RESEARCH ARTICLE

The Climate-Driven Adaptation of Mixotrophic Green *Noctiluca scintillans* in the Arabian Sea

Alisa M. D'Souza
Peekskill High School, 1072 Elm Street, Peekskill, New York, 10566, USA; alisamdsouza@gmail.com

ABSTRACT: Green *Noctiluca scintillans* (*Noctiluca*) is an unusually large mixotrophic dinoflagellate that forms widespread blooms in tropical coastal marine ecosystems. Its recent emergence as the dominant winter-monsoon bloom-forming organism in the Arabian Sea has been attributed to the unique ability of its endosymbionts, *Protoeuglena noctilucae*, present within its symbiosome to photosynthesize more efficiently under suboxic conditions. What also distinguishes green *Noctiluca* from most mixotrophs and other bloom-forming organisms is its ability to persist and thrive as large blooms, even under the most unfavorable nutrient conditions. In this study, we report the results of 1) nitrogenous nutrient enrichment experiments undertaken with a laboratory strain of green *Noctiluca* isolated from the Arabian Sea, and 2) nitrogenous nutrient uptake experiments undertaken with natural populations of green *Noctiluca* sampled off the coast of Oman. Both investigations revealed that this organism has a greater preference for regenerated nitrogenous nutrients, i.e., urea and ammonium (NH_4), as compared to new nitrogen, nitrate (NO_3). What was particularly surprising about this mixotroph, however, was its ability to grow and survive for extended periods, even in the absence of nitrogenous nutrients. In the field, green *Noctiluc*a bloom outbreaks were invariably preceded by extremely high concentrations of urea and NH_4. We also observed that irrespective of the nitrogenous nutrients available to them, green *Noctiluca* cells always accumulated large amounts of NH_4 within their symbiosome, which resulted in sharp increases in seawater NH_4 concentrations (30 to 200 μM) following their demise.

KEYWORDS: Earth and Environmental Sciences; Environmental Effects on Ecosystems; Climate Change; Mixotrophs; *Noctiluca scintillans*.

Introduction

The Arabian Sea (AS) is one of the world's fastest-warming ocean ecosystems. It harbors a permanent oxygen minimum zone (OMZ) that is roughly three times the size of Texas.[1] Warming and circulation processes in the AS are under the control of monsoonal winds which change their direction from southwesterly in summer (June to Sept.) to northeasterly in winter (Nov. to Feb.). The strength of these monsoonal winds is controlled by land-sea pressure gradients, hence the warming and cooling of the land and sea. Therefore, circulation processes and biological productivity in the AS are especially susceptible to global warming and climate change.[2,3] The AS marine and the surrounding terrestrial land biomes are habitats for a rich and diverse array of organisms, from phytoplankton and zooplankton at the base of the food chain to fish and larger marine mammals; it sustains the livelihoods of millions of humans living along the coasts of bordering nations of Oman, Iran, Pakistan, and India (see Figure 1 for AS region map). As such, the AS provides an important source of food and economic opportunity for the littoral populations (both tourism and fisheries are a substantial part of coastal livelihood). Recent research is providing conclusive evidence of alterations in the dynamics of this traditional ecosystem of the AS that impact not only the oceanic processes and organisms that live within the sea but the base (and consequently the rest) of the food chain, with serious socioeconomic implications.

Figure 1: The Arabian Sea region on the map.

Altered nutrient levels and ratios, processes involving certain nutrients and lower trophic level organisms, and food chains are among some of the early ramifications of climate change and other anthropogenic-related pressures in oceans around the world.[4] What makes the AS particularly susceptible to global warming is that it is inherently warm and nutrient impoverished, and its biological productivity is tightly coupled to the strength of monsoonal winds and circulation processes. The OMZ expansion can be largely accredited to 1) ocean stratification, as it inhibits the diffusion of atmospheric oxygen (O_2) into the water column and 2) the overall warming of the sea, because as waters warm, the solubility of O_2 decreases, resulting in a reduced dissolved O_2 content.[5] As a result of O_2 loss, bacteria that rely on dissolved O_2 in seawater rely on the O_2 from the NO_3 dissolved in seawater,

DOI: 10.36838/v4i6.5

causing subsequent NO_3 loss and NH_4 accumulation as the conventional nitrogen cycle is disrupted. In the AS, the accumulation of NH_4 in seawater is compounded by inputs from rivers. These nutrient inputs include both NH_3 and urea from fertilizer runoff, wastewater or sewage treatment plant effluents from large coastal cities, such as Karachi, Mumbai, Aden, Salalah, and Muscat.[6]

Typically, winter monsoonal winds coming from the Himalayan mountains cool the surface of the AS and the surrounding landmasses, which promotes circulation through intensified cooling of surface waters and convective mixing. However, with increased Earth temperatures, the winds coming off the Himalayan mountains have become warmer because of the decline in snow accumulation in the Himalayan mountains. These warmer winds dampen convective mixing, warm the surface waters and further stratify the upper water column, causing a decline in nutrients brought up to the surface waters.[3] Additionally, warmer waters are already observed as climate change increases land surface temperatures, and this, as mentioned before, reduces the amount of O_2 the AS can hold and expands the OMZ.

In addition to the abnormal patterns of convective mixing, water column stratification and OMZ expansion, the increasing presence of harmful algal blooms (HABs) forms yet another threat to the sea and the surrounding area. HABs are caused by the rapid growth of algae composed of either cyanobacteria, dinoflagellates, or diatoms that coat water surfaces; their impacts can range from being small disturbances of the food chain to massive fish mortality when they are toxic. In the AS, the spatial distribution and growth of HABs are influenced by climate change, freshwater sources, monsoonal patterns, changes in convective mixing patterns/currents, seasonal temperatures, wind direction, and velocity.[6-9]

HAB transportation and growth are also heavily determined by the nutrient levels and temperatures of the water, tying in climate change and human activities. Natural dust deposition, monsoons, and cold eddies can impact nutrient levels as well. Climate-related changes can also break down natural biogeographical barriers with intensified storms, a higher frequency of hurricanes, and more severe floods. These barrier breakdowns can lead to increased HAB outbreaks and transportation as well.[10] HABs have been seen across the Arabian Sea since 1908, and cause several problems for power plants, local schools and children, the region's tourist rates and economy, and the environment and ecosystem that host the blooms.[6]

HABs can affect the surrounding area in 3 major ways: the water quality, economy/socioeconomics, and of course the ecosystem. Water quality: The water quality of an area can be compromised as the blooms thrive and potentially clog desalination and other water treatment plants. The toxins that some HABs release can harm, or even kill, fish and other animals, including humans if they consume something that has been contaminated. Not all blooms are toxin-producing, though, and can instead harm water quality and the ecosystem by blocking light from passing through the surface, clogging fish gills and desalination plants, and providing burdens for the traditional organisms in the ecosystem through its dominance[11]

Economy: This can include tourism and fisheries. For tourism, HABs are unattractive with their foul smell, and their coating of water surfaces can prevent recreational swimming, fishing, and other activities. For fisheries, HABs are linked to fish mortalities and less fish diversity (either through deoxygenation or toxin releases), various health issues in humans, as well as an increase in fish prices because of less availability.[1] The presence of HAB– and their impacts- thus provide challenges for the area's socioeconomic system, especially for those who rely on tourism and fishing for livelihood, which are primarily the countries and coastal cities that border the AS (known as "rim countries").[9]

Ecosystem: If HABs persist, they have the potential of "taking over" the region's ecosystem and worsening their impacts. With an unusual abundance of phytoplankton at the base of the food chain comes disruption of the balance of the rest of the ecosystem; the amplification of toxins that some HABs contain (as previously mentioned) can also harm the ecosystem's health. HAB decay draws out oxygen from the water, and HABs thrive off certain nutrients, so there is also a nutrient disruption that affects other organisms and processes in the ecosystem.[12]

One of the most prominent HABs in the Arabian Sea is green *Noctiluca scintillans* (*Noctiluca*), which has had an increasing presence there during the winter monsoons since the early 2000s (see Figures 2a-c). There is also the red *Noctiluca scintillans* (red *Noctiluca*), which is a heterotrophic grazing organism, that consumes microzooplankton and does not photosynthesize. Red *Noctiluca* thrives in higher salinity waters, at 10-25°C.[13]

As a mixotroph, green *Noctiluca*, in contrast to the red variety, has a competitive advantage over most other lower trophic organisms, because it can sustain itself 1) by feeding on other organisms because of its innate heterotrophic trait, and 2) through photosynthesis and nutrient cycling provided by its autotrophic endosymbiont, *Protoeuglena noctilucae*.[14]

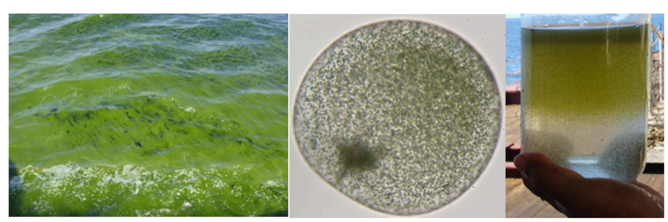

Figure 2a: Bloom image of natural populations of *Noctiluca*.
Figure 2b: A *Noctiluca* cell under the microscope with algal endosymbiont *P. noctilucae*.
Figure 2c: Sampled *Noctiluca* from a bloom.

It is interesting to note that *P. noctilucae* evolved in a low oxygenated (oxygen, O_2), highly carbonated (carbon dioxide, CO_2), environment 1.3 billion years ago, whereas *Noctiluca* is a rather new organism (dating back to only a few hundred years ago). *Noctiluca* thus depends on this *P. noctilucae* to help it adapt to a changing world.[1] These two organisms live in a mutualistic symbiosis, and the unique nutrition acquisition and recycling of *P. noctilucae* allows *Noctiluca* to survive and thrive in conditions where other organisms would not. The mixotrophy of *Noctiluca* has been studied under variable prey types and light concentrations, showing that *Noctiluca* can sustain life for prolonged periods with its endosymbionts. The study showed that without an external prey, *Noctiluca* growth rates were less than when fed with a prey.[14] *Noctiluca* endosymbionts also had carbon fixation rates between 25 and 300% higher in waters with low dissolved O_2 concentrations (i.e., near hypoxic conditions) than in ambient O_2 concentrated water. These results point to *Noctiluca* having a competitive advantage over other (non-mixotrophic) marine organisms which cannot keep up with the trend toward low O_2 levels in the AS.

However, there is no clear consensus regarding the specific environmental drivers for the annual blooms of green *Noctiluca* in the AS. For instance, a study conducted in the Northeastern AS (NEAS) found that the blooms of *Noctiluca* are not related to hypoxia, but are rather stimulated by intensified surface water stratification due to warmer waters from climate change.[15] The authors used in situ and satellite algorithm data to analyze bloom locations and cell abundance populations of *Noctiluca* and diatoms. In their analysis of water column environmental characteristics (temperature, salinity, dissolved O_2, *chl-a*), the authors found neither hypoxic nor anoxic waters in the sea, but rather comparatively high dissolved O_2 levels. This refuted the link between *Noctiluca* and low O_2 levels, even saying that "the suggestion by Gomes *et al*. (2014) that this linkage [between cultural eutrophication and blooms of *Noctiluca*] also can extend far out into oceanic waters is questionable". It may be noted that, unlike the contrasting studies, these findings were not based on *Noctiluca* with photo-physiological experiments but casual presence or absence of *Noctiluca* at different locations measured from space and few shipboard measurements. *Noctiluca* are free-floating organisms and can be easily transported by ocean currents to a different location from their point of origin.

Another study conducted in the NEAS also refuted the possibility of *Noctiluca* being tied to hypoxia, as well as sewage/anthropogenic outputs as a foundation for the blooms. The authors stated that convective mixing and stratification arising from climate change is the bigger and more likely issue than hypoxia, and hypoxia was found to be nonexistent in the sea.[16] Contradicting methods and results of studies on *Noctiluca* blooms prevent a full and ubiquitous understanding of overgrowth, specifically as they relate to causes and effects. These research gaps are not exclusive to *Noctiluca*; research has found that there are gaps in current knowledge about the overall effects of climate change and climate change-related pressures on marine ecosystems, most notably in responses to ocean acidification and O_2 levels.[17]

Despite the dispute over the origins of *Noctiluca*'s domination, it is clear that its relatively recent abundance during the winter monsoons has led to a shift in the base of the food chain (traditionally dominated by diatoms) and further depletion of O_2 in the sea, through its rapid decay and decomposition by bacteria.[1] These studies also show that as the sea may be shifting towards scenarios that are often not to the aid of conventional organisms, whatever they may be, green *Noctiluca* instead proliferates as a mixotroph.

To help better understand the environmental drivers behind the massive blooms of *Noctiluca*, a study was conducted from the end of August 2019 to February 2020, using previously collected mentor-provided research data from Oman and student-done lab data and analysis at the Lamont-Doherty Earth Observatory. Its research questions were: what are the nitrogenous nutrient preferences that allow *Noctiluca* to survive for extended periods? Does mixotrophy offer *Noctiluca* a competitive advantage for growth and survival over other phytoplankton especially when nitrogenous nutrients are unavailable? The goal was a better understanding of the blooms and future consequences for the ecosystem. This would help solve the problem of dangerous HAB overgrowth disrupting marine food chains, threatening fisheries (which increases economic pressures and food insecurity), and harming the water quality of oceanic ecosystems.

More specifically, this study aimed to examine how green *Noctiluca* is impacted by changes in nitrogen types, and which nitrogen type was preferred (new versus recycled/regenerated), because nitrogen pertains largely to other algae bloom growth and the changing of ecosystems. Embedded in the broad purpose of this study was to draw attention to the role of cultural eutrophication and whether NH_4 and urea from agricultural fertilizer runoff and sewage treatment plans could be contributing to *Noctiluca* bloom outbreaks. Concurrent with typical HAB behavior and research on other HABs, I hypothesized that *Noctiluca* grows faster in the presence of NH_4 and urea than in naturally occurring $NaNO_3$. I also hypothesized that in addition to the rapid growth of *Noctiluca*, its propensity to accumulate NH_4 made its presence as blooms unfavorable for the growth of other phytoplankton.

Statement of Purpose/ Societal Relevance:

To further note the importance of the results of this study and the impact *Noctiluca* has, especially in a changing environment, the focus of this section will be the societal relevance and connections of these laboratory and field results to the world *Noctiluca* and HAB research. Firstly, one must take worldwide HABs into consideration, both including and asides from *Noctiluca*. *Noctiluca* predominates mainly in warm, tropical waters. Blooms of *Noctiluca* appear with an increasing presence in the Yellow Sea, East China Sea, and the Arabian Sea/Oman region, and interestingly enough save proven to be detrimental to ecosystem health in all three of these regions.

The East China Sea *Noctiluca* HABs, though not inherently toxic, release large amounts of NO_4 as they consume toxic algae. High levels of NO_4 can be toxic to other phytoplankton. These blooms of *Noctiluca* can affect the waters as a traditional HAB does, and the reduced O_2 levels from aerobic respiration further threaten marine life. Although it is not certain, this phenomenon is believed to be linked to anthropogenic activities and outputs, as nutrients from sewage runoff fuel blooms further, similar to the growth patterns of *Noctiluca* in the AS.[18] Similarly, the Yellow Sea faces blooms of *Enteromorpha prolifera*, where the algae are also not toxic, but the sheer size and development of the blooms over the sea is detrimental to eco-

DOI: 10.36838/v4i6.5

system health. This bloom is yet another example of sewage outputs and nutrient disruptions leading to negative impacts on a body of water and its surrounding area due to subsequent HAB ououtbreaks[9] Perhaps more widely known are the blooms of Florida and Mexico, Massachusetts, and the Long Island Sound, composed of cyanobacteria and toxic dinoflagellates. The blooms around the Florida/Gulf of Mexico region stem from anthropogenic septic tanks and fertilizer runoff; those in Massachusetts have sources such as warmer waters and disrupted nutrient levels. Similarly, the suffocating Long Island Sound "red tide" bloom is linked to warmer waters and increased nutrient concentrations, such as nitrogen, phosphorus, and silicate.[20]

As discussed above, major HABs are linked to the same causes, what do all of these bloom examples mean for the world, and why do they matter? These HABs, much like green *Noctiluca* in the AS, often draw out the O_2 levels in the water and, because they live off of output nutrients, disrupt the nutrient levels further. In general, blooms are linked to low O_2 in the region, leading to the suffocation of marine animals and the detriment of other organisms such as lobsters, crabs, squids, and coccolithophores, especially paired with ocean acidification.[21] As aquatic species are put at risk, the humans in the surrounding areas suffer a loss in food sources and economic opportunity, especially in areas that heavily rely on the sea for tourism, fishing, or similar activities. HAB-contaminated water can also lead to various illnesses and poisoning from toxins, limiting the amount of non-polluted water available, as rivers, lakes, streams, and ponds are already filled with chemical outputs.

These are only a few examples of algae blooms; HABS coat the surface layers of water globally, and while they have been noticed throughout history (dating to 1908 as previously stated), knowledge is even more imperative as existing nutrient ratios and climate conditions worsen. The described ecosystems suffer calamity from the blooms, and all of them are linked to some form of pollution/anthropogenic outputs and warmer waters. Knowledge on the formation and presence of some HAB blooms, the effects they have on us humans, and information on how to limit the recurrences of the blooms should be well spread to create a more informed and conscientious society. This study was done to help increase research on the now annual blooms of *Noctiluca*, as contradicting research, as well as the ubiquity of *Noctiluca* and other HABs themselves, prevent consensus and provide concern for the future if not remedied.

■ Methods

Study Area:

Student laboratory work ("we"; "I") was done in the Lamont-Doherty Earth Observatory research unit of Columbia University, where two labs were used throughout the experiment. One was for most of the experiment (incubating cells, counting cells, FIRe, data, and graph analysis), and the other was for more of the "prep" work (such as filtering and microwaving seawater). My mentor collected data from sampling in the field (off the coast of Oman) during the Jan-Feb *Noctiluca* bloom of 2018 which allowed me to compare my laboratory results with the field observations. These data included microscopic counts of *Noctiluca* and other phytoplankton, nutrients (NH_4, urea, $NaNO_3$, phosphate), and chlorophyll.

The area sampled by researchers during the time of collection (23rd Jan-10th Feb 2018) is known for having stable waters, abundant nutrients, optimal growth temperatures for *Noctiluca* within the 5 months of *Noctiluca* blooms (roughly 26-27 ºC), and good salinity because of the antecedent winter monsoonal period. Samples were gathered from the peak *Noctiluca* bloom, and Figure 3 shows the details of the collection. 3A shows the chlorophyll levels, and the red areas indicate a high level of chlorophyll, which determined where the researchers sampled. 3B shows cyclonic eddies in the red swirls. As they move in a counterclockwise motion, this is a cyclonic eddy that brings up nitrogen-rich and low-oxygen waters, which prompted researchers to examine how the nutrients impact *Noctiluca* growth, which was then further proved by the lab results.

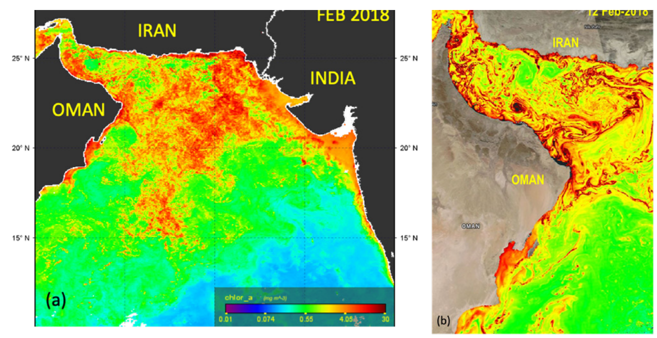

Figure 3: *Noctiluca* blooms as viewed from space by the Ocean Color Satellites (a) Monthly composite- NASA MODIS-Aqua (ocean color satellite) and (b) Daily image- NOAA VIIRS.

Cell Culture and Counts:

As an intern, I used lab-cultivated cells of *Noctiluca* previously collected from the Arabian Sea and placed them into 4 media types, 3 of which were a form of nitrogen and 1 was a control group. We used NH_4 chloride, sodium nitrate ($NaNO_3$), and urea as the tested variables, with urea and NH_4 as "regenerated" nitrogen types and $NaNO_3$ as "new" nitrogen; microwaved filtered seawater was the control to compare "normal" *Noctiluca* growth versus the nitrogen-impacted cells. CAUTION: Low concentrations of each of the hazardous chemicals (the tested/independent variables) were used, so there was no safety risk. Although, in the case of overexposure, irritation can occur to the eyes, skin, and respiratory system. NH_4 chloride can be the cause of all three, $NaNO_3$ for the eyes, and urea for the respiratory system. Chemicals were properly disposed of using mentor and SDS guidance, and nitrile gloves and standard laboratory attire (long pants, closed shoes, hair pulled back) were worn. Each nitrogen type (and the control) was used as a media in which the cells were allowed to grow for the duration of the experiment. The experimental flasks were labeled based on their nitrogen content, with three replicate treatments (3 separate flasks per nitrogen type) each labeled from A-C (Figure 4). The flasks were not hooked up to any gas chambers, as the only variable in the experiment was the nitrogen type that was in the media. In total, twelve bottles were filled with their respective media and placed in an incubator maintained at around 27 degrees Celsius with a 300 mE m^{-2} s^{-1}, 14:10 (Light: Dark) cycle. When ready for analysis, we took cell counts and photosynthetic electron transport rates to assess the cell health and growth over four sampling days, and below are the details of the measurements.

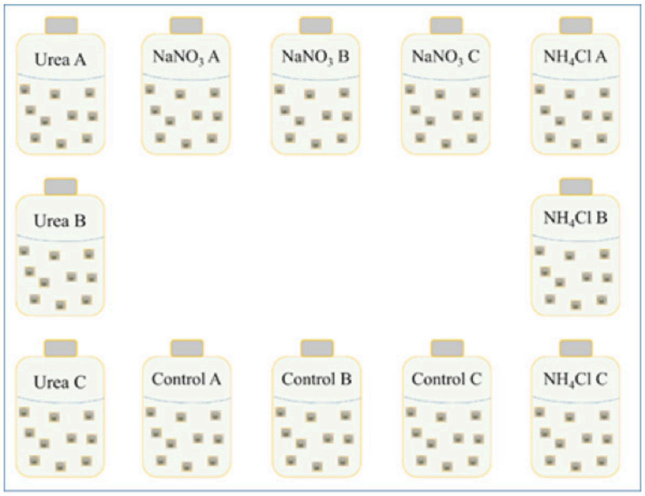

Figure 4: Experimental bottle setup of the cultures of *Noctiluca*; NH_4Cl represents NH_4 chloride, $NaNO_3$ is sodium nitrate, and urea is urea.

For the lab experiments: *Noctiluca* was enumerated manually in a 5mL pipette with the naked eye on sampling days.

Field: *Noctiluca* cells were counted after being collected from the bloom.

Changes in Noctiluca and Protoeuglena photo-physiology using Fluorescence Induction and Relaxation (FIRe) measurements:

At the start and throughout the experiment, I measured the photo-physiology and growth rates of *Noctiluca* and the free-living endosymbionts. For *Noctiluca*, 5 cells were picked up carefully with a dropper, washed with filtered seawater, and then transferred into 3ml of media. In the case of *P. noctilucae*, we used 3ml of only media from which all the *Noctiluca* cells were carefully removed. The FIRe provides a measure of bulk fluorescence (F_m) which can be related to the chlorophyll content of the *Noctiluca* cells or the endosymbionts that are released into the medium. Variable fluorescence (F_v/F_m) provides a measure of the health of the cells.

After the samples were processed in the FIRe, the cells of *Noctiluca* and that of the *P. noctilucae* were filtered on Whatman brand glass filters separately. After filtration, the filters were carefully stored in labeled histocaps in a -80ºC freezer until the time of analysis. When ready for analysis, the filters were placed in a small 5 ml tube that contained cold 90% acetone to extract the chlorophyll from the filters. CAUTION: acetone can irritate the eyes, skin dryness or crack in the case of repeated exposure, drowsiness and dizziness from vapor exposure, and– in high concentrations– harm to the nervous system. Students took precautions by doing all the transfers of tilters into acetone in a fume hood and did not experience any of the symptoms of overexposure or irritation related to the acetone.

Results

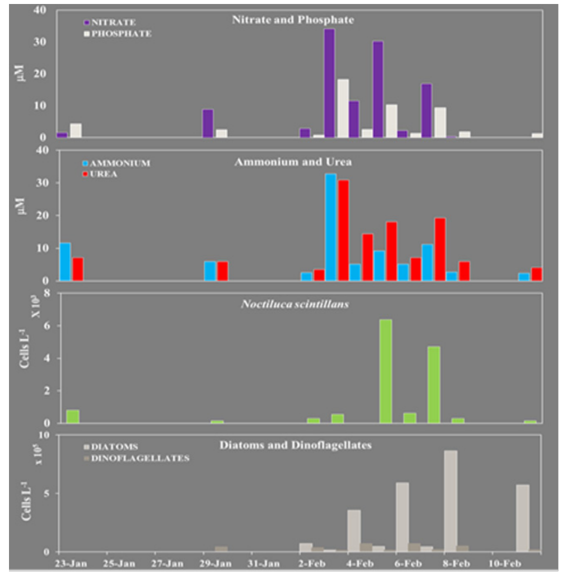

Figure 5: *Noctiluca* bloom peaks with increases of NH_4 and urea in the water column, and there was an increased diatom presence after *Noctiluca*'s demise.

Figure 5 depicts the cell counts from the field studies in Oman of populations of *Noctiluca* and other diatoms and dinoflagellates, against the $NaNO_3$, phosphate, NH_4, and urea (in µm) of the area surrounding the blooms. The collection was from January 23 to February 10, 2018.

The field ecological studies undertaken along the coast of Oman revealed peaks of *Noctiluca* blooms (of 4 and 6-7 x 10^3 cells per liter) when there was an increase in NH_4 and urea in the water column (around 30µm). We also see that after the demise of *Noctiluca* blooms, there is an increased presence of diatoms. Diatom presence was around 6 cells per liter (February 6) after the demise of the 6 cells per liter *Noctiluca* peak, about 8 x 10^3 cells per liter (February 8) after the demise of the 4 x 10^3 cells per liter *Noctiluca* peak, and about 5 x 10^3 cells per liter just around when *Noctiluca* existed at near 0 cells per liter levels (February 10). Other dinoflagellates (represented with the darker grey) do not seem to be particularly affected by the presence of *Noctiluca* or NH_4 and urea.

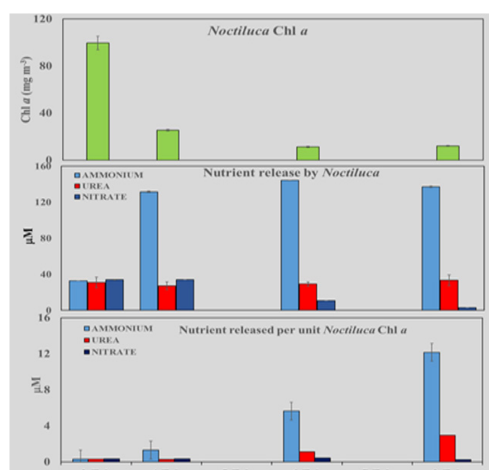

Figure 6: *Noctiluca* cells released a substantial amount of NH_4 upon their demise.

DOI: 10.36838/v4i6.5

The chlorophyll-a (*chl-a*) content of *Noctiluca* about the nutrient concentrations of NH_4, $NaNO_3$, and urea by *Noctiluca* (measured in μm), as well as the nutrient release in comparison to the unit chl levels of *Noctiluca*.

Figure 6 of the *Noctiluca* cells collected from the field bloom in Oman shows that the cells released a substantial amount of NH_4 (that accumulates in their central cytoplasm) upon their demise and decay. Before *Noctiluca*'s decay, released NH_4 was as nearly as high as 160μm, whereas released urea and $NaNO_3$ were only at most near 40μm.

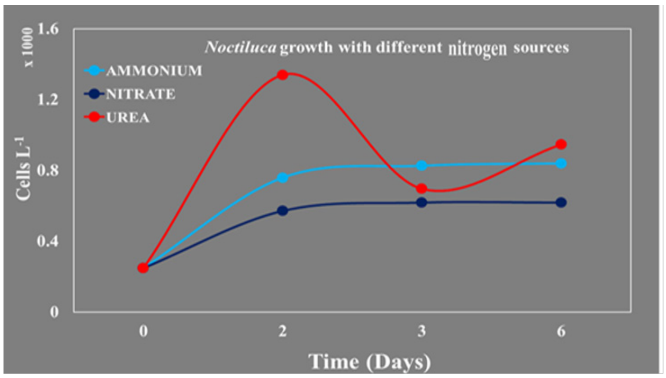

Figure 7: The laboratory cell counts of *Noctiluca*, under each of the three, tested nitrogen variables (NH_4, $NaNO_3$, and urea) in cells per liter, over time (each of the sampling days).

In the laboratory culture experiments, cells treated with urea generally had the highest growth, while $NaNO_3$ witnessed the least. Urea-treated cells show a maximum growth of over 1.2 x 10^3 cells per day, whereas NH_4-treated cells and urea-treated cells only reached a maximum of about 0.8 and 0.6 x 10^3 cells per day, respectively. Though not included in the graph, the control group faltered on the last day (Figure 7).

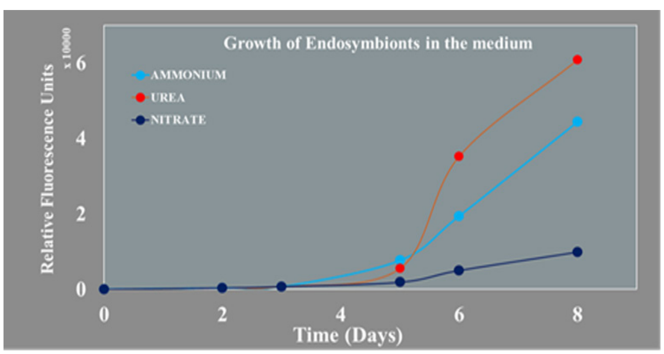

Figure 8: The growth of the endosymbionts, *Protoeuglena noctilucae*, is measured by relative fluorescence, in each media type (the variable nitrogen types: NH_4, $NaNO_3$, and urea) over time (each of the sampling days).

Figure 8 shows that when grown in urea and NH_3, the endosymbionts left the host *Noctiluca* cells and grew at a significantly better rate than when grown in $NaNO_3$. On day 8, the relative fluorescence of *Noctiluca* reached 6 units when treated with urea, 4 units when treated with NH_4, and not even 2 units when treated with $NaNO_3$. When paired with Figure 8, we see that both the host and the endosymbionts have higher growth rates in the presence of urea and NH_4.

Discussion and Conclusion

These experiments showed that 1) *Noctiluca* has a preference for "regenerated nitrogen" (urea and NH_4) in comparison to "new nitrogen" (nitrate) both in the lab and in the field, and 2), *Noctiluca* was able to grow and survive for extended periods, 3) green *Noctiluca* cells accumulated large amounts of NH_4 within their symbiosome, which resulted in sharp increases in seawater NH_4 concentrations (30 to 200 μM) following their demise. Our findings suggest that *Noctiluca* has a stronger preference for regenerated nitrogenous nutrients, i.e., urea and NH_4 over $NaNO_3$, in terms of its growth and NH_4 accumulation.

Figure 5 suggests that the *Noctiluca* blooms are triggered by NH_4 and urea and that diatoms and *Noctiluca* may live in an inverse relationship, where *Noctiluc*a may be able to flourish during times of low diatom presence, and vice-versa is true for diatoms. When you pair Figure 6 with Figure 5, which showed that *Noctiluca* blooms are succeeded by diatoms, we can connect this to the stability of the water column and its relation to the nutrient outputs. If the water column is not stable (meaning there is more convective mixing), that would make the nutrients less concentrated in the area and therefore allow for the diatoms to flourish after *Noctiluca*'s demise.

As for the laboratory results, Figure 7 depicts *Noctiluca* growth being most efficient in the urea and NH_4 treatments, coincident with the growth responses of the endosymbiont *P. noctilucae* in Figure 8. What is important to note about Figure 8 is that those endosymbionts measured were from the media, indicating that the endosymbionts may prefer the external conditions to what is provided inside of the host. Particularly surprising about this mixotroph and the results as a whole is the ability of *Noctiluca* to grow and survive for extended periods even in the absence of nitrogenous nutrients. It is possible that *Noctiluca* rejected the endosymbionts, though, considering the advantages *P. noctilucae* provides for the host *Noctiluca*, it may not be so.

These results also lend credence to the idea that urea and NH_4 released from land-based activities by humans may be contributing to outbreaks of *Noctiluca*. Further studies will be needed to ascertain this hypothesis.

As mentioned previously, contradicting research is a common issue in marine and HAB research, so steps should be taken to limit the conflicting results from different studies, both through focused laboratory experiments on *Noctiluca* and other HABs. Having a plethora of "significant results" from multiple studies that lead science in miscellaneous directions is no better than only a few elements of research. The short time frame of this laboratory work may provide a limitation specifically for this research study. Moving forward, we would like to reassess the patterns noticed by including more sampling dates to assess patterns over a longer period—potentially 2-3 months, like that around the duration of natural *Noctiluca* blooms—as well as evaluate the different methods used in HAB and *Noctiluca* research and see if implementing different methodologies to this study would lead to a significantly different outcome. Additionally, the field and lab experiments' observations and data provided valuable insight into *Noctiluca* and its endosymbionts, and how growth and physiological processes are affected by changing oceanic conditions and nitrogenous levels, but stable isotopes should also be taken into consideration to examine more *Noctiluca*'s internal processes.

HABs coat the surface layers of water globally, and while their overgrowth has been observed throughout history, knowledge of their impacts on ecosystems is even more imperative as existing nutrient ratios and climate conditions worsen. The described ecosystems suffer calamity from the blooms, and all of them are linked to some form of pollution/anthropogenic outputs and warmer waters. As for *Noctiluca*, the sources of the nutrients studied, including urea and NH_4, could include anthropogenic activity, such as fertilizer runoff and effluents from wastewater and sewage treatment plants.

The year-on-year increase in *Noctiluca* blooms in the AS appears to also be tied to climate change, because of the relationship between the OMZ and water warming, then the OMZ and the nitrogen fluxes, and then the nitrogen changes within *Noctiluca*'s responses. Knowledge on the formation and presence of some HAB blooms and the effects they have on us is well-studied and information should be well spread to limit the recurrences of the blooms. This is especially important for the populations around the AS, who need to be informed of the impact of their daily lives on the environment, and consequently themselves with the occurrence of *Noctiluca*. Together the scientific community, the public, and governmental agencies can develop plans to prevent further damage to the delicate balance of the Arabian Sea and local land ecosystems.

Acknowledgments

I would like to thank Lamont Doherty Earth Observatory at Columbia University for the opportunity to conduct this research. I am very grateful to Ms. Kali McKee, Dr. Helga does Rosario Gomes and Dr. Joaquim Goes for mentoring me during this study, and they were all a great help in both this experiment and previous ones. Additional thanks to Mrs. Erum Hadi, Science Research Teacher at Peekskill High School, for guiding me in my work throughout the school year. Thank you also to Khalid Al-Hashmi, Sultan Qaboos University, and the Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman for their help with this work and field data. We are grateful to NASA Applied Sciences, the Gordon Betty Moore Foundation, and the Sultan Qaboos Cultural Centre for their support of this research study.

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Author

Alisa D'Souza graduated from Peekskill High School in 2021 with advanced designation and math and science mastery; she won 4 total awards at the Westchester Science and Engineering Fair and the Tri-County Science Fair for this research. Alisa will attend Stony Brook University, majoring in marine science.