Letters in
General Microbiology

The Roles of Ambient Light on Gymnodinium breve Algae Population Growth

Bushra Khawar, Heather Gallagher, and Alex Gaughan

Department of Biology, Rutgers University, Camden NJ 08102

Abstract

Red tide is a natural occurrence that can be harmful to both humans and marine life. The species *Gymnodinium breve* causes red tide in the Gulf of Mexico. It has been speculated that many environmental factors contribute to the accumulation of algae that cause harmful algae blooms, but very few studies have delved into the effects of light. In the current study, we tested the roles of ambient light on the growth of *G. breve* algae, and found that different spectrum of light play differential roles in the algae growth. The three light conditions tested were red light, blue light, and dark light. The outcome of the experiment demonstrated that blue light resulted in the highest cell count.

Introduction

In many parts of the world, a natural phenomenon often referred to as red tide causes harmful algal blooms (HAB). These blooms can occur year round, but they are more prominent in the late summer months, where the vast amount of algae that accumulates causes the sea water to appear a reddish brown color (Tester 1997). One of the main toxic species of red tide algae called *G. breve* is an autotrophic marine dinoflagelatte that has been found off the coasts of West Florida and the Gulf of Mexico. *G. breve* blooms can be deadly to sea life such as fish, shellfish, birds and other marine organisms as well as the human population due to the brevetoxins that are released in the ocean. In neurons, the toxin binds to voltage-gated sodium channels which lead to depolarization and the continuous activation of cells causing improper nerve communication (Pierce 2008). These toxins essentially damage the fishes' nervous system.

Humans can inadvertently consume these toxins through contaminated seafood and sea spray. Neurotoxic Shellfish Poisoning and Paralytic Shellfish Poisoning can both result from the consumption of infected seafood. People inhale brevetoxins through ocean turbulence and the toxin is released into the air by a bubble-mediated transport (Pierce 2008). A study from 1954 to 2002 observed *Gymnodinium brevis* blooms along the southwest coast of Florida that concluded that the presence of *G. breve* was 13-18 times more plentiful through 1994 to 2002 than the years of 1954-1963 (Brand 2007). Therefore, the harmful blooms have increased significantly over the years.

The cause of red tide is unknown. Scientists have speculated that environmental factors play a role in the occurrence such as temperature and salinity. In a study relating the growth of red tide algae to the temperature and salinities, the red tide dinoflagellate was able to endure high temperatures and salinities, while another red tide species preferred lower temperatures (Kelley 1997). Being aware of the mechanisms and requirements of red tide organisms can predict the severity of HABs and possibly create methods to constitute precautionary measures.

Algae obtain energy through photosynthesis. The harmful algal blooms essentially diminish photosynthesis for these autotrophs (Miao 2012). Photosynthesis is obtained through different light and dark reactions. Each color on the visible light spectrum penetrates to different depths of the ocean. When light hits phytoplankton, it absorbs the warmer colors on the visible light spectrum at the surface of the ocean. Blue light has a higher frequency and a shorter wavelength than green, red, and yellow light. Since the longer wavelengths are absorbed first, the higher intensity and frequency of light such as blue light penetrates the farthest down the ocean. Moreover, the red tide algae are usually present at the top of the ocean surface. Therefore, we propose that light impacts the growth of *G. breve* algae.

Over the years, these harmful algal blooms have been occurring more frequently and the causes of them are still unknown. Most studies performed involved fluorescent and ultraviolet light. These studies tested different algae with LED lights and demonstrated the impact LED lights had on algae behavior (Miao 2012). A study testing the relationship of ambient light with the growth of *G. breve* algae and their toxin production has yet to be performed. Samples of the algae will be placed under different visible

light conditions to see if the number of cells have increased or decreased. This experiment is the first step in understanding how light impacts the production of brevetoxins.

Materials & Methods

Media and Culture

Two test tubes of *G. breve* containing 30 milliliters of the algae were obtained from a company called Carolina, which supplies biological materials. Six containers were autoclaved to assure no other organisms were present. The algae were separated into six containers of purified seawater media. One cup of the sea salt mixture, made by TOPFIN™, makes one gallon of purified seawater. Since only one-fourth of a gallon of the sea salt was required, one-eighth of a cup was mixed in a separate flask. Each of the six containers had 200 milliliters of the purified seawater and 10 milliliters of *G. breve*. Multiple trials are performed by dividing the six containers into two sets of three.

Algae Growth

Algae were grown at room temperature (about 22 degrees Celsius) for seven days (Eng-Wilmot 1977). The second set of containers remained growing at room temperature for another seven days while the first set was placed under the different ambient light conditions. The second set of containers was placed under the light chambers after fourteen days. Two containers were placed under red light, blue light, and no light. The no light was covered entirely by foil.

Growth Rate Measurement

Cell count was performed by extracting a sample from each container with a 20µL micropipette into a hemacytometer. The containers were mixed for approximately thirty seconds, because the algae had a tendency to stay at the bottom of the containers. Three samples were obtained and counted by three separate individuals.

Results

To test the hypothesis that ambient light impacted the growth of *G.breve* algae, the number of cells were counted each day on a hemacytometer. For the first trial, days 5, 6, and 7 were not obtained as well as days 2, 3, and 4 for trial 2. Four containers were placed under red light and blue light and two containers had no exposure to light by foil coverage.

The cell count averages of the three individuals were graphed for each day. The error bars were measured by calculating the standard deviation. The trend for blue light demonstrated an increase in growth. The trend for red light showed a delay of growth, and the algae count decreased when exposed to darkness.

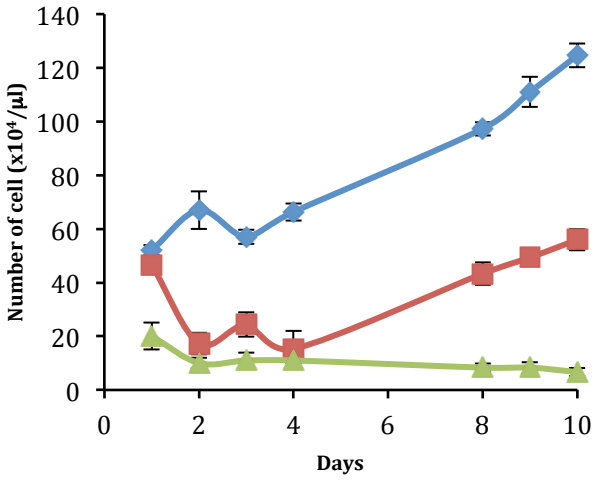

Figure 1. Trial 1. The scatter plot correlates the number of days to the number of cells x10⁴/µl. The blue diamonds represent the cell counts taken under the blue light. The red squares represent the cell counts taken under the red light, and the green triangles represent the cell counts taken in dark conditions. Error bar represents one standard deviation.

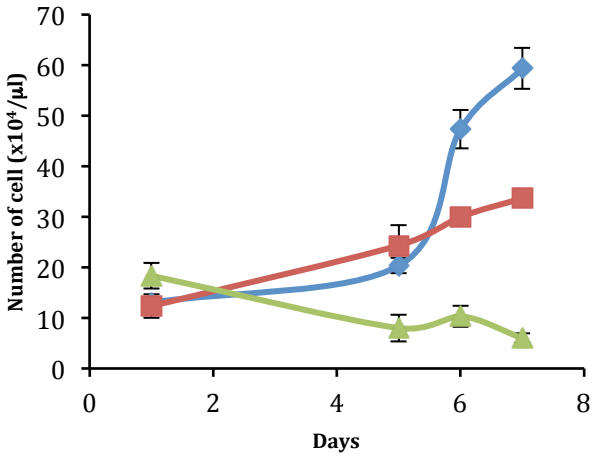

Figure 2. Trial 2. The scatter plot correlates the number of days to the number of cells x10⁴/µl. The blue diamonds represent the cell counts taken under the blue light. The red squares represent the cell counts taken under the red light, and the green triangles represent the dark conditions. Error bar represents one standard deviation.

Discussion

This study found that blue light promoted the most growth, red light promoted a slower growth rate, and dark conditions impaired the growth of *G. breve* algae.

Observations

Under the blue light, there was more movement of the algae, while under red light clumping of the cells was

observed. The "clumping" of cells appeared to be clusters of dead cells. Conversely, in the absence of light, the algae did not show any movement, which can indicate decaying of the cells. The quality, color, and shape of the cells also differed between the conditions. The vast quantity of cells of the blue light exhibited a distinct green color while the cells from the red light were not as distinguishable.

Environmental Errors

Although the repeated experiments showed similar results, there was an inconsistency in the growth under red light. This could be due to experimental errors such as improper mixing of the containers. Also, the cell count differed in the two trials due to the fact that trial 2 was left at room temperature to grow for an additional week.

Throughout the experiment, the contact of red light containers was much colder than the others and the blue light containers were much warmer. Thus, temperature may have affected the experiment. From previous studies, we know that lower temperatures slow down the growth of algae. Light can have a direct relationship with temperature. In order to improve this experiment, temperature would be completely excluded from the equation and the light would be the only factor. If it were possible for the containers to be placed at a constant temperature for the duration of the experiment, the results would have been more effective.

G.breve tended to stick to the bottom of the glass container, which could reveal ways in which the algae invade a fishes' nervous system. It could use this action to stick inside the fishes' gills and cause irritation and eventually death (Watkins 2008). The containers were mixed by hand for 30 seconds each. The duration and speed of the mixing could have varied for each sample, leading to variances in the samples. If the containers were mixed with a machine for exactly 30 seconds each, a more accurate cell count could have been attained. The improper mixing of the cells led to a less accurate cell count averages.

Drawbacks

A major drawback of this study was that it was a class project and only a short amount of time was given. Not all of the experimental conditions were tested. A larger variety of light conditions could have been explored with a number of repeated trials. For the most part, the results were as predicted. Initially, it was assumed that red light might decrease the cell count of the algae due to its low frequency, but red light had actually increased the growth in both trials gradually while blue light showed an immediate growth within twenty-four hours.

Assumptions

Based on results of this experiment, We concluded that light plays a major role in the growth of *G. breve* algae. It can be presumed that the *G. breve* will have more growth on the surface of the ocean, because the visible light frequency is stronger at the ocean's surface. In the current study, blue light exposure resulted in the highest growth rate of *G. breve* and the dark revealed a decrease in the growth. Since the dark container reduced the growth of *G. breve* algae, we can assume that light is necessary for the accumulation of *G.breve* algae along with other factors such as nutrients found in the ocean, salinity, pH, etc. The determination of the environmental factors that cause red tide is difficult to narrow down. Therefore, it is important for future studies to experiment on a combination of environmental conditions.

Future Studies

To further investigate the experiment, there are several ways to analyze the production of brevetoxins such as the ELISA test and receptor binding assay method (Watkins 2008). *G. breve* algae secrete these brevetoxins that can be deadly when ingested by fish and shellfish and can be very harmful to humans. This can shed some light on the relationship between the amount of algae produced and the toxin secretion.

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