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SYMBIOSIS UNDER STRESS: UNRAVELING THE INTERPLAY OF OCEAN ACIDIFICATION AND RISING TEMPERATURES ON ACROPORA SAMOENSIS (STAGHORN CORAL)

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ABSTRACT

SYMBIOSIS UNDER STRESS: UNRAVELING THE INTERPLAY OF OCEAN ACIDIFICATION AND RISING TEMPERATURES ON ACROPORA SAMOENSIS (STAGHORN CORAL)

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This experiment explores the response of marine organisms to the combined effects of ocean acidification and rising temperatures, with a focus on coral reef ecosystems. As global climate change threatens profound declines in coral reefs, understanding the multifaceted impacts of these stressors becomes crucial. The study observes cellular changes in A. samoensis tissues in four different groups, including control, Temp, pH, and Temp and pH. Results indicate significant breakdown of membrane compartmentalization and cell junctions, with notable degradation and calcium carbonate crystallization in pH-stressed samples. Additionally, chlorophyll extraction data support coral bleaching due to the expulsion of zooxanthellae. These findings underscore the severe impact of pH and temperature variations on coral health, with pH conditions exhibiting a stronger effect. The study concludes by proposing a follow-up experiment involving the addition of carbon-fixing plants to mitigate environmental stressors and enhance coral resilience, aiming to contribute to the conservation of coral reef ecosystems in the face of climate change challenges.

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BACKGROUND

Amidst the backdrop of contemporary challenges facing our world's oceans, our research project investigates the intricate dynamics between rising ocean temperatures and ocean acidification, two primary consequences of human-induced climate change. Oceans, often likened to expansive deserts due to their vastness, exhibit diverse ecosystems concentrated primarily within coral reefs. These reefs, teeming with life, serve as ecological linchpins. Corals, as keystone species within them, contribute significantly to the formation and sustenance of these vibrant habitats. Thus, we've chosen coral as our model organism for this study, given their pivotal role as builders of reef ecosystems and their essential contribution to the biodiversity and health of marine environments. Our project's central objective is to meticulously observe and analyze how these species respond to the multifaceted challenges presented by ocean acidification and rising temperatures. In doing so, we aim to shed light on the intricate interplay between these stressors and their collective impact on marine organisms within reef ecosystems.

Global climate change is poised to drive profound declines in coral reefs in the foreseeable future (Dove et al., 2020). Accordingly, it is crucial to understand the multifaceted impacts of climate change on these vital ecosystems. One informative study delves deep into the intricacies of this challenge (Dove et al., 2020). By meticulously examining experimental coral reefs, this research explores the consequences of elevated temperature and ocean acidification under conditions projected for the year 2100, assuming unabated CO_2 emissions (Dove et al., 2020). The study unveils a disconcerting interruption between calcifier biomass and calcification processes within the reefs (Dove et al., 2020). This disconnect arises from the synergistic effects of warming and acidification, which, in turn, accelerate the loss of essential carbonate frameworks that are the bedrock of coral reef ecosystems (Dove et al., 2020). Such degradation impedes the capacity of coral reefs to keep pace with rising sea levels, with potentially dire consequences that may persist for millennia. The alarming outcome is that rather than transitioning into states that could safeguard coastlines, these reefs confront the looming threat of submersion, resulting in substantial losses of ecosystem services (Dove et al., 2020). This comprehensive research sheds light on the intricate dynamics of climate change impacts on coral reefs, emphasizing the urgency of immediate attention and concerted action to mitigate their decline.

Ocean acidification due to elevated CO2 is hypothesized to advantage macroalgae over corals, contributing to shifts in marine ecosystems (Del Monaco et al., 2017). Recent scientific research provides concrete evidence supporting this hypothesis (Del Monaco et al., 2017). Studies have shown that three common macroalgae are more damaging to corals when they compete under CO2 concentrations predicted for 2050 and 2100 than under present-day conditions (Del Monaco et al., 2017). Furthermore, investigations have revealed that two of these macroalgae damage corals through allelopathy, with one of them exhibiting allelopathy primarily under conditions of elevated CO2 (Del Monaco et al., 2017). For instance, lipid-soluble, surface extracts from the macroalga *Canistrocarpus cervicornis* were found to be significantly more damaging to corals growing in the field when these extracts were obtained from thalli grown under elevated CO2 conditions, as opposed to ambient levels (Del Monaco et al., 2017). These findings demonstrate that increasing ocean acidification indeed advantages seaweeds over corals, shedding light on the mechanisms through which algal allelopathy can mediate coral-macroalgal interactions, especially in the context of ocean acidification (Del Monaco et al., 2017). It's worth noting that other mechanisms also influence these interactions under ocean acidification, and this acidification further suppresses the resilience of coral reefs when confronted with macroalgal blooms (Del Monaco et al., 2017). This evidence emphasizes the urgency of addressing the impact of ocean acidification on coral reefs and the need for conservation efforts to mitigate its consequences.

The most conspicuous consequences of elevated carbon dioxide levels come to light when we scrutinize the alterations in seawater chemistry. As experts in the field have previously stated, the oceans are progressively becoming more acidic due to the absorption of excess CO2 from the atmosphere (Turley, 2013). Insights into the realm of ocean acidification highlight a concerning decline in surface ocean pH levels. While this may appear to be a minor shift, it is essential to recognize that the pH scale operates logarithmically, signifying a substantial 30 percent increase in acidity with each 0.1 pH unit decrease (Turley, 2013). This research offers a valuable perspective on the logarithmic nature of pH and underscores the significance of even seemingly minor pH fluctuations. As explored in this body of work, these shifts in seawater chemistry spell dire consequences for marine life, especially for those organisms' dependent on calcium carbonate to construct their shells and skeletons (Turley, 2013). For these organisms, the ongoing acidification of their habitats resembles a gradual yet inexorable erosion of their protective structures, a looming threat to their survival, with ramifications extending to the broader ecological balance and the communities reliant on these marine resources.

Moreover, an insightful study by Russell et al. (2013) focuses on the complex interplay of ocean acidification and rising temperatures within marine ecosystems. This research, conducted through a controlled microcosm experiment in the UK intertidal system, specifically examines the combined impacts of elevated CO2 and temperature on key components: biofilms and their primary consumers, *Littorina littorea* (Russell et al., 2013). The study's approach surpasses short-term laboratory experiments by incorporating two different pre-exposure periods, lasting two weeks and five months, to assess how acclimation influences outcomes (Russell et al., 2013). Surprisingly, the results defy predictions rooted in metabolic theory, revealing a decrease in grazer consumption of primary productivity under a five-week exposure to elevated temperature and CO2, while biofilm abundance rises. However, the influence of exposure duration emerges as a pivotal factor; with a five-month pre-exposure, grazer consumption surpasses the two-week scenario (Russell et al., 2013). These findings stress the importance of considering long-term exposure and the potential for acclimation in understanding the complex and interactive impacts of climate change on marine ecosystems. They call for a comprehensive approach that combines laboratory investigations of physiological responses with large, long-term experiments to predict the future of marine ecosystems more accurately as organisms adapt over extended periods (Russell et al., 2013). This research provides a deeper understanding of the intricate dynamics of marine ecosystems in the face of climate change.

Ocean acidification, a grave consequence of escalating atmospheric carbon dioxide levels, poses a substantial threat to marine ecosystems (NOAA, 2020). With the ocean absorbing approximately 30% of the excess CO2 released into the atmosphere, it undergoes chemical transformations that increase hydrogen ion concentration, making seawater more acidic (NOAA, 2020). This acidity surge is particularly concerning due to its detrimental effects on marine life, especially calcifying organisms that rely on carbonate ions for the formation of structures such as seashells and coral skeletons (NOAA, 2020). This poses a significant challenge for these species as they contend with the scarcity of carbonate ions, impacting their ability to maintain vital structures. Furthermore, ocean acidification doesn't solely affect calcifying organisms. Non-calcifying species, including certain fish, face their own set of challenges. They experience compromised predator detection abilities in increasingly acidic waters, which can have cascading effects throughout the marine food web (NOAA, 2020). What makes the situation even more urgent is the pace at which ocean acidification is occurring, surpassing the rates of past natural events (NOAA, 2020). If left unchecked, seawater pH could plummet by an additional 120% by the end of the century, creating oceanic conditions more acidic than any observed in the past 20 million years (NOAA, 2020). The rapidity of this change, coupled with other climate-related threats, compounds the challenges facing marine life and ecosystems, accentuating the vital importance of addressing its extensive consequences within the broader context of climate change's impact on the stability and functioning of marine ecosystems.

Despite growing awareness of ocean acidification and its dire consequences, notable gaps in our knowledge remain. As elaborated in a pertinent study, extensive research is warranted to discern the precise mechanisms and dynamics governing the interactions between ocean acidification and marine life (Kroeker et al., 2013). The research highlights the significance of investigating the effects of ocean acidification on calcifying organisms like corals, mollusks, and phytoplankton, which are particularly susceptible to increasingly corrosive waters (Kroeker et al., 2013). As these organisms confront conditions of reduced carbonate saturation, they struggle to construct and uphold their calcium carbonate structures. The study emphasizes the need for additional research to gain a deeper understanding of the underlying mechanisms by which ocean acidification affects calcifying organisms (Kroeker et al., 2013). Furthermore, it underscores the necessity of comprehending how different types of corals, encompassing solitary, colonial, zooxanthellate, and non-zooxanthellate species, respond to these shifting environmental conditions (Kroeker et al., 2013). These pertinent questions propel ongoing scientific inquiries, stressing the urgency of deepening our comprehension of ocean acidification to address the ecological and socioeconomic challenges it poses.

Rising global sea temperatures are a pressing environmental issue with extensive ramifications for marine ecosystems. As the effects of climate change intensify, our planet's oceans are experiencing a substantial and enduring increase in temperature, which affects various ecosystems, including coral reefs. Coral reefs are biodiverse and productive ecosystems but are threatened by local and global stresses (Hoegh-Guldberg et al., 2019). Climate projections suggest that coral reefs will continue to undergo major changes even if we take substantial preventative measures in the near future (Hoegh-Guldberg et al., 2019). The significant warming of our oceans accentuates the urgency of addressing climate change on a global scale. As a response to this warming, marine life contends with multifaceted challenges, including shifts in distribution patterns, altered reproductive cycles, and a heightened prevalence of diseases. The intricate interplay between rising sea temperatures and the well-being of marine organisms constitutes a complex realm requiring thorough examination to comprehensively understand its ecological impacts (Hoegh-Guldberg et al., 2019). Furthermore, the study highlights the economic significance of these changes, especially for industries reliant on fisheries and tourism (Hoegh-Guldberg et al., 2019). As we navigate this era of swift climate change, understanding the implications of rising ocean temperatures isn't solely an ecological imperative but also a socio-economic necessity.

A core assertion of one relevant study is the imperative need to comprehend the repercussions of cumulative stressors on coral reef vulnerability, a vital consideration for effective reef conservation (Anthony et al., 2014; Nogales et al., 2011). The authors contend that strategically focusing on bolstering ecological resilience, characterized by the capacity to withstand stress and recover, can mitigate coral reef vulnerability up to a certain extent. Additionally, to address these challenges, the study introduces an innovative operational framework known as Adaptive Resilience-Based Management (ARBM) (Anthony et al., 2014). This framework provides guidelines for augmenting resilience and offers a comprehensive understanding of the biological and ecological processes underpinning resilience within various environmental and socio-economic contexts. By distinguishing between "press-type" stressors (e.g., pollution, ocean warming) that erode resistance and recovery and "pulse-type" stressors (e.g., storms, bleaching events) that heighten the demand for resilience, the study highlights the challenges that both types pose (Anthony et al., 2014). To illustrate the practical application of the framework, the authors employ it to address scenario-based problems in Caribbean and Indo-Pacific reefs. Their analysis advocates for a combined strategy that includes risk reduction and resilience support, with decisions guided by specific management objectives and a deep understanding of the processes governing reef ecosystems (Anthony et al., 2014).

Evidently, coral reef health is a critical concern, as these ecosystems face threats like disease, temperature changes, and coral bleaching (Gove et al., 2023). One study focusing on early warning indicators for coral stress probes the importance of recognizing stress or illness in corals at an early stage to enable timely conservation measures. To specify, researchers monitored various chemicals involved in coral physiology and immune systems to detect chemical signatures that appear when corals are impaired or stressed (Hansel et al., 2019). One of the key chemical signatures of interest is the reactive oxygen species (ROS) superoxide, which both corals and their zooxanthellae produce (Su et al., 2019). Under normal conditions, superoxide plays important roles in cell signaling, reproduction, and tissue repair (Ito et al., 2019; Hansel et al., 2019; Su et al., 2019). However, excessive intracellular superoxide produced in response to sudden stress can lead to DNA and biomolecule breakdown and coral death. At heightened levels, ROS exhibit widely recognized toxic characteristics, such as modifying the redox status of vital enzymes or causing damage to essential biomolecules like membranes and proteins; for instance, superoxide initiates oxidative stress in both macro- and microorganisms, induces programmed cell death, enhances the ichthyotoxicity of phytoplankton blooms, and contributes to the bleaching of symbiotic corals (Hansel et al., 2019). To address this, they developed a submersible sensor, DISCO, for in-situ measurement of superoxide levels (Grabb et al., 2019). Using this sensor, the team recently showed that healthy corals release superoxide outside of their cells, which they predict is providing essential physiological roles for the coral and symbionts (Grabb et al., 2019). By establishing baseline ROS values for different coral species, scientists can detect when stressors cause an elevation in ROS levels, even before visible signs of stress or disease appear.

To obtain a better understanding of the method of attack of oxidative stress due to the synergistic effects of ocean acidification and rising temperatures, it’s essential to examine the relationship between corals and zooxanthellae. Specifically, symbiosis plays a crucial role on coral reefs, with the most vital symbiotic relationship being between corals and the microalgae known as zooxanthellae residing within their cells (Campoy et al., 2020; Charles et al., 2017; US Department of Commerce, National Oceanic and Atmospheric Administration, 2019; Zandonella, 2016). Relevant research delved into the diversity of zooxanthellae, highlighting their connection to coral calcification and the nutritional aspects of this symbiotic relationship, particularly the transfer of photosynthetically fixed carbon to corals and the conservation and recycling of vital nutrients, notably nitrogen and phosphorus (Campoy et al., 2020; Charles et al., 2017; Zandonella, 2016). Furthermore, it discussed the establishment and disruption of this symbiosis, particularly in the context of thermal stress, which can lead to coral bleaching (Campoy et al., 2020; Charles et al., 2017; Zandonella, 2016). The implications of this vulnerability are essential to understand the objective way that the synergistic effects of ocean acidification and rising temperatures on corals are analyzed.

On another note, rising ocean temperatures are inherently linked to the alarming phenomenon of coral bleaching, a topic comprehensively investigated in a specialized study. Coral reefs, among the most biodiverse ecosystems on Earth, are notably sensitive to temperature increases. The research underlines the pivotal role of symbiotic algae known as zooxanthellae in coral health. It expounds on how elevated sea temperatures induce stress in corals, leading them to expel these vital symbionts, resulting in the loss of their vibrant colors and, ultimately, their health (Prada et al., 2017). The study furnishes an extensive analysis of the coral bleaching events witnessed in diverse regions, emphasizing that recurrent or severe bleaching can culminate in coral mortality. It also highlights that coral bleaching isn't solely an environmental concern but also a significant economic one, impacting tourism and fisheries across many regions. The findings emphasize the urgency of global efforts to mitigate climate change and reduce the frequency and severity of coral bleaching events, a collective endeavor essential to safeguard the ecological and economic values of coral reefs and the myriad species reliant on them for survival.

The researchers in this study on ocean warming and acidification employed a two-way ANOVA to explore the influence of seawater temperature (SWT) and pH on coral mortality and net calcification rates (Prada et al., 2017). The research found that ocean warming and acidification collaboratively elevate coral mortality rates, leading to increased polyp mortality across all species and augmented coenosarc-polyp tissue mortality in *A. calycularis* under low pH and high temperature conditions (Prada et al., 2017). Notably, the interaction term showed non-significance, indicating an additive rather than synergistic effect of SWT and pH on net calcification rates (Prada et al., 2017). These findings underscore the complex interplay of ocean warming and acidification in influencing coral mortality and accentuate the necessity for effective management strategies to mitigate the repercussions of these stressors on coral reefs, emphasizing their critical conservation in the face of escalating environmental challenges.

Within the context of rising ocean temperatures, the impact on specific marine organisms, such as sea anemones, is a subject of mounting concern. Recent research examined the effects of temperature stress on sea anemone populations, particularly those dwelling in vulnerable habitats (Nicholson et al., 2022). This study utilized controlled experiments to replicate elevated sea temperatures, simulating conditions expected under future climate scenarios (Nicholson et al., 2022). It accentuates the diverse responses observed in sea anemones, ranging from physiological stress to alterations in behavior. Notably, the research parallels findings from the subpolar Southern Ocean, where storms drive the outgassing of CO2, resulting in rapid changes in ocean chemistry (Nicholson et al., 2022). Importantly, the research identifies potential adaptive mechanisms in certain sea anemone populations, suggesting they may possess the ability to acclimate to higher temperatures over time (Nicholson et al., 2022). These findings accentuate the complexity of species' reactions to temperature stress and the necessity for further studies to predict the long-term viability of various marine populations in the face of ocean warming. Comprehending the specific responses of sea anemones and similar organisms is fundamental to assessing their ecological and conservation implications. Furthermore, considering that sea anemones and corals belong to the same phylum (cnidaria) it is worth considering that they may react to such stressors in similar ways. Therefore, this knowledge underpins efforts to devise effective strategies for safeguarding these pivotal components of marine ecosystems from the escalating threat of climate change.

As previously mentioned, understanding the health of sea anemones within reef ecosystems holds significant ecological importance and is integral to understand the health of corals. Sea anemones play multifaceted roles in these environments. Specifically, they are recognized as one of the first organisms to inhabit reefs, paving the way for greater biodiversity in marine ecosystems, rendering them indispensable to the well-being and diversity of these critical marine ecosystems (Siedlecki et al., 2021). Additionally, they serve as habitat providers, offering shelter to various fish species and invertebrates, and they serve as primary food sources for certain marine organisms, contributing to the intricate food web of coral reefs (Siedlecki et al., 2021). Furthermore, sea anemones participate in nutrient cycling within the ecosystem. Therefore, assessing the health and resilience of these organisms is crucial for comprehending the overall well-being of reef environments and directly correlational to the health of corals. As such, this study emphasizes an often-overlooked aspect of coral reef health, shedding light on the understudied sea anemones' responses to the interplay of multiple stressors. Such insights can significantly impact reef conservation and management (Siedlecki et al., 2021). Sea anemones are sentinel species whose health reflects the broader challenges faced by coral reefs. Their responses to acidification and ocean warming can offer valuable insights into the complex, interconnected dynamics of these ecosystems, influencing the strategies employed in their conservation and protection (Siedlecki et al., 2021). Previous research that focused on the intricate dynamics of ocean acidification in the Gulf of Maine (GOM) ecosystem illuminated the region's vulnerability to this global issue (Siedlecki et al., 2021). The GOM, characterized by its seasonal variability and unique confluence of various processes impacting the carbonate system, is particularly sensitive to ocean acidification due to influences like low alkalinity river discharge, atmospheric deposition of acidic and alkaline compounds, sedimentary processes, and coastal eutrophication. Using a combination of high-resolution simulations with a focus on coastal processes, the study projects ocean acidification conditions for the GOM in 2050 (Siedlecki et al., 2021). The findings reveal a concerning trend as the aragonite saturation state (Ωa) is predicted to decline across the entire GOM by 2050, with the nearshore and subsurface environments facing the most significant impacts (Siedlecki et al., 2021). The study underscores the imperative need to comprehend the intricate interactions between ocean acidification and other stressors within marine ecosystems and advocates for the development of effective management strategies to mitigate the impending effects of ocean acidification on the GOM (Siedlecki et al., 2021). In essence, this paper underlines the pressing concern surrounding ocean acidification in the Gulf of Maine and the necessity for proactive measures to safeguard its delicate ecosystem (Siedlecki et al., 2021).

Contemporary research places significant emphasis on understanding the combined and potentially synergistic effects of various stressors, particularly ocean acidification and rising sea temperatures, on marine ecosystems. Scientists are actively working to construct a comprehensive theoretical framework that accounts for the intricate interplay of these stressors. They investigate how elevated CO2 levels influence species' responses to warming waters (Hassoun et al., 2022). Through the analysis of these multi-stressor scenarios, their primary objective is to cultivate a holistic understanding of how diverse facets of climate change intersect and amplify the challenges confronting marine life (Hassoun et al., 2022). This integrated approach facilitates a more precise assessment of potential impacts, recognizing that marine organisms may concurrently face multiple stressors, resulting in consequences that extend beyond what can be understood by studying each stressor in isolation (Hassoun et al., 2022). Such a theoretical framework is essential for forecasting the future health of ocean ecosystems and shaping effective management strategies. It underscores the critical importance of holistic conservation efforts in effectively mitigating these collective threats.

Efforts to comprehend the concurrent impacts of multiple stressors on marine ecosystems, particularly ocean acidification and rising sea temperatures, are at the forefront of contemporary research. Rising atmospheric CO2 levels, largely resulting from activities like fossil fuel combustion, deforestation, and various land-use practices, are leading to substantial increases in seawater CO2 and inorganic carbon levels (Doney et al., 2020). This, in turn, results in reductions in pH and changes in the acid-base chemistry of estuarine, coastal, and open-ocean waters (Doney et al., 2020). Through laboratory experiments and field studies in naturally elevated CO2 marine environments, it has become evident that human-driven ocean acidification has far-reaching biological consequences (Doney et al., 2020). These consequences range from shifts in organism physiology and population dynamics to alterations in communities and ecosystems (Doney et al., 2020). When coupled with other climate-related stressors, especially under future climate change scenarios and increasing atmospheric CO2 levels, these effects pose a significant risk to essential ecosystem services provided by the ocean, including fisheries, aquaculture, and shoreline protection (Doney et al., 2020). This holistic approach to research is indispensable for forecasting the future health of ocean ecosystems and guiding management strategies (Doney et al., 2020). It underscores the necessity of comprehensive conservation efforts to effectively mitigate these collective threats, recognizing that marine organisms may simultaneously face a convergence of stressors (Doney et al., 2020).

Similarly, another significant review emphasizes the urgency of understanding the mechanisms that enable corals to endure high-temperature stress (Baker et al., 2018; Carballo-Bolaños et al., 2019; Goulet et al., 2020). It explores various strategies, such as forming associations with thermally tolerant endosymbionts, acclimatization, and adaptation processes, which vary among coral species (Carballo-Bolaños et al., 2019). The findings indicate that warming to 31°C led to a 60% reduction in the coral holobiont's net primary productivity due to increased respiration (Baker et al., 2018). This decrease in host carbon by 15% did not incur a discernible cost to the symbiont (Baker et al., 2018). Simultaneously, Symbiodinium demonstrated a 14% increase in carbon assimilation, a 32% increase in nitrogen assimilation, and a 15% boost in their mitotic index (Baker et al., 2018). However, the hosts did not experience a commensurate increase in translocated photosynthates. These results point to a disparity in benefits and costs between the partners, indicating evidence of symbiont parasitism in the coral symbiosis (Baker et al., 2018). The reviews underscore that, for the survival of coral reefs, substantial reductions in greenhouse gas emissions are imperative (Carballo-Bolaños et al., 2019; Goulet et al., 2020). It also highlights the physiological diversity within the coral holobiont and the complex interplay of host and microbial endosymbionts (Carballo-Bolaños et al., 2019; Goulet et al., 2020). These insights are invaluable for developing conservation strategies and ensuring the continued existence of coral reef ecosystems. Additionally, the resultant rise in sea temperatures could render marine organisms more susceptible to the toxic effects of ocean acidification. This concern is especially relevant when we consider our model organism, the staghorn coral, which is a reef builder, which paves the way to more biodiversity in marine environments. As an integral component of marine environments, the coral's susceptibility to both rising temperatures and ocean acidification highlights the urgency of our research.

In the face of dire threats confronting coral reef ecosystems in the Gulf of Mexico (GoM) and the western Caribbean Sea, this study plays a crucial role within the broader context of climate change and marine conservation, highlighting the urgency of understanding and addressing these challenges (Lawman et al., 2022). These threats primarily result from rising temperatures and ocean acidification linked to anthropogenic climate change (Lawman et al., 2022). The substantial decline in the structural complexity of these reefs since the 1970s, with only a limited number still maintaining a significant live coral cover, highlights the urgent need for our investigation (Lawman et al., 2022). The research from this study employs climate model simulations spanning the years 2015 to 2100, projecting ocean temperature increases of 2°C–3°C throughout the

21st century, exceeding established regional bleaching thresholds by mid-century (Lawman et al., 2022). Although ocean acidification is anticipated, the speed and extent of temperature increases surpass the significance of changes in aragonite saturation state (Lawman et al., 2022). Thus, the study reveals the critical importance of substantial mitigation measures to avert the severe stress projected for existing corals in the GoM and the Caribbean, with subsequent far-reaching economic and ecological consequences (Lawman et al., 2022). While this research primarily focuses on these specific regions, it underlines the urgency of a multidisciplinary approach to safeguarding marine life and coastal communities in the face of unprecedented environmental change, considering the broader implications for marine conservation.

This research aims to address the identified gaps in the literature through a comprehensive investigation into the responses of corals to the simultaneous pressures of ocean acidification and rising sea temperatures. Specifically, the study seeks to elucidate the mechanistic underpinnings of these responses. The research hypothesizes that corals may exhibit resilience or vulnerability under these combined stressors, depending on various factors, such as their physiological adaptations and acclimation capacity. By conducting a collection of controlled experiments and observations, the research will provide valuable insights into the dynamic responses of staghorn corals. Additionally, the findings may have broader implications for the conservation and management of coral reef ecosystems and can inform strategies to enhance their resilience in the face of climate change.

It is imperative to emphasize the collaborative nature of this research, as it involves a multidisciplinary team of scientists with diverse expertise, including marine biology, toxicology, and climate science. This interdisciplinary approach is essential for addressing the complex and multifaceted interactions between ocean acidification and rising sea temperatures, which affect not only individual species like staghorn corals but entire marine ecosystems. By pooling their knowledge and resources, the research team can provide a more comprehensive and nuanced understanding of the challenges posed by climate change. The collaborative nature of this study is a testament to the interconnectedness of scientific research and the necessity of integrating various disciplines to tackle complex, real-world environmental issues.

Furthermore, the inclusion of diverse perspectives in this research aligns with the global call for collaborative efforts to address climate change and its impacts on marine ecosystems. As international organizations and governments recognize the urgent need to reduce greenhouse gas emissions and safeguard the oceans, research like this is a testament to the global commitment to understanding and mitigating the challenges posed by climate change. It highlights the interdependence between scientific inquiry and global conservation efforts, emphasizing the imperative of working collectively to protect the world's oceans for future generations.

In conclusion, this research project, focusing on the responses of corals to the combined pressures of ocean acidification and rising sea temperatures, represents a pivotal contribution to our understanding of the complex challenges posed by climate change. Through an interdisciplinary approach, this study seeks to elucidate the ways through which corals respond to these multiple stressors, ultimately contributing to the broader knowledge of coral reef ecosystems and their resilience. As we confront the escalating threats of climate change, this research highlights the interconnectedness of scientific inquiry, conservation, and global efforts to protect marine life and coastal communities. It accentuates the urgency of acting collectively to address the unprecedented environmental changes that imperil the world's oceans and the countless species that call them home. With a multidisciplinary perspective and a commitment to exploring the multifaceted interactions within marine ecosystems, this study aims to further the scientific and practical understanding necessary for effective conservation and management strategies in a changing world. As we navigate the era of climate change, such research stands as a testament to our shared responsibility to protect and preserve the oceans, one of our planet's most vital and vulnerable ecosystems.

METHODS AND MATERIALS

Animals and Treatment

Individual colonies of A. *samoensis* were housed in the Animal Care Center of St. John's University in isolated 38-liter static tank systems, with a 12-hour photoperiod. Water quality parameters including pH, salinity, and temperature were monitored daily. After a 24-hour acclimation period, the animals were exposed to their respective conditions. The control was kept at a pH of 8.0, 25 °C, and salinity of 32 ppt. The groups which were placed in the "ocean acidification" and "rising temperature and ocean acidification" conditions were exposed to a 12-hour drip of HCl. Additionally, the groups which were placed in "rising temperature" and "rising temperature and ocean acidification" conditions were exposed to a 12-hour period to increase temperature 3 degrees Celsius (25 °C to 28 °C). After a 48-hour exposure period, animals were euthanized, processed, and appropriately stored for analysis.

Decalcification

Samples were decalcified by the Formic Acid - Sodium Citrate Method. The calcified coral samples were placed in equal portions of solution A (50 grams sodium citrate and 250 mL distilled water) and Solution B (125 mL of 90% formic acid and 125 mL of distilled water) (Armed Forces Institute of Pathology (U.S, 1968, p. 19).

Light Microscopy

Tissue collected from animals for light microscopy was immediately fixed in 2% seawater buffered glutaraldehyde for 3 hours at 4°C, washed in seawater buffer, and postfixed in 1% seawater buffered osmium tetroxide for 1 hour at 4°C. Tissue was washed in buffer and dehydrated in a series of water/ acetone mixtures to 100% acetone. The tissue was then infiltrated in LX112- Araldite, embedded in BEEM capsules, and placed in a 60°C oven for 4 d. Using a Dupont Sorvall Porter-Blum MT-1 ultramicrotome, tissue blocks were sectioned, and the sections were collected and mounted on slides. The methylene blue/azure B-stained sections were viewed on an Olympus BH2 micro- scope and images were captured digitally using a Spot insight camera.

Scanning Electron Microscopy

Tissue collected from animals was immediately fixed in 2% seawater buffered glutaraldehyde for 3 hours at 4°C , washed in seawater buffer, and postfixed in 1% seawater buffered osmium tetroxide for 1 hours at 4°C. Tissue was washed in buffer and dehydrated in a series of water/acetone mixtures to 100% acetone. Tissue was critical-point dried using CO2 as the transition fluid. Specimens were mounted on steel stubs, attached with silver paint, and coated with 40 nm platinum. The tissue was observed using a goniometer stage on a Hitachi S-530 scanning electron microscope (SEM) at 25 kV coupled with Evex analytical software. Images were captured digitally.

Chlorophyl Extraction

Samples were soaked in 90% acetone (15 mL per sample) for 30 minutes and then analyzed by spectroscopy. Absorbance was measured at 470 nm for the temperature, pH, temperature and pH, control, and dilutions 1-4 from the control. The control was used as the standard.

Statistical Analysis

One-way analysis of variance (ANOVA) and two-tailed *t*-tests were performed to determine statistical significance among and between groups. All statistical analysis was performed using GraphPad Prism version 10 (Graph- Pad Software, Inc.). The criterion for significance was set at $p < 0.05$.

RESULTS

Figure 1: Light Micrograph of Control Sample (A) at 20 X Magnification. The sample is of digestive glandular tissue.

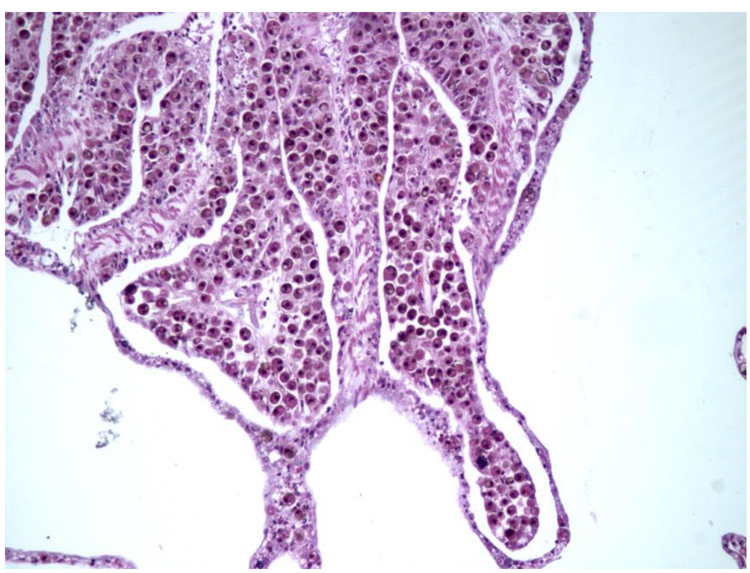

Figure 2: Light Micrograph of Control Sample (B) at 20X Magnification.

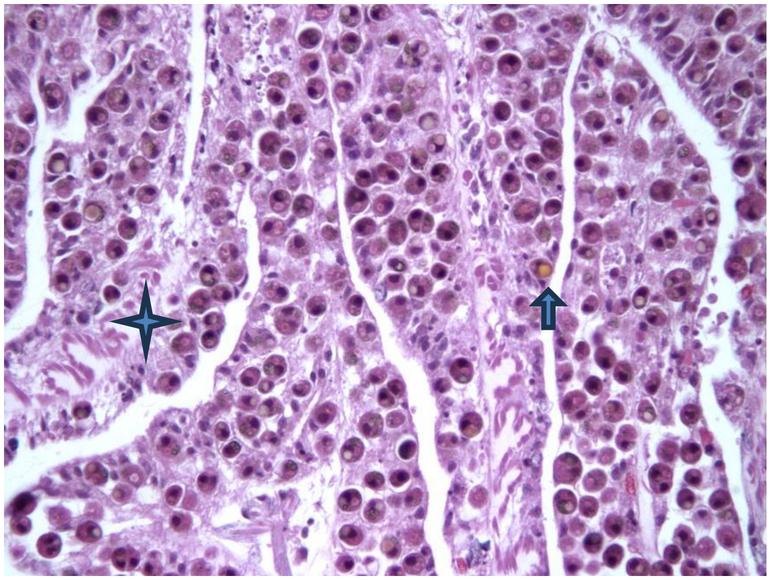

Figure 3: Light Micrograph of Control Sample at 40X Magnification. The arrow is pointing to a zooxanthellate. The four-point star is pointing to musculo-epithelial tissue.

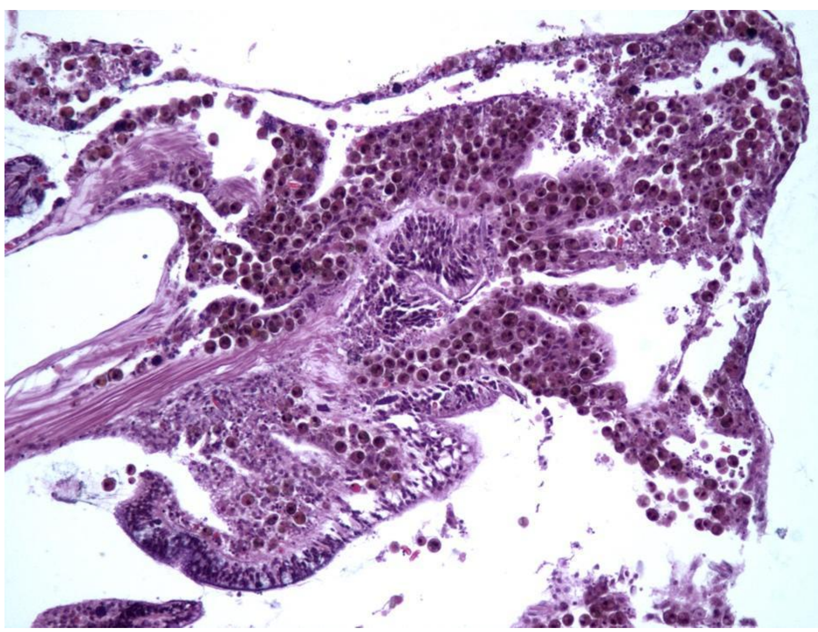

Figure 4: Light Micrograph of Temp Sample at 20X Magnification. The Temperature (Temp) samples show extensive breakdown of membrane compartmentalization.

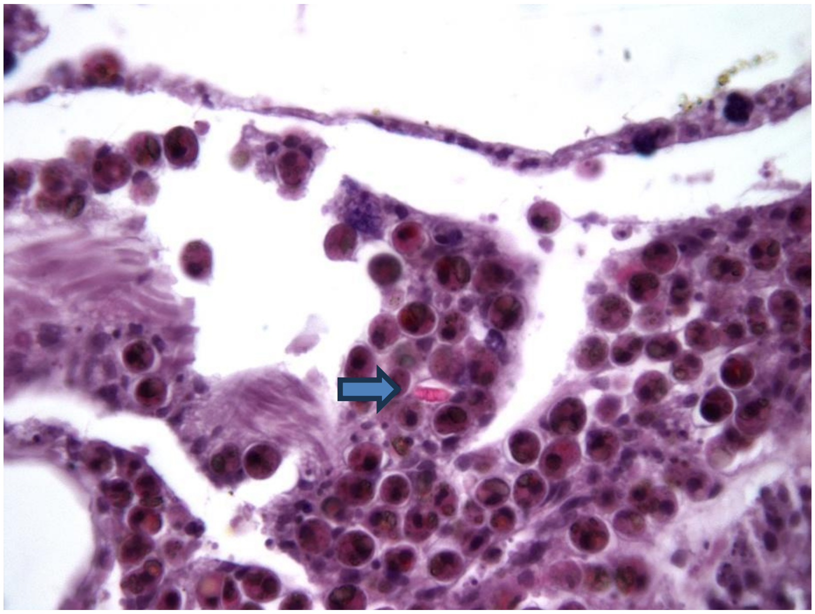

Figure 5: Light Micrograph of Temp Sample at 40X Magnification. The arrow is pointing to a cnidocyte. A loss of cell junctions is observed in this figure.

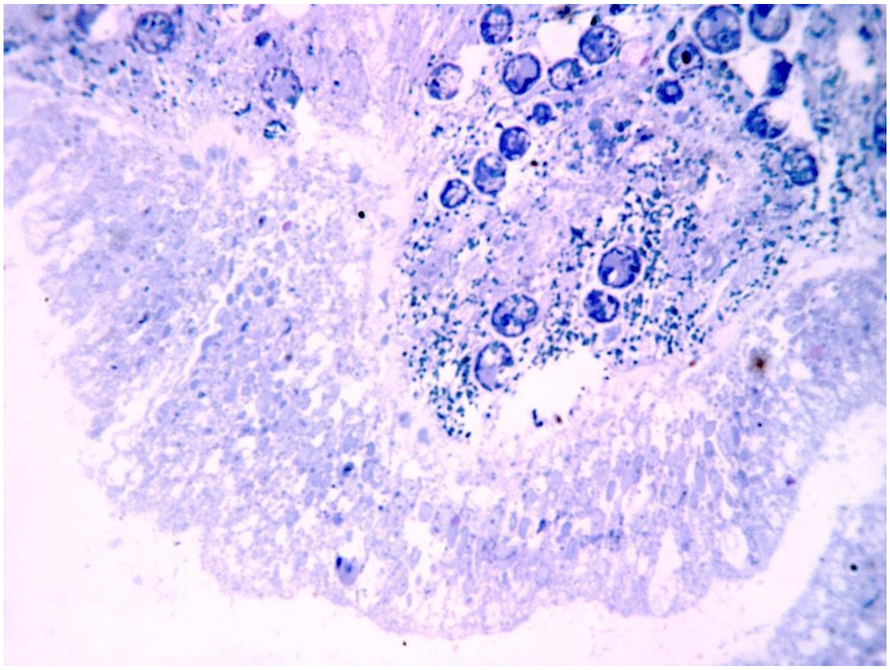

Figure 6: Light Micrograph of pH Sample (A) at 40X Magnification. Due to the composition of the tissue, the staining isn't as vibrant in these samples as it is in the other samples.

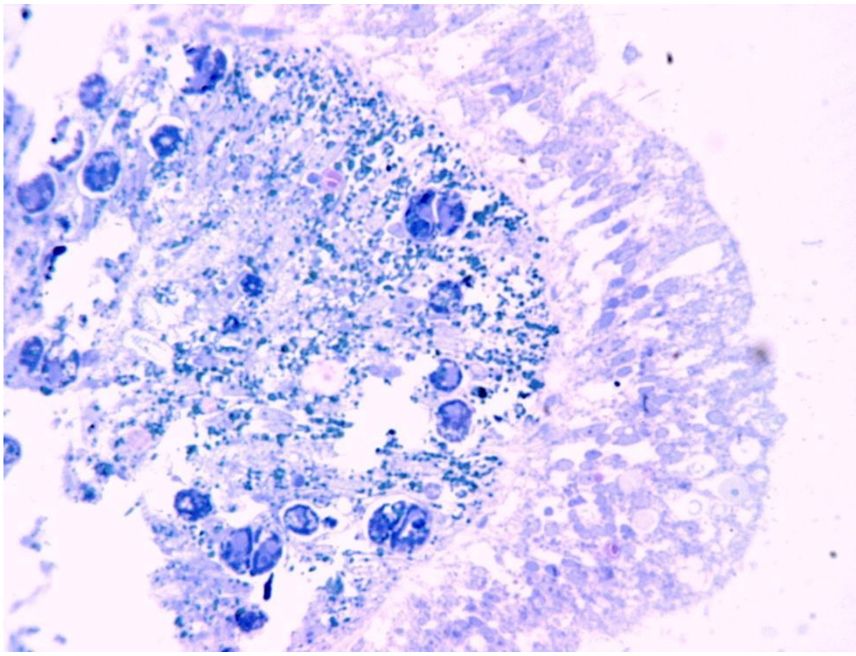

Figure 7: Light Micrograph of pH Sample (B) at 40X Magnification. There is a visible breakdown of membrane tissue.

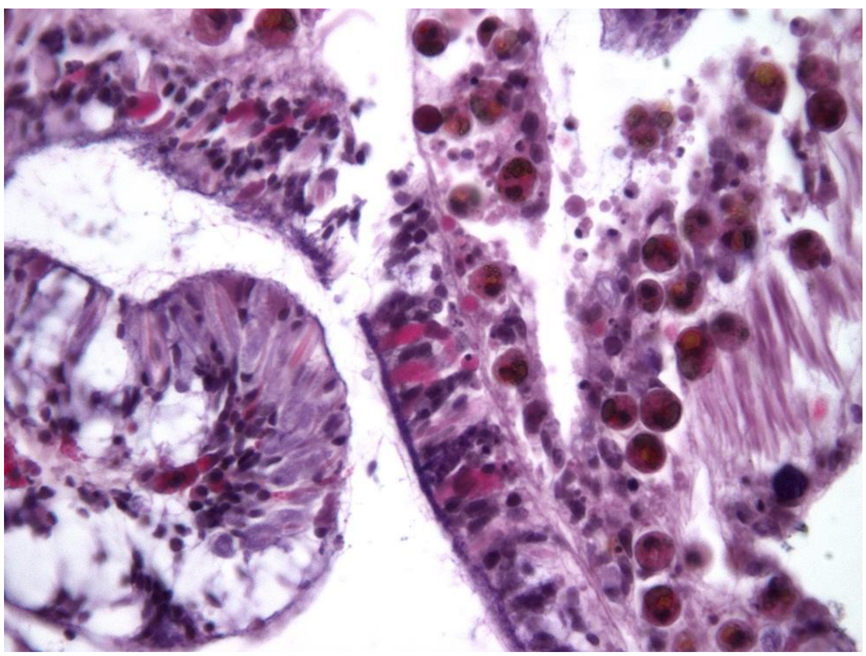

Figure 8: Light Micrograph of Temp and pH Sample (A) at 40X Magnification.

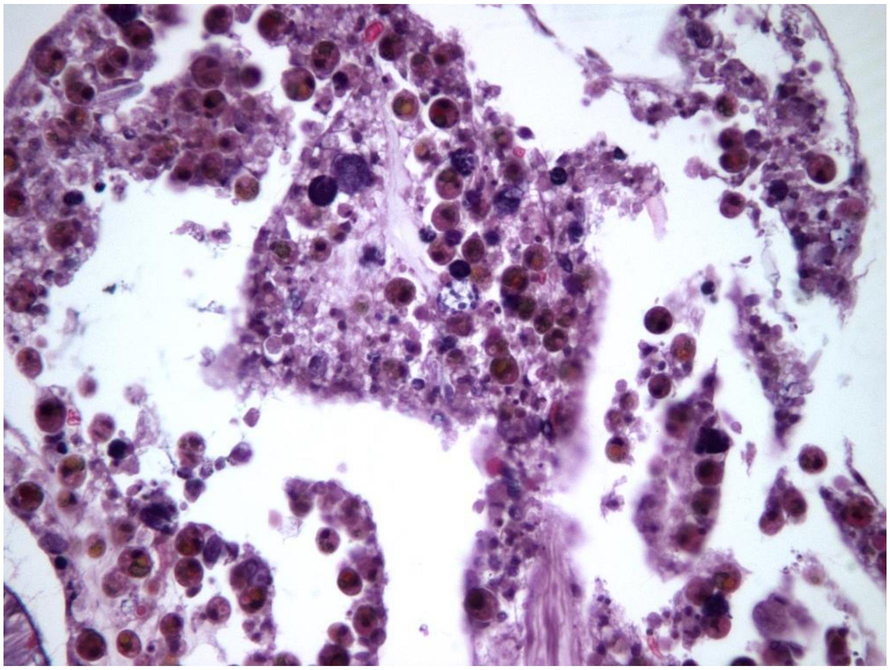

Figure 9: Light Micrograph of Temp and pH Sample (B) at 40X Magnification. These samples exhibit extensive exfoliation, rounding up of cells, loss of cell junctions and the breakdown of membrane compartmentalization.

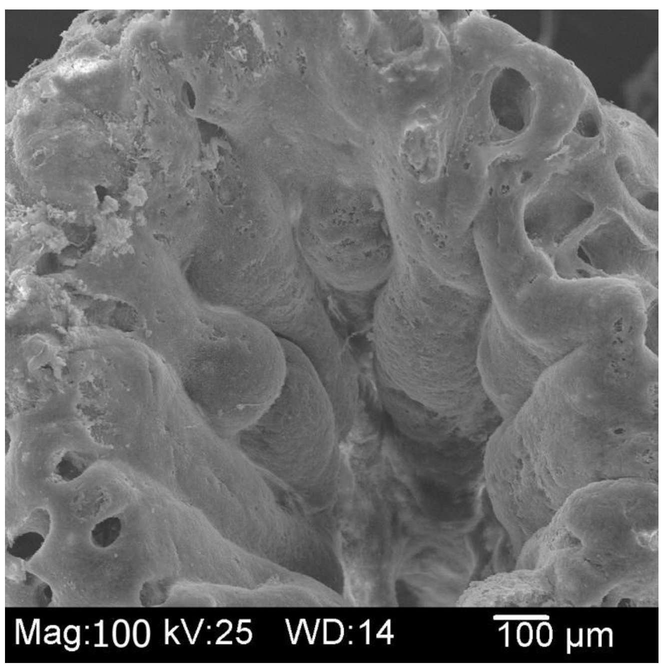

Figure 10: Scanning Electron Micrograph of Control Sample at 100X Magnification.

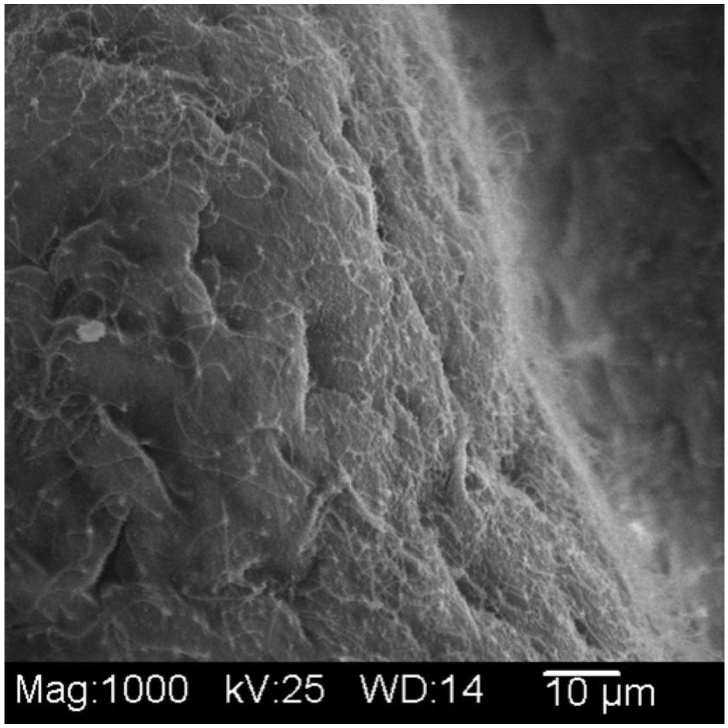

Figure 11: Scanning Electron Micrograph of Control Sample at 1000X Magnification.

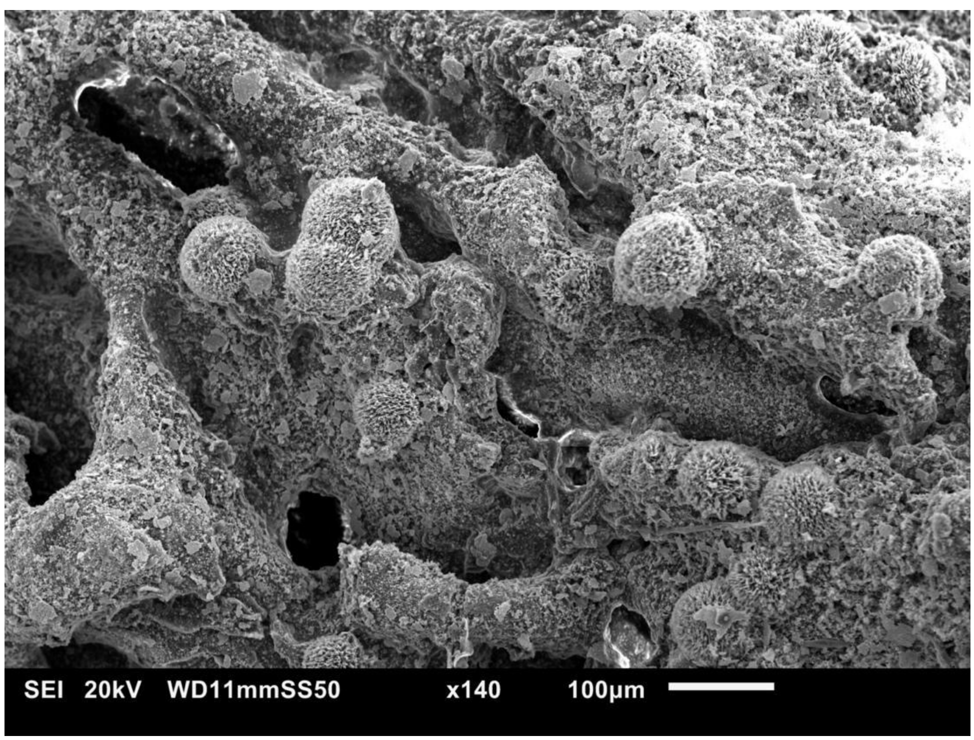

Figure 12: Scanning Electron Micrograph of Temp Sample at 140X Magnification. The figure shows a basement membrane with exfoliation occurring. Protein aggregation and the rounding up of cells are also visible in the micrograph.

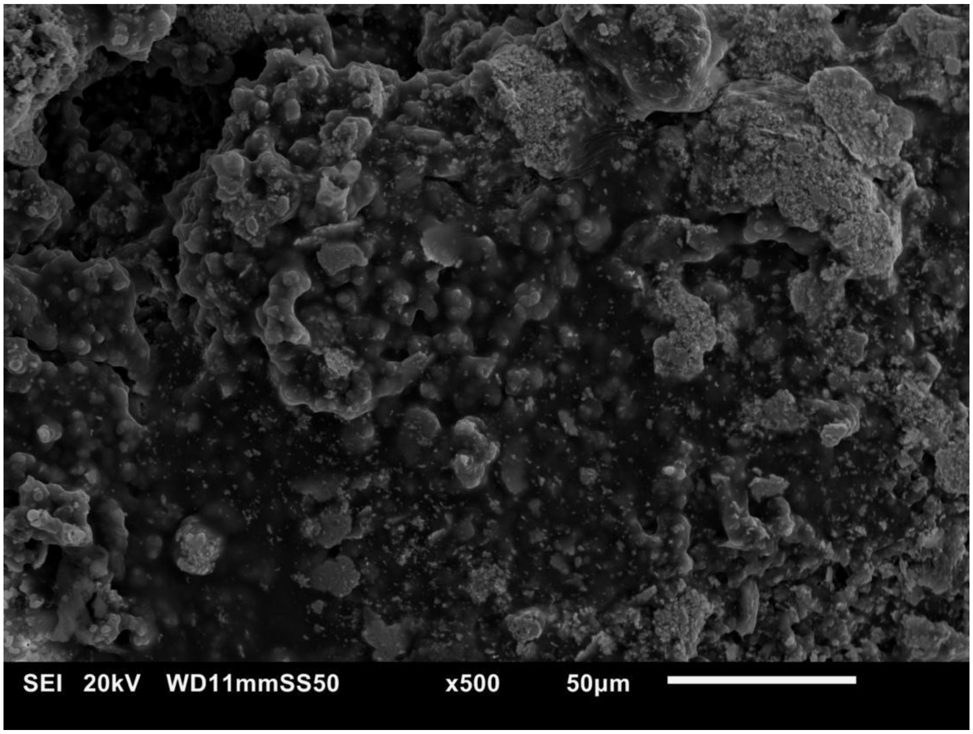

Figure 13: Scanning Electron Micrograph of Temp Sample at 500X Magnification. There is degradation of the outer epithelium tissue. Also, there is visible exfoliation along with protein aggregation.

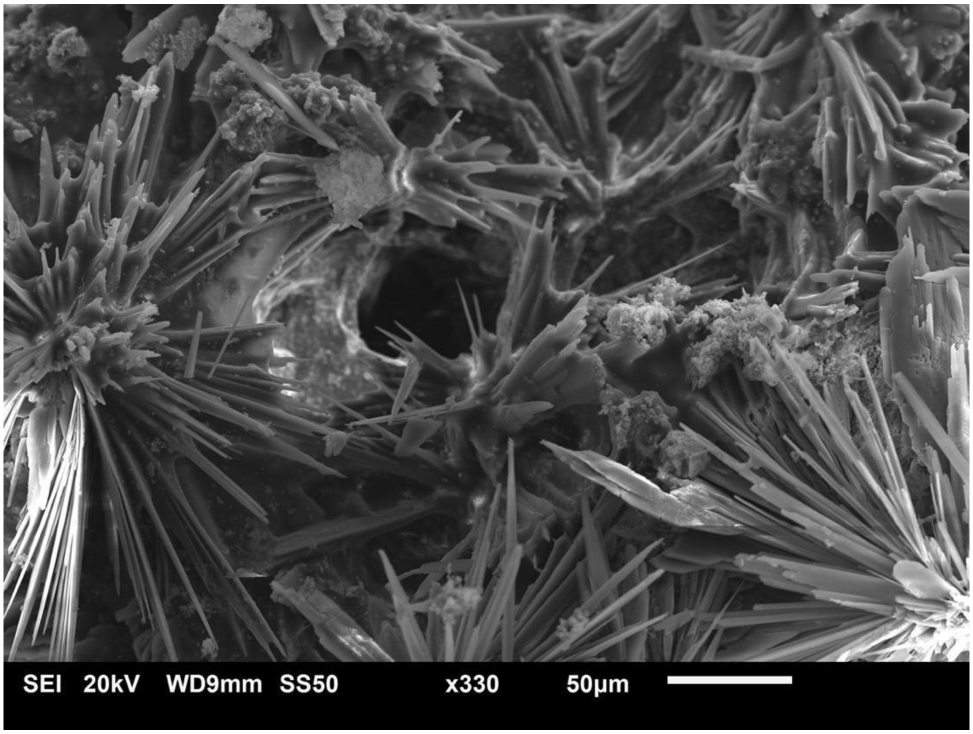

Figure 14**:** Scanning Electron Micrograph of pH Sample at 330X Magnification. Evidently, there is extensive difference in the morphology between the pH and Control Sample. The normally smooth and flat epithelium is now characterized by prominent exfoliation, calcium carbonate crystallization and degradation.

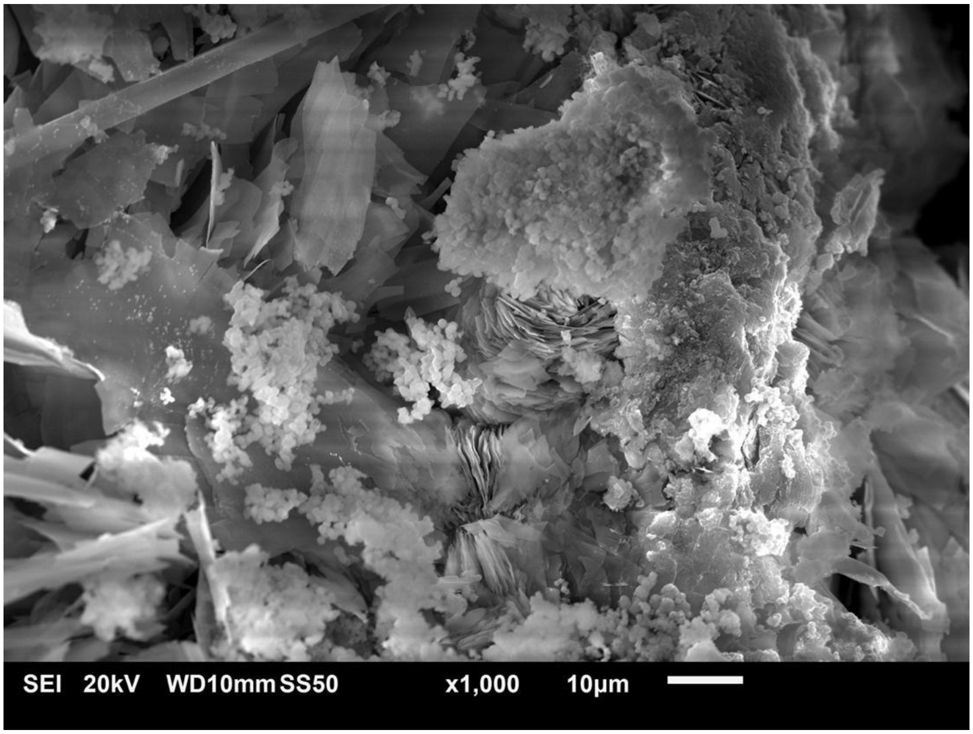

Figure 15: Scanning Electron Micrograph of pH Sample at 1000X Magnification. Rounding up of the tissue, along with protein aggregation are present.

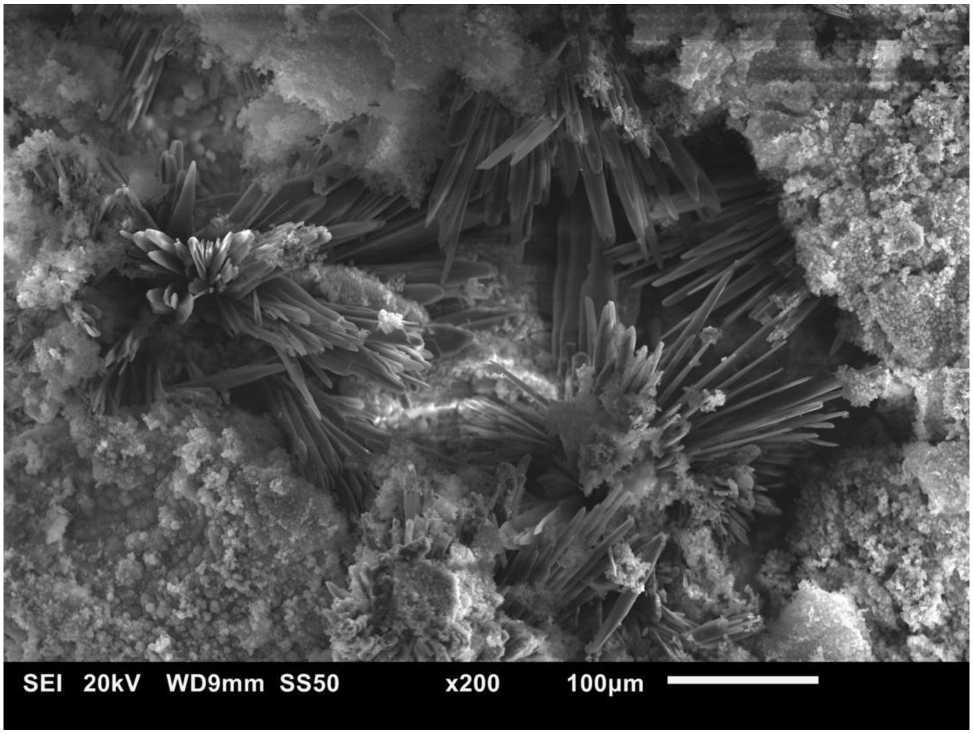

Figure 16: Scanning Electron Micrograph of Temp and pH Sample at 200X Magnification. Crystallization, degradation of epithelial tissue, and exfoliation extensive in this sample.

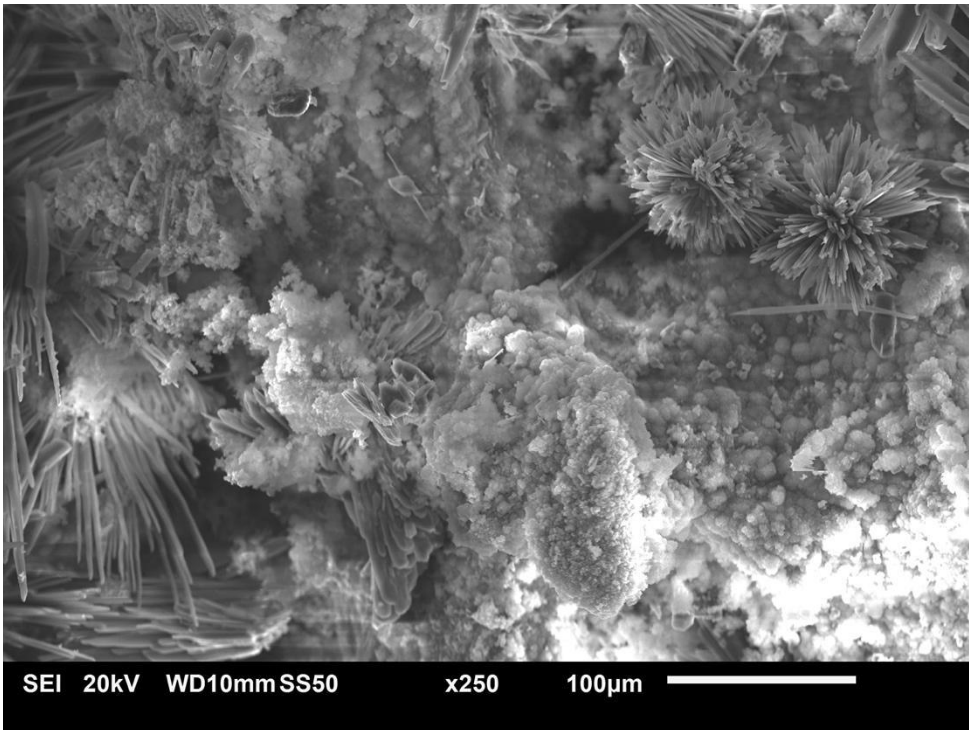

Figure 17: Scanning Electron Micrograph of Temp and pH Sample at 250X Magnification. Crystallization, degradation of epithelial tissue, exfoliation, rounding up of cells, and protein aggregation are prominent throughout the image.

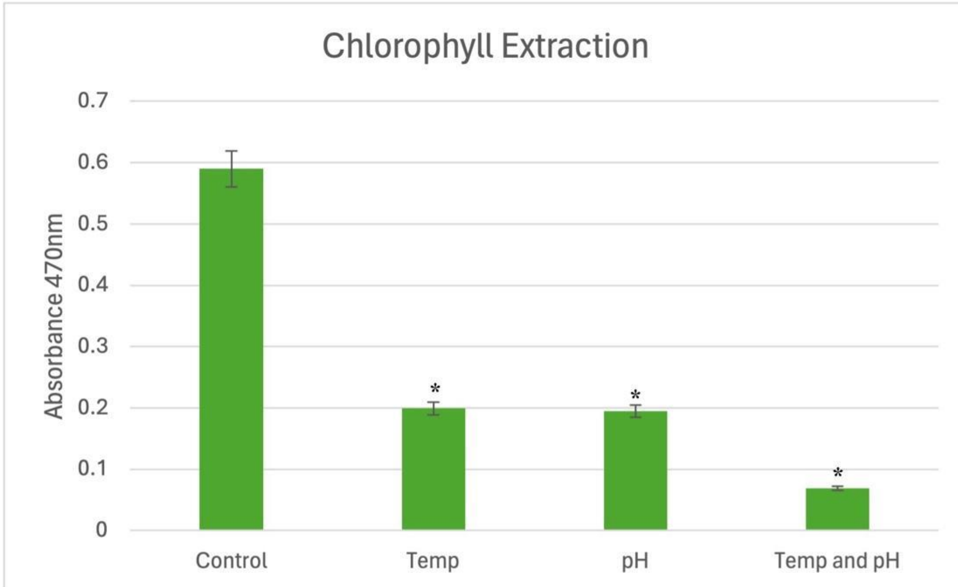

Figure 18: Chlorophyll extraction in control, Temperature (Temp), pH, and Temperature and pH (Temp and pH) samples measured by spectrometry at 470 nm. The results depict that the Control sample had an absorbance of 0.590, the Temp sample had an absorbance of 0.199, the pH sample had an absorbance of 0.195, and the Temp and pH sample had an absorbance of 0.069. The criterion for significance was set at $p < 0.05$.

DISCUSSION

The early events leading to the progression of cellular degradation following exposure to environmental stressors play a critical role in risk assessment. Specifically, damage due to peroxidative stress is an event that occurs early and leads to pathology. From the data that has been collected in this experiment, it's evident that there were morphological changes in tissue samples of *A. samoensis* following exposure to the three tested environmental stressor groups (Temp, pH, and Temp and pH). These results support the hypothesis that upon exposing the organism to an environment resembling that of projected ocean acidification and rising temperatures by the end of the century, photobleaching will occur and the corals will be unable to adapt to the changes in the environment.

As seen in figures 1A- 4B prominent degradation of different tissue types is present. In Figure 2A, at 20X magnification, the Temp samples depict significant breakdown of membrane compartmentalization, suggesting disruption in cellular integrity. This observation is further corroborated in Figure 2B at 40X magnification, where a loss of cell junctions is evident. Moving to Figures 4A and 4B, representing Temp and pH samples at 40X magnification, a similar pattern emerges, with extensive exfoliation, rounding up of cells, and loss of cell junctions observed. Additionally, the breakdown of membrane compartmentalization is highlighted, further underscoring the disruptive effects of combined temperature and pH stressors on cellular morphology. These findings collectively emphasize the significant impact of environmental stressors on cellular structure and organization, with implications for understanding the physiological responses of organisms to changing environmental conditions.

These observations are consistent with the findings that were detected in the SEM micrographs. To clarify, while figure 6A depicts intact bare calcium carbonate skeletal structures with concentrated areas of protein aggregation and rounding up of tissue, the Temp samples lack the calcium carbonate crystallization that is characteristic of pH conditions. The fact that they lack this crystallization indicates that the ocean acidification condition had a stronger impact on the corals than the increased temperature. To clarify, the crystallization suggests that not only are the living cells being destroyed, but the existing skeletal structure as well, which prevents further growth and signifies a disastrous future for our coral reefs. Furthermore, figures 7A and 7B show much more prominent exfoliation and degradation along with calcium carbonate crystallization in various areas. This trend is also seen in the Temp and pH samples, except to a visibly extensive degree. These observations collectively underscore the significant impact of pH and temperature variations on tissue morphology, with increased severity correlating with combined temperature and pH stressors.

The completion of the chlorophyll extraction supports the findings and observations previously mentioned because there is an evident and statistically significant change in the chlorophyll absorbance in comparison to the control group. This indicates that the zooxanthellate were expelled from the corals, which led to them losing their colors and the decrease of chlorophyll witnessed in the groups held under the tested conditions. This supports previous findings linking coral bleaching as a result of the loss of the symbiotic algae.

Therefore, the results and findings of this experiment suggest a dire outlook for coral reefs under projected ocean acidification and rising temperatures by the end of the century. The morphological changes observed in *A. samoensis* tissues exposed to various environmental stressors, including pH, temperature, and combined pH and temperature stressors, provide valuable insights into the early events of cellular degradation. Notably, the micrographs illustrate the detrimental effects of pH conditions, particularly evident through extensive exfoliation, degradation, and calcium carbonate crystallization, which signify significant damage to both living cells and calcium carbonate skeletal structures. While temperature stressors also induce tissue damage, including the rounding up of cells and protein aggregation, the absence of calcium carbonate crystallization in temperature samples highlights the severe impact of ocean acidification on coral health. The chlorophyll extraction data further supports these observations, indicating the expulsion of zooxanthellae and subsequent coral bleaching under stress conditions. To address these alarming findings and mitigate the effects of ocean acidification and rising temperatures on coral reefs, a follow-up experiment focusing on the addition of carbon-fixing plants presents a promising avenue. By exploring the potential of these plants to alleviate environmental stressors and enhance coral resilience, future research endeavors aim to contribute to the conservation and preservation of coral reef ecosystems in the face of climate change-induced challenges.

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