineralogy introduce complexity. Thus, while Mg/Ca ratios cannot yet serve as a robust quantitative proxy in *C. tulipa*, this work contributes original empirical data and methodological insights to advance understanding in this field.

6.1 Recommendation

To enhance understanding of Mg/Ca ratios as a proxy for water temperature in tropical estuarine environments, several future research avenues are suggested:

1. Controlled Laboratory Experiments: Conduct experiments using *Crassostrea tulipa* under controlled conditions to isolate temperature effects on Mg/Ca ratios. This includes manipulating temperature while keeping salinity and pH constant, and vice versa, to understand their individual and combined impacts.

2. Expanded Shell Analysis: Examine different parts of the oyster shell, such as growth bands and hinges, which might record environmental conditions differently. This could identify areas that more reliably record temperature signals. Long-Term Monitoring: Implement continuous monitoring programs to track water chemistry and oyster shell composition over time. This involves using

automated sensors to gather high-resolution environmental data paired with regular oyster shell samples, capturing seasonal and interannual variability.

3. Comparative Studies Across Estuaries: Replicate the study in other estuarine environments with varying salinity regimes, climate patterns, and biological communities. This helps identify common trends and unique factors affecting Mg/Ca ratios

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APPENDICES

A table showing the Mg/Ca ratios in the water across a period of 12 months at the three stations

Mg/Ca RATIOS IN WATER						
	Stations	Mg Conc(ppm)	Mg (mmol/l)	Ca Conc(ppm)	Ca(mol/L)	Mg/Ca(mmol/mol)
Mar-23	S1a	1063.98	43.78	660.00	0.016	2.66
	S1b	1062.19	43.70	510.56	0.013	3.43
	S1c	1052.00	43.28	508.72	0.013	3.41
	S2a	1064.95	43.82	532.33	0.013	3.30
	S2b	1038.31	42.72	1067.08	0.027	1.60
	S2c	1055.33	43.42	554.93	0.014	3.14
	S3a	1227.29	50.50	657.78	0.016	3.08
	S3b	1238.84	50.97	663.93	0.017	3.08
	S3c	1250.31	51.44	607.31	0.015	3.39
Apr-23	S1a	377.79	15.544	162.18	0.004	3.84
	S1b	381.12	15.681	115.10	0.003	5.46
	S1c	392.62	16.154	138.59	0.003	4.67
	S2a	518.33	21.326	155.91	0.004	5.48
	S2b	515.00	21.189	152.95	0.004	5.55
	S2c	514.11	21.152	157.13	0.004	5.40
	S3a	589.61	24.259	199.23	0.005	4.88
	S3b	594.47	24.459	219.50	0.005	4.47
	S3c	596.03	24.523	219.93	0.005	4.47
May-23	S1a	274.09	11.277	229.92	0.006	1.97
	S1b	229.29	9.434	176.15	0.004	2.15
	S1c	279.36	11.494	232.78	0.006	1.98
	S2a	315.43	12.978	285.47	0.007	1.82
	S2b	384.51	15.820	460.20	0.011	1.38
	S2c	414.05	17.036	447.31	0.011	1.53
	S3a	844.67	34.753	818.79	0.020	1.70
	S3b	864.53	35.570	791.73	0.020	1.80
	S3c	860.03	35.385	822.39	0.021	1.72
	S1a	747.04	30.736	742.49	0.019	1.66
	S1b	775.48	31.906	700.93	0.017	1.82
	S1c	791.28	32.556	749.37	0.019	1.74

Jul-23	S2a	464.24	19.101	607.33	0.015	1.26
	S2b	453.40	18.655	639.52	0.016	1.17
	S2c	463.85	19.085	627.82	0.016	1.22
	S3a	536.53	22.075	669.08	0.017	1.32
	S3b	467.19	19.222	650.51	0.016	1.18
	S3c	535.54	22.034	732.69	0.018	1.21
Sep-23	S1a	119.65	4.923	57.94	0.001	3.41
	S1b	290.82	11.965	134.85	0.003	3.56
	S1c	124.79	5.134	83.31	0.002	2.47

	S2a	387.05	15.925	182.48	0.005	3.50
	S2b	330.92	13.615	166.89	0.004	3.27
	S2c	314.61	12.944	163.29	0.004	3.18
	S3a	543.62	22.367	298.17	0.007	3.01
	S3b	561.54	23.104	293.73	0.007	3.15
	S3c	544.43	22.400	273.63	0.007	3.28
Oct-23	S1a	278.55	11.461	196.63	0.005	2.34
	S1b	293.63	12.081	200.86	0.005	2.41
	S1c	315.65	12.987	207.72	0.005	2.51
	S2a	306.91	12.627	303.23	0.008	1.67
	S2b	309.17	12.720	251.56	0.006	2.03
	S2c	318.17	13.091	289.12	0.007	1.81
	S3a	502.32	20.667	375.33	0.009	2.21
	S3b	556.65	22.903	396.01	0.010	2.32
	S3c	574.86	23.652	387.64	0.010	2.45
Nov-23	S1a	227.52	9.36	229.01	0.006	1.64
	S1b	223.53	9.20	221.45	0.006	1.66
	S1c	194.20	7.99	166.18	0.004	1.93
	S2a	239.38	9.85	273.83	0.007	1.44
	S2b	215.70	8.87	228.59	0.006	1.56
	S2c	206.75	8.51	207.17	0.005	1.65
	S3a	355.66	14.63	355.78	0.009	1.65
	S3b	378.49	15.57	526.38	0.013	1.19
	S3c	363.98	14.98	398.93	0.010	1.50
	S1a	222.12	9.14	221.13	0.006	1.66
	S1b	239.01	9.83	267.08	0.007	1.48

Dec-23	S1c	236.67	9.74	274.51	0.007	1.42
	S2a	385.94	15.88	358.11	0.009	1.78
	S2b	384.44	15.82	353.38	0.009	1.79
	S2c	370.63	15.25	397.43	0.010	1.54
	S3a	555.78	22.87	531.73	0.013	1.72
	S3b	595.77	24.51	564.04	0.014	1.74
	S3c	556.75	22.91	542.97	0.014	1.69
Jan-24	S1a	306.36	12.60	254.86	0.006	1.98
	S1b	317.59	13.07	293.57	0.007	1.78
	S1c	237.08	9.75	227.79	0.006	1.72
	S2a	456.77	18.79	323.50	0.008	2.33
	S2b	366.90	15.10	250.02	0.006	2.42
	S2c	358.65	14.76	269.80	0.007	2.19
	S3a	530.94	21.84	314.59	0.008	2.78
	S3b	559.94	23.04	298.28	0.007	3.10
	S3c	487.99	20.08	258.21	0.006	3.12
Feb-24	S1a	112.93	4.65	348.27	0.009	0.53
	S1b	114.23	4.70	362.77	0.009	0.52
	S1c	128.28	5.28	453.47	0.011	0.47
	S2a	194.74	8.01	476.24	0.012	0.67
	S2b	199.83	8.22	575.02	0.014	0.57
	S2c	200.35	8.24	555.93	0.014	0.59
	S3a	219.17	9.02	686.35	0.017	0.53
	S3b	207.38	8.53	567.09	0.014	0.60
	S3c	201.95	8.31	593.87	0.015	0.56
Mar-24	S1a	190.86	7.85	540.18	0.013	0.58
	S1b	181.47	7.47	514.46	0.013	0.58
	S1c	191.14	7.86	546.18	0.014	0.58
	S2a	169.84	6.99	573.18	0.014	0.49
	S2b	182.96	7.53	577.80	0.014	0.52
	S2c	166.02	6.83	567.94	0.014	0.48
	S3a	162.71	6.69	634.63	0.016	0.42
	S3b	171.56	7.06	678.65	0.017	0.42
	S3c	159.28	6.55	643.29	0.016	0.41
	S1a	164.25	6.76	127.62	0.003	2.12

Apr-24	S1b	135.67	5.58	106.95	0.003	2.09
	S1c	132.27	5.44	122.52	0.003	1.78
	S2a	134.86	5.55	123.62	0.003	1.80
	S2b	237.87	9.79	189.09	0.005	2.07
	S2c	253.41	10.43	193.73	0.005	2.16
	S3a	251.18	10.33	205.44	0.005	2.02
	S3b	248.61	10.23	200.73	0.005	2.04
	S3c	235.11	9.67	192.63	0.005	2.01

A table showing the Mg/Ca ratios in the water across a period of 12 months at the three stations

Mg/Ca RATIOS IN OYSTER SHELLS						
	Stations	Mg Conc(ppm)	Mg (mmol/l)	Ca Conc(ppm)	Ca(mol/L)	Mg/Ca(mmol/mol)
Mar-23	S1a	17751.36	730.36	649272.88	16.200	0.05
	S1b	13577.25	558.62	500350.36	12.484	0.04
	S1c	20371.18	838.15	1283898.70	32.035	0.03
	S2a	12289.60	505.64	731498.88	18.252	0.03
	S2b	10422.31	428.81	580905.14	14.494	0.03
	S2c	12733.76	523.92	644374.29	16.078	0.03
	S3a	17328.44	712.96	920321.48	22.963	0.03
	S3b	35326.95	1453.48	715172.41	17.845	0.08
	S3c	10138.59	417.14	645640.11	16.110	0.03
Apr-23	S1a	18904.82	777.816	418788.10	10.449	0.07
	S1b	14727.84	605.959	613528.14	15.308	0.04
	S1c	10913.89	449.039	165295.24	4.124	0.11
	S2a	23274.05	957.583	799667.77	19.953	0.05
	S2b	45907.42	1888.806	1447709.52	36.122	0.05
	S2c	26176.40	1076.997	1259929.72	31.437	0.03
	S3a	21145.73	870.016	885139.41	22.085	0.04
	S3b	12505.36	514.518	384833.08	9.602	0.05
	S3c	13333.66	548.597	680772.64	16.986	0.03
	S1a	47002.09	1933.844	3673694.43	91.664	0.02
	S1b	14964.08	615.679	1396481.45	34.844	0.02

May-23	S1c	26003.56	1069.885	2192016.72	54.694	0.02
	S2a	31613.56	1300.702	2065564.71	51.539	0.03
	S2b	25526.79	1050.269	2097369.98	52.332	0.02
	S2c	26183.01	1077.268	1655470.29	41.306	0.03
	S3a	36835.48	1515.552	3708253.09	92.526	0.02
	S3b	53264.79	2191.516	1360522.19	33.947	0.06
	S3c	42345.69	1742.262	1467345.25	36.612	0.05
Jul-23	S1a	10614.57	436.724	350507.87	8.746	0.05
	S1b	15740.61	647.628	655182.20	16.348	0.04
	S1c	13146.51	540.897	513320.01	12.808	0.04
	S2a	16313.00	671.179	676951.16	16.891	0.04
	S2b	14343.61	590.151	659929.88	16.466	0.04
	S2c	18829.56	774.720	118933.82	2.968	0.26
	S3a	11382.82	468.332	1016330.25	25.359	0.02
	S3b	15549.37	639.760	583602.99	14.562	0.04
	S3c	24409.18	1004.286	1690008.16	42.168	0.02
Sep-23	S1a	9710.92	399.544	777743.06	19.406	0.02
	S1b	17377.15	714.962	339281.42	8.466	0.08
	S1c	46705.15	1921.627	122020.72	3.045	0.63
	S2a	14966.86	615.793	1117855.51	27.892	0.02
	S2b	25969.94	1068.502	1211125.50	30.219	0.04
	S2c	14351.87	590.490	1124834.15	28.066	0.02
	S3a	14351.87	590.490	849106.66	21.186	0.03
	S3b	20803.94	855.953	1345802.41	33.580	0.03
	S3c	3295.84	135.603	1128989.63	28.170	0.00
Oct-24	S1a	44179.38	1817.707	674010.86	16.817	0.11
	S1b	59295.63	2439.647	3300075.22	82.341	0.03
	S1c	20760.81	854.179	1319483.14	32.923	0.03
	S2a	14199.44	584.219	1023414.82	25.536	0.02
	S2b	20037.41	824.415	880764.79	21.976	0.04
	S2c	15752.77	648.129	1585041.67	39.549	0.02
	S3a	54008.66	2222.121	1991018.63	49.679	0.04
	S3b	7250.65	298.319	665736.89	16.611	0.02
	S3c	13235.06	544.541	848413.75	21.169	0.03
	S1a	14514.46	597.180	1290783.33	32.207	0.02
	S1b	12974.66	533.827	1466097.85	36.581	0.01

Nov-23	S1c	17067.60	702.226	1702445.19	42.478	0.02
	S2a	8154.27	335.498	771556.55	19.251	0.02
	S2b	24393.36	1003.635	2818756.42	70.332	0.01
	S2c	21825.48	897.983	2093564.01	52.237	0.02
	S3a	5807.83	238.956	820045.70	20.461	0.01
	S3b	12834.45	528.058	1260125.72	31.442	0.02
	S3c	13545.68	557.321	1727385.02	43.101	0.01
Dec-23	S1a	8463.08	348.203	1145880.06	28.591	0.01
	S1b	13639.29	561.172	2071312.05	51.682	0.01
	S1c	11201.82	460.885	1662534.55	41.482	0.01
	S2a	13584.24	558.907	1819169.78	45.391	0.01
	S2b	25079.04	1031.847	1925730.02	48.050	0.02
	S2c	87940.66	3618.213	3670280.65	91.578	0.04
	S3a	20659.76	850.021	2347769.92	58.580	0.01
	S3b	29984.39	1233.672	2823768.39	70.457	0.02
	S3c	21604.35	888.885	2619858.61	65.369	0.01
Jan-24	S1a	645821.68	26571.556	9152961.58	228.379	0.12
	S1b	4310296.91	177341.984	37353063.61	932.009	0.19
	S1c	1509278.64	62097.455	19052549.06	475.387	0.13
	S2a	65521.89	2695.819	3000735.46	74.872	0.04
	S2b	1394212.78	57363.208	11871522.77	296.210	0.19
	S2c	3949133.33	162482.342	30051099.70	749.815	0.22
	S3a	4053060.51	166758.301	40331130.66	1006.316	0.17
	S3b	291454.14	11991.530	5361535.90	133.778	0.09
	S3c	997564.78	41043.603	11376324.83	283.855	0.14
Feb-24	S1a	448487.13	18452.464	7377928.20	184.089	0.10
	S1b	154886.22	6372.607	3281616.72	81.881	0.08
	S1c	12018667.35	494493.617	156004175.82	3892.514	0.13
	S2a	1110550.62	45692.270	24330956.05	607.090	0.08
	S2b	272799.38	11224.002	2701403.43	67.404	0.17
	S2c	2332171.74	95954.402	17923438.23	447.214	0.21
	S3a	382332.29	15730.602	7140213.54	178.158	0.09
	S3b	374795.03	15420.491	4296942.85	107.215	0.14
	S3c	412009.42	16951.632	10806028.20	269.625	0.06
	S1a	687944.06	28304.631	7647314.98	190.811	0.15

Mar-24	S1b	306587.95	12614.193	4294759.75	107.160	0.12
	S1c	134118.93	5518.162	5837133.45	145.644	0.04
	S2a	6150613.37	253059.591	45415983.22	1133.190	0.22
	S2b	25231.63	1038.125	3353383.16	83.671	0.01
	S2c	409399.45	16844.248	7070152.40	176.410	0.10
	S3a	100387.17	4130.309	2969257.74	74.087	0.06
	S3b	287045.18	11810.129	6561178.94	163.710	0.07
	S3c	450372.41	18530.031	14417297.71	359.731	0.05
Apr-24	S1a	1627023.25	66941.915	25121440.95	626.814	0.11
	S1b	437152.81	17986.127	14219622.78	354.799	0.05
	S1c	263765.50	10852.314	10296904.32	256.922	0.04
	S2a	1487286.79	61192.627	39825283.65	993.694	0.06
	S2b	82668.88	3401.312	1835422.38	45.796	0.07
	S2c	385136.40	15845.974	15545600.88	387.884	0.04
	S3a	1013522.00	41700.144	42573556.39	1062.267	0.04
	S3b	366322.32	15071.891	6208678.40	154.915	0.10

	S3c	2236971.14	92037.488	4612539.65	115.089	0.80

A table showing the physico-chemical across a period of 12 months at the stations

Lagoon-fed (S1)			
Date	Temperature (°C)	Salinity (ppt)	pH
Mar-23	33.12	18.04	7.75
Apr-23	30.37	7.76	7.45
May-23	30.79	10.05	7.30
Jul-23	28.75	9.62	8.06
Sep-23	30.69	14.09	7.12
Oct-23	30.43	11.83	7.21
Nov-23	30.71	9.91	7.25
Dec-23	30.77	13.79	7.21
Jan-24	31.90	9.84	7.68
Feb-24	33.6	5.25	7.21

Mar-24	33.03	4.30	8.57
Apr-24	32.31	3.64	7.50

Midstream (S2)			
Date	Temperature (°C)	Salinity (ppt)	pH
Mar-23	33.00	14.19	7.96
Apr-23	31.38	6.41	7.72
May-23	31.36	4.45	7.69
Jul-23	28.87	6.66	8.37
Sep-23	30.79	8.47	7.44
Oct-23	30.35	7.16	7.07
Nov-23	30.87	5.07	7.63
Dec-23	31.35	9.11	7.65
Jan-24	32.82	5.56	8.03
Feb-24	31.17	5.17	7.82
Mar-24	33.60	4.53	7.80
Apr-24	33.10	6.23	7.67

Ocean-fed (S3)			
Date	Temperature (°C)	Salinity (ppt)	pH
Mar-23	30.50	14.91	7.67
Apr-23	31.49	4.50	7.31
May-23	31.52	3.67	7.77
Jul-23	29.12	9.91	7.95
Sep-23	29.55	2.95	6.95
Oct-23	29.83	5.61	7.20
Nov-23	30.60	6.13	7.77
Dec-23	30.46	6.25	7.43
Jan-24	31.78	6.79	7.86
Feb-24	30.66	3.38	8.03
Mar-24	31.87	5.75	8.44
Apr-24	32.88	6.07	7.88