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BLOOMS OF THE COLONIAL GREEN ALGAE, *Botryococcus braunii* Kützing, IN PAOAY LAKE, LUZON ISLAND, PHILIPPINES

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ABSTRACT

Blooms of the colonial green algae, *Botryococcus braunii,* have been widely known to exert toxic effects on a variety of aquatic organisms and have been noted to cause fish deaths in some environments. In this study, a monitoring of the abundance and distribution of *B. braunii* in Paoay Lake was done in 2006. Samples were taken from the surface and deep portions in the lake from 4 sampling sites. The density of *B. braunii* was found to increase with time from an average of 9,670 colonies/L in May to 24,656 colonies/L in June. The highest recorded density was 44,732 colonies/L near the area surrounding the town of Nagbacalan in July. It was also found that the abundance of some zooplankton species was lowered when the abundance of *B. braunii* was elevated in the lake. This study showed that the *B. braunii* bloom has likewise caused a decrease in dissolved oxygen and might have attributed to the toxic effects exerted by this green alga on the other organisms found in the lake. This is the first reported occurrence of a *B. braunii* bloom in Paoay Lake.

Keywords: Algal bloom, *Botryococcus braunii,* Paoay Lake

INTRODUCTION

The occurrence of algal blooms is a natural phenomenon which may be increased by anthropogenic eutrophication (Engstrom-Ost, 2002) and have adverse effects on the health of people, organisms, the ecosystems, and social economy. These range from cell damage and organism mortality through toxin production to financial losses in tourism, and aquaculture (Bushaw-Newton and Sellner, 1999). Phytoplankton blooms become aesthetic nuisances when dense occurrence happens in open water or beaches. Aside from this, anoxia occurs as a result of decaying algal blooms due to high microbial activity. This leads to mortality among a variety of aquatic organisms, including fish and zooplankton

(Engstrom-Ost, 2002; Chiang *et al*., 2004; Ikawa, 2004). Some toxic substances, such as polyunsaturated fatty acids, produced by algae, may inhibit the growth and occurrence of other algal species (Chiang *et al*., 2004) and lysis of stressed phytoplankton cells (Wu *et al.*, 2006).

The colonial *Botryococcus braunii* (Chlorophyceae) is a bloom-forming alga well known for its ability to produce and accumulate long chain hydrocarbons (Huang *et al.*, 1988; Chiang *et al*., 2004; Ikawa, 2004), which comprise 30-75% of the biomass (Metzger *et al.*, 1991; Qin, 2005). It may be best characterized by rust colored algal blooms that develop as large floating mats on the surfaces of infested water bodies (Huang *et al.*, 1988; Qin, 2005); its colonies appear as clusters of semi-transparent cells embedded in a dense matrix with transparent threads attaching several clusters together. The intensity of the red pigmentation due to the presence of carotenoids present in the colony is directly related to the amount of lipid produced (Aaronson *et al.*, 1983). The matrix is made up of chemically resistant biopolymers of lipids. It is widely distributed in freshwater, brackish and saline lakes, reservoirs or small pools situated in temperate, tropical and continental zones (Metzger *et al.*, 1991; Qin, 2005). It has been recognized as one of the possible sources of renewable liquid hydrocarbons (Ranga Rao *et al.*, 2007). However, it has also been noted to have toxic effects on fish and zooplankton during bloom formation, this may be attributed to allelochemicals produced by the algae, which include a mixture of free fatty acids (Chiang *et al*., 2004; Wu *et al*., 2006). Strains like wild populations differ in the type of hydrocarbons they synthesize and accumulate. These include *n*-alkadienes and trienes, triterpenoid botryococcenes, methylated squalenes, or the tetraterpenoid, lycopadiene. They were also found out to produce ether lipids closely related to hydrocarbons (Metzger and Largeau, 2005). Production of these constituents has also been found to be under the influence of salinity (Ranga Rao *et al.*, 2007).

There has been neither report nor study on *B. braunii* blooms in Philippine lakes. In this paper, we present a 5 month-long observation on *B. braunii* blooms in Paoay Lake, Luzon Is., Philippines.

MATERIALS AND METHODS

Description of the study area

Paoay Lake (Latitude: 18° 7' 16 N, Longitude: 120° 32' 18 E) is situated in the Municipality of Paoay, Ilocos Norte. It is the only lake in this province (Guerrero, 2001), being bounded by the towns of Suba in the north, Nanguyudan in the northeast, Pasil in the east, Sungadan in the south and Nagbacalan in the north. It is one of the most scenic and historical places in the entire region. Small-scale aquaculture has been developed in some parts of the lake to add to the income of the local townsfolk resided in this area. It has been declared as a protected area by the national government for easy monitoring and control of human activity

in the lake and its environs. The lake is devoid of tributaries; its source of water comes from the ground water flow and surface run-off during rainy seasons from the surrounding hills. Aquatic plants like *Ipomoea aquatica* (water spinach) and *Eichhomia crassipes* (water hyacinth) can be found along its shallow and muddy portions. Paoay Lake is also home to populations of *Clarias batrachus* (catfish), *Oreochromis* sp. (tilapia) and *Neochanna* sp. (mudfish).

Methods

For this study, we used samples collected from four sites- Nagbacalan (I), Suba (II), Nanguyudan (III) and Sungadan (IV) from May to September 2006 (Fig. 1). During sampling, rust-colored aggregates of the algae was frequently observed on the lake surface. Microscopic examination verified that it was of the oil-rich colonial green alga, *Botryococcus braunii*.

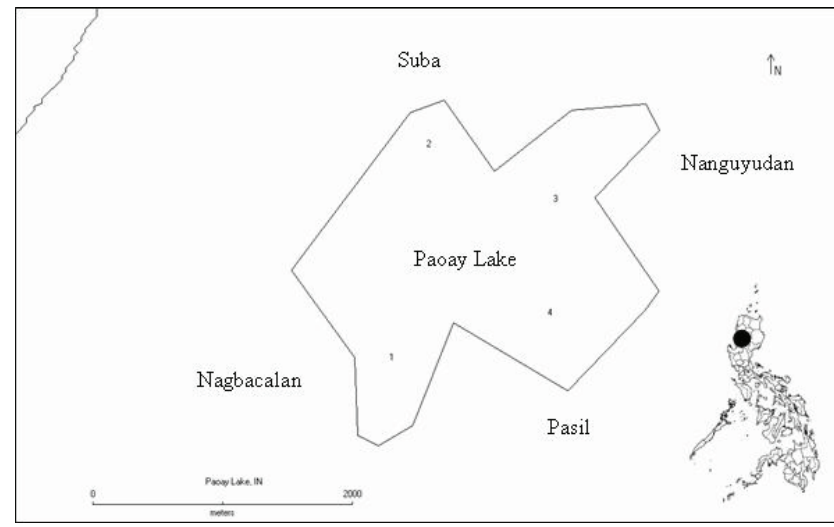

Figure 1. Map of Paoay Lake, Ilocos Norte, Luzon Is., Philippines, showing the sampling sites 1-4.

To assess its density, three 1-mL replicates were taken using a 1 mL Pipette from the collection bottles. These were placed in 1-mL Sedgewick-Rafter counting chambers and were counted with low magnification (100 x) under a microscope. Total count (TC) was determined by multiplying the average number of *B. braunii* colonies per counting chamber by the volume of sample in the collection bottle. This was later divided by the total volume of sample filtered per site to determine *B. braunii* density (colony/L).

Data on zooplankton density as well as physico-chemical variables such as depth, Secchi disc transparency, water temperature, dissolved oxygen (DO) and pH were correlated with the densities of *B. braunii* over the study time using Pearson-Product Moment Correlation (MS Excel).

RESULTS AND DISCUSSION

Blooms of *B. braunii* in Paoay Lake

Analysis of the collected samples revealed that *B. braunii* colonies were present in all of samples collected from the four sampling sites during study time. Changes in density were noticeable for the different sampling sites in the different months. The lowest mean density of this alga was observed in the samples collected in May (9,670 colonies/L). An increase in algal density was observed for the samples collected in June (24,656 colonies/L) and in July (23,085 colonies/L). Then, the density of *B. braunii* decreased to 17,086 colonies/L in August, though this was still higher than that recorded for May. The month of September saw another increase in *B. braunii* density to 20,689 colonies/L.

Densities of *B. braunii* at all sampling sites for the month of May were noted to be low. Samples from site 3 (6,591 colonies/L) had the lowest density of *B. braunii* colonies for the entire sampling period. The highest densities were observed for May at site 4 (12,164 colonies/L). In June, the number of colonies was elevated, when compared with that in the previous month. The elevation in algal density was observed in all of samples collected from sites 1, 2, and 3 except site 4. Even, the 2nd highest density of *B. braunii*, 27,657 colonies/L, over the entire study time was revealed at site 2.

In July, the density of *B. braunii* increased at site 1 and reached the highest density, 44,732 colonies/L, recorded for the entire sampling period. In contrast, a decrease in algal density was observed at sites 2 and 3, though it was still higher than that collected in May. The density of *B. braunii* in the samples from Site 3 in May were comparable to those collected from the same sampling site for July, while the density of *B. braunii* from Site 4 was comparable to the collections made at the same site from the two previous sampling months.

Collections made during August revealed a decrease in density for site 1, while no noticeable increase or decrease was obtained for the rest of the sampling sites. In September, samples showed a decrease in the number of *B. braunii* colonies at site 1, when compared with the previous collections at sites 2 and 4. At site 3, algal density increased when compared with that of August (Fig. 2).

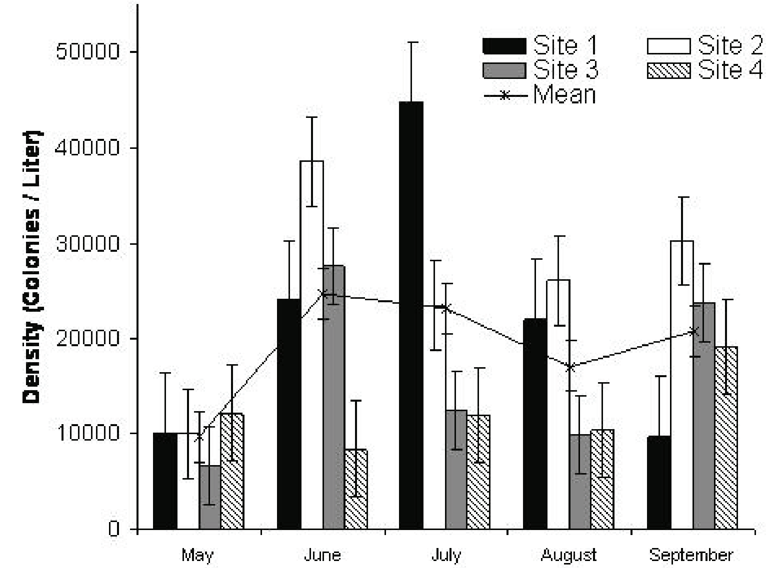

Figure 2. Variations in the density of *Botryococcus braunii* at the 4 sampling sites and its mean density in Paoay Lake over the study time from May to September 2006.

Correlation of *B. braunii* densities with mean densities of the major zooplankton groups and individual species

There were 27 zooplankton species present in Paoay Lake. Variations in species composition and mean densities were noticeable over the study time. The relationship between the blooms of *B. braunii* and the densities of each of the said species was likewise analyzed. Results revealed that 9 species (5 rotifers, 3 cladocerans and 1 copepod) showed a negative correlation with *B. braunii*. Of these, strong negative correlations were observed with the rotifers *Keratella procurva* (-0.85) and *Brachionus falcatus* (-0.7) and was highest with the cladoceran *Bosmina longirostris* (-0.92). The remaining 18 species present in Paoay Lake either had a weak or moderate positive correlation with *B. braunii* (Fig. 3).

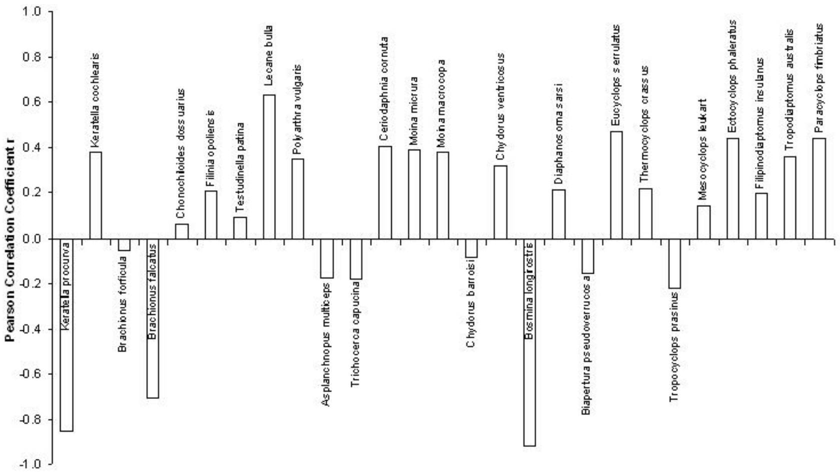

Figure 3. Pearson correlation coefficients (r) between mean densities of *B. braunii* and individual zooplankton species in Paoay Lake over the study time from May to September 2006.

Meanwhile, correlations between the mean densities of each of the three major zooplankton groups and *B. braunii* densities revealed that highest negative correlation existed between the rotifers and *B. braunii* densities (-0.95) (Table 1).

Table 1. Pearson correlation coefficients (r-value) and interpretation between the density of *Botryococcus braunii* and mean densities of the three major zooplankton groups in Paoay Lake over the study time from May to September 2006.

Taxonomic Group	r-value	Interpretation
Copepoda	0.39	Low
Cladocera	0.32	Low
Rotifera	-0.95	Very Strong

Correlation of *B. braunii* densities with physico-chemical variables

Analysis of the correlation between the densities of *B. braunii* and physico-chemical variables, namely depth, pH, temperature, Secchi disc transparency (SDT), and dissolved oxygen (DO) was also done. Results showed that there was moderate positive correlation between lake depth and *B. braunii* density (r = 0.60). The density of *B. braunii* exhibited very weak correlation with pH and temperature,

r = -0.06 and -0.4, respectively, whereas moderate negative correlation with SDT (r = -0.4). A strong negative correlation was observed between *B. braunii* density and DO levels in the lake (r = -0.80) (Table 2).

Discussion

This is the first reported *B. braunii* bloom in Paoay Lake which was present over the entire five months of study. It is likewise the first recorded bloom of this alga in the Philippines. *B. braunii* blooms have been recorded in places such as Liyu Lake (Taiwan) (Chiang *et al*., 2004), Darwin River Reservoir (Australia) (Wake and Hillen, 1979), Bear Shola Falls (India) (Dayananda *et al.*, 2007), and a number of other freshwater, brackish and saline lakes.

Variations were apparent in the distribution and abundance of *B. braunii* collected from the different sampling sites. The densities were lowest in the month of May. There was also an almost equal distribution of *B. braunii* colonies among the four sampling sites. This was the only month wherein such was observed. The following months showed a drastic increase in the mean number of colonies. Aside from this, there were now one or two sampling sites which harbored a greater number of *B. braunii* colonies as compared to the others. The formation of large conspicuous orange / rust-colored mats on the water surface by these blooms was also more apparent during the succeeding months, because the intensity of hydrocarbon content (which causes the orange pigmentation) of the algal colonies is directly related to the density of the bloom (Huang *et al.*, 1988; Chiang *et al*., 2004; Ikawa, 2004). From the months of June-September, the mean number of colonies of *B. braunii* present in Paoay Lake ranged from 13,000 – 20,000 colonies/L. The highest mean numbers of colonies per liter was also observed for the months of June, July and September (three of the five sampling months). Sites 1 and 2 had the highest number of *B. braunii* colonies present for the months of June and July while it was sites 3 and 4 where this has occurred for September. *Botryococcus braunii* has been known to grow under a wide spectrum of conditions (Komárek and Marvan, 1992). Studies in Australia revealed optimum temperature for growth was 23^0C, a photoperiod of 12 hours light and 12 hours dark and salinity of 8.8‰ however, tolerance to temperatures up to 33^0C have been recorded, suggesting a wide temperature tolerance by *B. braunii* (Qin, 2005). It was also observed to have wide pH adaptability (Dayananda *et al.*, 2007). High concentrations of nutrients, conductivity and alkalinity were likewise observed during a *B. braunii* bloom in Banglang reservoir, Thailand (Ariyadej *et al.*, 2004). The presence of *B. braunii* blooms in the eutrophic lakes Liyu (Taiwan) (Chiang *et al*., 2004) and Tomahawk Lagoon No. 2 (New Zealand) (Mitchell, 1975) also suggests that the trophic status of the lake may indicate its susceptibility to bloom formation. The physico-chemical parameters in this study also show that conditions were favorable for *B. braunii* blooms to form. Temperature and pH were within the tolerable ranges. An almost constant photoperiod of 12 hours light and 12 hours dark was observed all throughout the sampling time; pH values were near alkaline and mean depths were noted to only be between 2 - 5 meters. All of these may have contributed to the development of the bloom in the lake.

The nine-hole golf course near Sites 1 and 2 may have also contributed to an increase in available nutrients in the lake due to fertilizer run-offs (Klein, 1999) and may also be the cause of the decrease in water depth since lake water is utilized for irrigation. Natural and artificial fertilizers provide principal chemical nutrients necessary for algal growth and reproduction (Conte, 2000). The presence of golf courses near bodies of water has long been a concern of many biologists and environmentalists because of its detrimental effects to aquatic organisms (Klein, 1999). Data on N and P availability in the lake may further support this; however this current study did not include an analysis of such nutrients in the lake. The presence of higher densities of *B. braunii* in Sites 1 and 2 as compared to Sites 3 and 4 may help prove that there is an increase in nutrients available due to run-offs in this part of the lake.

The results of the Pearson correlation between *B. braunii* densities, zooplankton mean densities and physico-chemical parameters measured in the lake revealed that *B. braunii* has varying levels of negative impacts on the previously mentioned parameters.

Keratella procurva, *Brachionus falcatus* and *Bosmina longirostris* were most affected by the high densities of *B. braunii* in the different sampling months. Patterns of increase in the alga's density influenced the corresponding decrease in the density of these three species. Likewise, rotifers were also observed to be the most susceptible of the three major zooplankton taxa to the patterns of increase in *B. braunii* density. A related study by Aquino *et al.* (in review) revealed that rotifers were the most abundant and diverse of the three major zooplankton groups. Comparison with data in this study revealed that this happened before the occurrence of higher densities of *B. braunii*. This affirms the earlier results by Chiang *et al.* (2004) on the negative impacts of allelochemicals from *B. braunii* on zooplankton mortality. This may also be attributed to the possible effect of the fatty acids which serve as defense mechanisms of some algae against grazers (Wu *et al.*, 2006). A study by Ianora *et al.* (2004) on *Calanus helgolandicus* feeding on blooms of *Skeletonema* revealed that inhibitory effects exerted by this algae was through the impairment of egg development after consumption. This was due to aldehydes released from the enzymatic cleavage of fatty acids immediately after cell damage to the diatom by the copepod. Long chain fatty acids have also been known to inhibit Na^+/K^+-ATPase activity in the brine shrimp *Artemia salina* (Morohashi *et al.*, 1991). Other zooplankton species showed moderate positive correlations with *B. braunii* density shifts probably because these species were absent or found in low densities throughout the sampling period. The high densities of *B. braunii* may still be a factor in the absence or low densities of

these species. The lack of a negative correlation just indicates that the pattern of increase of *B. braunii* was not able to cause a correlated pattern of decrease in their density.

Levels of water clarity (in terms of Secchi disc transparency values) and dissolved oxygen in the lake were also affected by high *B. braunii* densities. These blooms, which can give rise to a film of oil forming on the surface of the water, decreases water clarity and prevent normal oxygenation of the water. This, in turn, may have helped cause the decrease in zooplankton density in Paoay Lake.

CONCLUSIONS

Botryococcus braunii blooms were present in Paoay Lake from May to September 2006. The densities of *Keratella procurva*, *Brachionus falcatus* and *Bosmina longirostris* were also highly affected by the elevated densities of *B. braunii*. Likewise, high numbers of *B. braunii* have also caused a decrease in water clarity and have also lowered dissolved oxygen values in the lake which may in turn have helped aggravate the negative impact of the blooms on the ecosystem.

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