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Adaptive significance and abundance of bioluminescent dinoflagellates in the Cuajiniquil Bay

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Abstract

Bioluminescence in nature is a phenomenon that is found in many different organisms, including fungi, fish and bacteria. Of bioluminescent organisms, dinoflagellates are some of the most common. Despite their abundance, the ecological function of the bioluminescence in dinoflagellates is not fully understood. In my study I attempted to explain the ecological function of this bioluminescence by observing dinoflagellate abundances at different times of day. I collected water samples in the Cuajiniquil Bay in Costa Rica, over the course of seven days, and counted the total number of organisms that I found.

This was done to try to find a relationship between time of day and abundance of dinoflagellates. In my study I found that dinoflagellate abundance is generally higher at night than during the day. This finding supports my hypothesis that bioluminescence is a predator defense. Their bioluminescence may be more effective when it's dark, making them less susceptible to predators.

Significancia adaptativa y abundancia de dinoflagelados bioluminiscentes en la Bahía de Cuajiniquil

Resumen

La bioluminiscencia en la naturaleza es un fenómeno que ocurre en muchos organismos. Entre los organismos bioluminiscentes, los dinoflagelados son algunos de los más comunes. A pesar de su abundancia, la función ecológica de la bioluminiscencia en dinoflagelados no se comprende completamente. En mi estudio, intenté explicar la función ecológica de esta bioluminiscencia, observando las abundancias de los dinoflagelados a diferentes horas del día. Durante el transcurso de siete días, recolecté muestras de agua en la Bahía Cuajiniquil en Costa Rica y conté el número total de organismos que encontré. Esto se hizo para tratar de determinar si existe una relación entre la hora del día y la abundancia de los dinoflagelados. En mi estudio, encontré que la abundancia de los dinoflagelados generalmente es más alta por la noche que durante el día. Este hallazgo apoya mi hipótesis de que la bioluminiscencia es una defensa contra los depredadores. Su bioluminiscencia puede ser más efectiva cuando está oscuro, lo que los hace menos susceptibles a los depredadores.

Introduction

Bioluminescence is the production of light by a living organism, and is a widespread phenomenon in the natural world. Multiple organisms, from deep-sea fish and marine bacteria, to fireflies and fungi, produce bioluminescence. Marine bioluminescence is particularly widespread, and attracts the study of many researchers. In the ocean, bioluminescence has evolved many times and spans all oceanic dimensions (Haddock 2009). There are examples of light production across a broad range of organisms including fish, squid, bacteria and protists (Haddock 2009). Marine bioluminescence is primarily blue, and therefore tuned to the wavelength that travels best in water (Valiadi 2013). According to Haddock (2009), Dinoflagellates are the main marine eukaryotic protists that are capable of bioluminescence. Despite the abundance of these dinoflagellates, the ecological function of their bioluminescence has not been fully studied.

The function of marine bioluminescence is best studied in deep-sea fauna and in bacteria. For these creatures it plays a range of important roles, including oxygen defense, predator avoidance, camouflage and courtship (Valiadi 2013). However, according to Valiadi (2013) "the function of bioluminescence in dinoflagellates has been less extensively assessed, and the theoretical concepts that have been put forward are supported by limited experimental evidence". The main theory put forward for dinoflagellate bioluminescence is that it acts as a predator defense. In a study by Esaias et al. (1972), rates of ingestion of dinoflagellates by copepods were measured in populations of dinoflagellates with differing bioluminescent capabilities. The study found that populations with higher bioluminescence were ingested less then the ones with lower bioluminescence. Esaias' experiment, while conclusive, is singular and needs further testing. Another example for dinoflagellate bioluminescence as a predator defense is that it acts as a kind of "burglar alarm" (Burkenroad 1943). The idea suggests that when a predating grazer stimulates a flash, it will attract a higher-level predator that will then consume the grazer. Both of these ideas support the theory of predator defense, but could benefit from additional testing.

For my study on dinoflagellate bioluminescence I did my research in the Cuajiniquil Bay, in Northwest Costa Rica. This area of Costa Rica has a population of bioluminescent dinoflagellates, most likely of the genus *Noctiluca. Noctiluca* are large (0.2-2mm in diameter) heterotrophic dinoflagellates. They are nonparasitic, free-living, and marine dwelling. They are a single celled protist, and produce light using spherical organelles known as scillitons. A key piece of information for my study is that *Noctiluca* can move through the water column. *Noctiluca* are non-motile, meaning that they do not actively swim; they do, however, move vertically through the water column by changing their buoyancy (Khan 1978).

My study attempted to answer the question, "what is the adaptive significance of dinoflagellate bioluminescence?" To address this question I hypothesized that bioluminescence is a predator defense. To test this hypothesis hypothesis, I considered what predictions would support it. Knowing that dinoflagellates have the ability to move throughout the water column, I reached another question in my study; is their abundance dependent on whether it's day or night? To support the hypothesis of bioluminescence being a predator defense, I predicted that I would find more dinoflagellates in the water column at night. If dinoflagellate abundances are higher at night, it may be because their bioluminescence is more effective as a predator defense in the dark.

Materials and Methods

In order to test my hypothesis, I collected concentrated dinoflagellate samples near the water's surface at different time intervals. All samples collected came from the water near the dock in Cuajiniquil, Costa Rica.

Collection- Between 18 Nov and 25 Nov 2017, I collected a total of 14 samples at different times of day and night. My samples fall in to 3 basic categories; morning (between 8 AM and 9 AM), afternoon (between 2 PM and 4 PM) and night (between 7PM and 8 PM).

Table 1: Dates and times of collections. Checkmarks indicate on what day and at what time a sample was taken

Date:	18 Nov	19 Nov	20 Nov	21 Nov	23 Nov	24 Nov	25 Nov
Morning	✔	✔		✔	✔	✔	✔
Afternoon	✔	✔	✔				
Night	✔	✔	✔		✔		✔

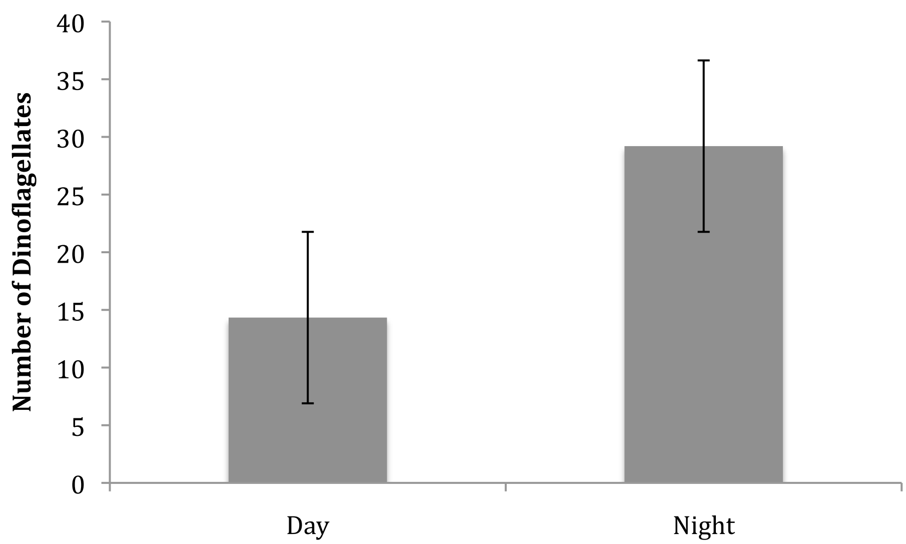
I standardized my collecting procedure to reduce variation from unknown sources. I started by marking out a 50m distance using buoys in the water near the dock. Then I took the kayak out, and while towing the plankton net with a sample cup attached, paddled four lengths between the buoys for a total of 200m. The plankton net I was towing always stayed near the kayak, within 30cm of the surface. I would then immediately cap the sample and bring it back to the study center (Casa Verde) for observation. While taking my samples, I made sure to note time of collection, tide and ocean temperature. With respect to tide, all samples were taken within 2 hours of high or low tide, allowing me to categorize them as either high tide or low tide samples. Ocean temperature data was collected from a device 2km from my study site that records temperature accurate to a tenth of a degree °C every 30 minutes. Temperature data was not available for 24 and 25 November.

Observation- Making sure the samples were fresh, I observed them using a dissecting microscope. I took my sample jars and transferred approx. 10ml of sample at a time to a petri dish for counting. For my searches I counted only organisms that I could identify as dinoflagellates with certainty. I used a grid on the scope to make sure that I performed a methodical search and did not miss any organisms. After going through an entire sample, I would record the total number of organisms found. Near the end of my study, I noticed that I was finding two different morphospecies that were producing bioluminescence. Once I realized this, I started separating my counts into two separate categories. The organisms that were clearly the genus *Noctiluca,* based on their shape and flagellum, I designated with the name “dinoflagellates”. The other species, which were more round, transparent and lacked the flagellum, I designated “flage-less”.

For my statistical analysis, I used both a T-Test and an ANOVA. I used these tests on the day and night sample populations because I wanted to see if there were similar to a t-test known as a Mann-Whitney test to see if I got any different results.

Results

On average, more dinoflagellates were found at night than during the day (Figure 1). In addition, regarding sample sets containing both a morning and night sample from the same day, three out of those four had higher dinoflagellate abundance at night. (Figure 2).



Average Abundance of Dinoflagellates at Day and Night

Figure 1. Comparison of abundance of day (morning only) samples and night samples. Average for day is 14.3 (n=6). Average for night is 29.2 (n=5).

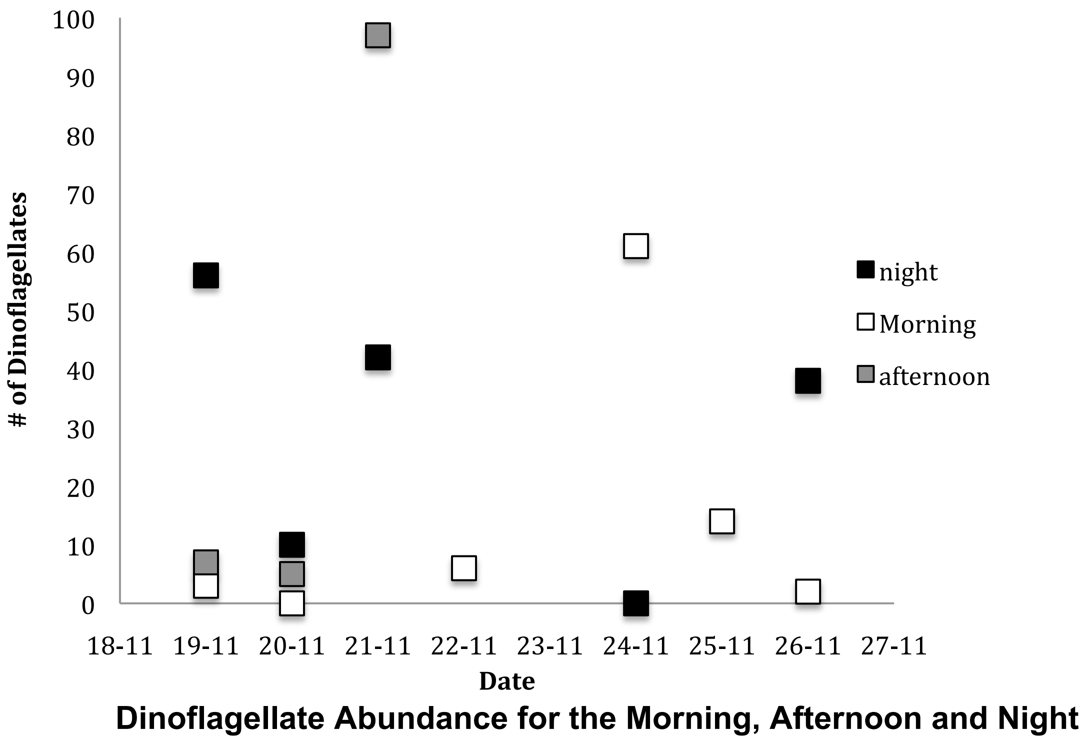


Figure 2. Per day comparison of dinoflagellate abundances. On 18 Nov, 19 Nov and 25 Nov counts were higher at night vs. during in the morning. On 23 Nov count was higher in the morning. Of afternoon counts, one was higher than night, while other two were lower.

Although the averages were higher at night vs. the day, they are not statistically significant. Both values from the ANOVA (F=0.606, p=.563) and T-Test (p=.321) show that the results are not significant. The non-parametric Mann-Whitney test gave me a U value of 10.5, which is also considered not significant.

I recorded the tide level at time of collection as well. There was no difference in the average number of dinoflagellates collected between low and high tide. Low tide had an average of 27.4 organisms per sample, and high tide had an average of 24.8 organisms per sample. On some days, low and high tide correlated with low abundance and high abundance (Figure 3), but on other days the opposite was true (Figure 4).

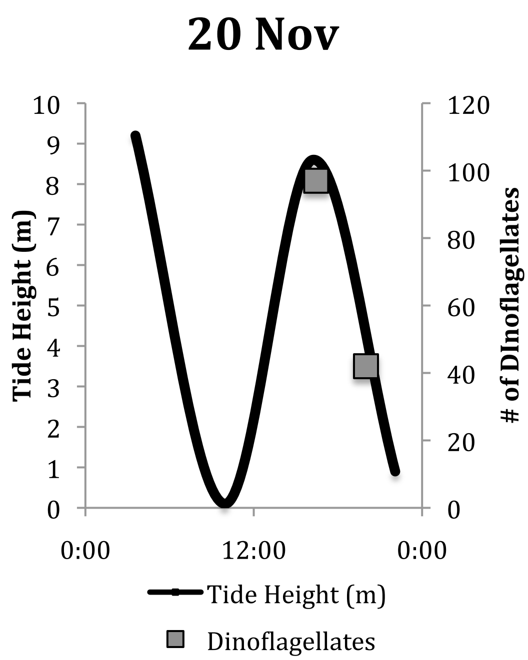


Figure 3. Relationship of tide and dinoflagellate abundance on 20 Nov 2017. High abundance at high tide, and low abundance at low tide.

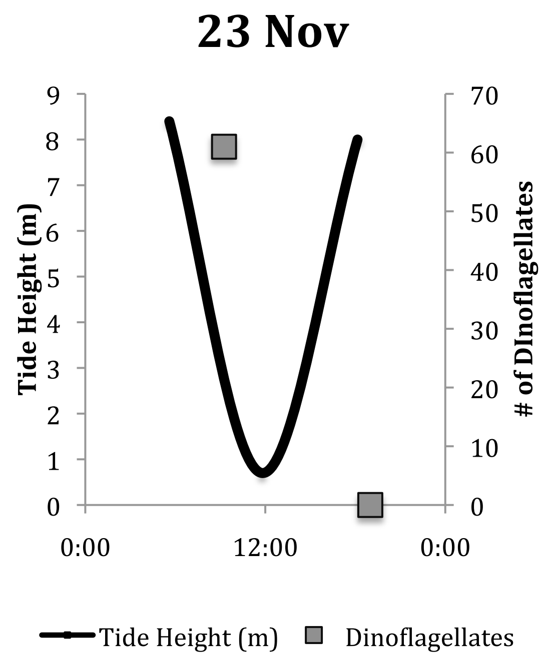
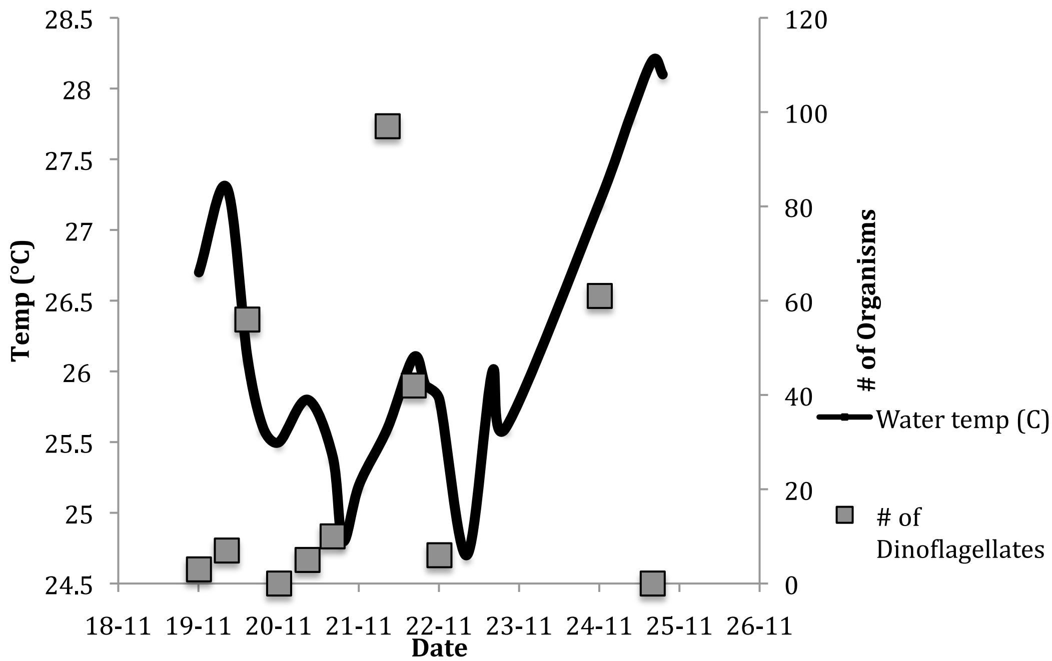


Figure 4. Relationship of tide and dinoflagellate abundance on 23 Nov 2017. High abundance at low tide, and low abundance at high tide

I also plotted dinoflagellate abundance and ocean temperature. Using data collected from the device, I was able to compare the ocean temperature on a daily basis (Figure 5). For about half of my samples temperature and abundance have a positive correlation (i.e. higher temp=higher abundance, lower temp=lower abundance). The other half of the samples doesn't agree with this correlation.



Dinoflagellate Abundance vs. Ocean water temperature

Figure 5. Relationship of ocean water temperature readings and number of dinoflagellates found.

Overall, there were no statistically significant relationships between dinoflagellate abundances and any of my tested variables. With that being said, the abundance graphs do show trends. Average abundance was higher at night, and more than half of night/day sample sets showed higher abundance at night. Tide showed me no clear correlation, but ocean temperature showed some interesting results. Ocean temperature had a positive correlation with abundance in about half of the samples.

Discussion

My results show abundance of dinoflagellates differs at different times of day and night. Though the abundances of dinoflagellates are variable, I found more on average in my night samples. The central question of my study was "what is the adaptive significance of bioluminescence in dinoflagellates?" This led me to a hypothesis; bioluminescence is a predator defense. I found higher average abundances in samples taken at night, which supports my hypothesis.

We already know that these organisms can control their buoyancy (Khan 1978), and theories have been put forward to suggest that bioluminescence in dinoflagellates is a predator defense (Esaias 1972, Burkenroad 1943). It is also known that dinoflagellates participate in diel vertical migration (Blasco 1978). Diel vertical migration is the movement of millions of creatures up and down the water column and is thought to be a way to escape predators during the day and feed at night. Since *Noctiluca* are heterotrophic, they likely participate in this migration for that reason. Bioluminescence would then become an advantageous trait, helping them avoid predation while they feed. A possible explanation for higher abundances at night is that their bioluminescence is more effective in the dark and allows them to feed near the surface, while simultaneously avoiding predators.

In a study by George and Quintana (2012), dinoflagellates were sampled from the same bay I used and tested for bioluminescence brightness during the day and night. They found that dinoflagellates did indeed glow during the day, but brightness was consistently higher at night. When discussing their study, they suggested that one possible explanation for lower brightness during the day is lower abundance during the day. My study shows that this explanation may be true.

When relating my abundance counts to other factors, like tide and ocean temperature, patterns were hard to find. There seems to be no observable pattern between tide and the abundance of dinoflagellates. Ocean temperature has been shown to affect the migration patterns of dinoflagellates (Kamykowski 1981). In his paper Kamykowski suggests that dinoflagellates are part of a diurnal vertical migration that is based on temperature gradients. In my study, I found on a few occasions, ocean temperature did positively correlate with the number of dinoflagellates found.

There are still questions about the function of bioluminescence in dinoflagellates, though there is evidence to suggest that it acts as a predator defense. This brings up some interesting questions that are worth further research. If bioluminescence is a predator defense, why are dinoflagellates present near the water surface at all during the day, when their light is less visible? Also, is the diel vertical migration of dinoflagellates related to their bioluminescent abilities? Another interesting discovery was the other morphospecies of bioluminescent dinoflagellate that I couldn’t identify. These “flage-less” organisms were bioluminescent, but were clearly not in the genus *Noctiluca.* It would be useful to identify what this species is, and if it interacts with *Noctiluca.* Overall, my study showed that dinoflagellates are more abundant at night, which supports the theory of bioluminescence as a predator defense.

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