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Effect of Lowered pH on Community Composition, Growth and Cell Morphology of Freshwater Phytoplankton

SHASWATI CHAKRABORTY | MAHIN AFROZ | RIYAD HOSSEN

Department of Botany, University of Barishal, Barishal-8254, Bangladesh

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Corresponding Author
Riyad Hossen
Contact: rhossen@bu.ac.bd

ABSTRACT: This experiment studied the probable effects of lowered pH (6.5 to 5.0) on four freshwater phytoplankton species *Chlorella vulgaris* Beyerinck, *Scenedesmus quadricauda* (Turpin) Brebbison, *Euglena granulata* (Klebs) Schmitz and *Gomphonema subtile* Ehrenberg. The initial community composition was 2:2:1:1 for the four taxa respectively, which changed to 1.7:1.6:1:1 in pH 6.0, 1.6:2:1.6:1 in pH 5.5 and 1.2:2:1.8:1 in pH 5.0. The two chlorophytes showed almost gradual decreasing in growth rates and cell densities with the decreases in pH values, while diatom *E. granulata* showed more tolerance in a wide range of pH (6.5 to 5.5). Conspicuous changes in total biomass first observed in pH 6.0 and it reached the drastic level in pH 5.0. Moreover, significant changes in cell size of *S. qaudricuada* and *E. granulata* were found in pH 5.0 only. This lowered pH range has no effects on cell shape of the studied phytoplankton species.

Keywords: Phytoplankton, Growth rate, Biomass, Cell size, pH effects.

World climate is changing as an act of nature and the changes might create many impacts on biodiversity which has been focused of extensive research nowadays. Climate change itself represents a complex amalgam of stressors including alterations in pH, salinity, temperature, and so on (1,2). Freshwater ecosystem is more vulnerable to climate changes, because many species within these ecosystems have limited abilities to cope up the changes (3). Fresh water ecosystem only occupy less than 1% of the earth's surface, but they have significant amount of living organisms in their ecosystems (4). Among the living organisms, phytoplankton are considered as the base of all food chains in freshwater ecosystems and provide oxygen, foods and energy for other aquatic organisms (5). These tiny organisms have been used for environmental assessment studies, because they are extremely responsive to environmental changes (6). Scientists expected that changing climate would modify aquatic ecosystems over the next century which would alter the phytoplankton standing stock (7). These changes could modify their community structure, growth rates and morphologies (8-10).

Variations in pH values can change the availability of essential nutrients needed for phytoplankton growth. It was reported that natural phytoplankton communities have shown altered species composition in response to lowered pH (11). On the other hand, some studies showed that effects of lowered pH on growth and productivity of phytoplankton are rare (12,13). According to Fabry et al., pH indeed may have an effect on phytoplankton growth, but extensive research on cell morphology of freshwater phytoplankton are not available (14). However, the occurrence of pH variations in marine waters may not be uncommon, but for freshwater ecosystem reports are sparse (10). Thereby, the changes can make serious ecological impacts in freshwater ecosystem in future.

Therefore, the study aimed to know the prospective changes happened in community structure, growth rate, and biomass and cell morphologies of some phytoplankton species in freshwater ecosystem by different pH and salinity concentrations.

MATERIALS AND METHODS

Isolation and pre-cultures

In this experiment, four phytoplankton taxa from two major groups of algae were used as model species (Table 1). These taxa were collected from the MS laboratory of the Department of Botany, University of Barishal.

Table 1. Model phytoplankton species with their isolation, collection and cell description information.

Phyt group & Taxon	Place of Collection	Date of Isolation	Cell dimension (μm)	Cell density (cell/ml)
Chlorophytes				
C. vulgaris	NIS Pond	07.09.20	4.0×4.5	40000±700
S. quadricauda	R Lake	16.09.20	10×3.5	38850±625
Diatom				
E. granulata	CR Pond	11.09.20	10×4.0	36500±880
G. subtile	KS Pond	11.09.20	36×12	25500±350

Note: Phyt= Phytoplankton, NIS= Nazrul Islam Sarak, R= Rupatoli, CR= College Road, KS= Kalushah Sarak.

During pre-culture or stock culture preparation, the initial community composition was random, based on natural availability of phytoplankton in this region (15,16). Pre-culture followed the ratio of 2:2:1:1 (2:1 ratio of chlorophytes and diatoms), respectively. Moreover, pre-culture were maintained in Erlenmeyer flask (100 ml) providing neutral pH (pH 7.0), normal freshwater salinity (0.2 PSU) and Bold Basal nutrient medium. The pre-culture was kept in 16±1°C temperature, 45 to 60 μmol photons m^{-2} s^{-1} light intensity with light:dark cycle of 14:10 h.

Experimental design

The cultures were performed in Pyrex bottles of 1L (culture volume was 600 ml) incubated in temperature (16±1 ^{0}C) and light (150 to 200 μmol photons $m^{-2}s^{-1}$) controlled growth chamber with light/dark cycle of 14/10 h including capacity to avoid gas exchange with the air. Bold's Basal medium were used as a basic nutrients requirement and bottles were placed following randomized block design with 5 cm distant to each. To create a homogenous light field, the bottles were illuminated from below and positions changed randomly during sampling. To prevent sedimentation, cell damage and loss of cellular content, the bottles were mixed by shaking thrice per day at 9.00 am, 1.00 pm and 5.00 pm. The goal was to determine at what level of lowered pH the growth rates, community composition and biomass of four taxa were affected. The used different pH concentrations were pH 7.0 (control culture), pH 6.50 (T1), pH 6.0 (T2), pH 5.50 (T3) and pH 5.0 (T4). In this experiment, 'T' is denoted for treatment culture and 'C' for control culture. All treatments were run in triplicate. The pH for the experiment was set with a regulation interval of 0.50 pH units. To maintain the steady pH in each treatment, 0.1 M HCL and/or 0.1 M NaOH was added to the medium after monitoring daily. Two to three weeks before setting up the experiment, the stock cultures were introduced and acclimated to a higher experimental light intensity. Salinity, temperature and dark/light cycle was similar in pre-culture to each treatment. During the last 1 to 4 days of acclimation to light intensity, pH was adjusted stepwise until the culture medium had reached the pH of the specific treatment. The experiment was conducted from 1st to 25th January, 2021.

Sampling and measurements

To count cell number and cell dimension, 2 ml of samples from each bottle were taken once after each 4 days and immediately fixed with Lugol's iodine into small vials. That time, same amount fresh culture was added to each bottle from the stock culture to maintain steady culture volume. Mixing was done before sampling to ensure homogeneity of the phytoplankton and counting was done by a Burker haemocytometer using light microscope equipped with camera. To measure cell dimension (length and width), measuring tool of Adobe Photoshop CS3 were used in the recorded micrographs. To count phytoplankton growth and average cell density, the cell number counted per ml of samples were calculated, and growth rate (Division/day) was measured based on the changes in produced cell number of each 4 days. Community composition was measured through counting the cells for each phytoplankton in the final week of the experiment. Algal samples were harvested by flocculant $Al_2(SO_4)_3$ and collected through filtration to measure total biomass (Fresh weight and dry weight). Biomass was expressed as g/L and cell size was expressed as µm.

Data analysis

All data were processed in MS Excel 10 version. Statistical analysis was carried out by the JMP software program and LSDs were calculated at 5% probability level.

RESULTS

Effects on community composition

In this experiment, the counted cell number at the end of the study said that the initial community composition was almost same in control culture, 2:2:1:1 ratio of the four taxa *C. vulgaris*, *S. quadricauda*, *E. granulata* and *G. subtile* or expressed as percentage 33.33, 33.33, 16.16 and 16.16 %, respectively. As a group, chlorophytes showed 66.66% and diatoms showed 33.33%. In T1 culture, the community composition slightly changed from the initial, though this change was statistically non-significant (<0.05 P). The T2 treatment showed 60 percent of cells possessed by chlorophytes and the rest 40 percent of cells possessed by diatoms. The individual ratio in this treatment was 1.7:1.6:1:1. The treatment T3 showed great variation in the community composition where *S. quadricauda* hold highest share (32%), while *G. subtile* contributed only 16% of the community. *C. vulgaris* and *E. granulata* both shared 26% respectively, thus the ratio was 1.6:2:1.6:1 (Figure 1). The T4 treatment witnessed the share of *C. vulgaris* reduced to 21 percent, while share of *E. granulata* increased to 29 percent. In this treatment, the observed ratio was 1.2:2:1.8:1.

Effects on growth

To calculate the growth status of the four phytoplankton species, average growth rate and total biomass was considered. In C and T1 culture showed same growth rates for each species near to 1.1 div/day. Consequently the individual cell density was almost same in those cultures, while T2 showed significantly different than C and T1 culture. Cell density reduced in T2 treatment in case of the chlorophytes (16500± 1420 and 15400± 695 cells/ml), whereas density of the diatom cells was steady, although the overall density was found in decreasing trends (Figure 2). T3 showed lowest rate (0.42 div/day) by *C. vulgaris* and the highest rate was in 0.91 div/day by *E. granulata*. Finally, T4 treatment showed decreasing pattern in growth rates for each species and showed almost same density in case of *S. quadricauda* and *E. granulata*. Growth rate of diatom cell dominated over the chlorophytes cells throughout the experiment (Figure 3). Total biomass was counted as fresh weight and dry weight of the finally harvested phytoplankton. The biomass was gradually decreased due to increase in pH intensity. Highest 7.6 and 7.3 g/L fresh weight as well as 5.1 and 5.0 g/L dry weight was found in control and T1 culture. Lowest 3.2 g/L fresh weight and 1.9 g/L dry weight was recovered from T4 culture (Figure 4).

Effects on cell morphology

In this experiment, the measured cell dimension of *C. vulgaris* slightly changed; however, the changes were statistically non-significant and the shape was almost rounded during the entire experiment (Table 2). It showed lowest dimension (4.0×4.0 µm) in T4 treatment, while highest dimension (4.5×4.0 µm) in T1 treatments. The rest of the chlorophytes, *S. quadricauda* showed gradual decrease in cell size in increasing pH intensity which reached the significant level (9.0×4.0 µm) when cultured in T4 treatment. The cell shape was also totally unchanged for all treatments. The diatom *E. granulata* was 10.0×4.0 µm initially and this species showed gradual increase in cell length throughout the experiment which touched the pick in T4 culture. Shape of the cell was almost same except cell was somewhat elongated in T4. *G. subtile* was comparatively longer species in this investigation, which was 31.2×9.0 µm in the entire research. This taxon was not observed any significant changes in its cell morphologies like *C. vulgaris*.

Table 2. Comparative cell dimension of the phytoplankton taxa in the study

SL	T	**Cell morphology** (Size and Shape)							
		CHL		**SCE**		**EUG**		**GOM**	
		Size (µm)	Shape	Size (µm)	Shape	Size (µm)	Shape	Size (µm)	Shape
1	C	4.3×4.0	Round	10.15×3.5	E. ellipsoidal	10.0×4.0	Spindle	31.0×9.0	Leaf
2	T1	4.5×4.0	Round	10.05×3.5	E. ellipsoidal	10.0×3.5	Spindle	31.2×9.0	Leaf
3	T2	4.2×4.2	Round	10.00×3.2	E. ellipsoidal	10.1×3.5	Spindle	31.5×9.0	Leaf
4	T3	4.2×4.0	Round	9.75×3.5	E. ellipsoidal	10.2×3.5	Spindle	31.2×9.0	Leaf
5	T4	4.0×4.0	Round	9.0×4.0**	E. ellipsoidal	10.75×3**	Spindle	31.2×9.0	Leaf

Note: CHL= *C. vulgaris*, EUG = *E. granulata*, SCE = *S. quadricauda*, GOM = *Gomphonema subtile*, T = Treatment, E=elongated, ** indicates significant at 5% level.

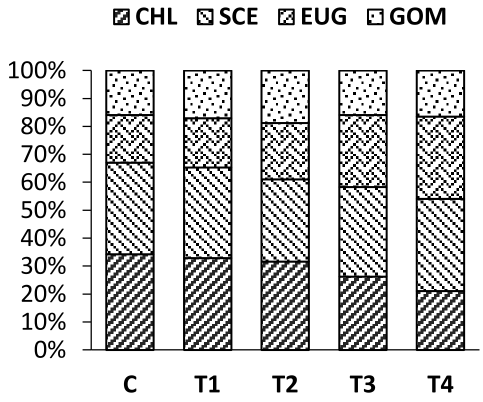

Figure 1. Changes in community composition.

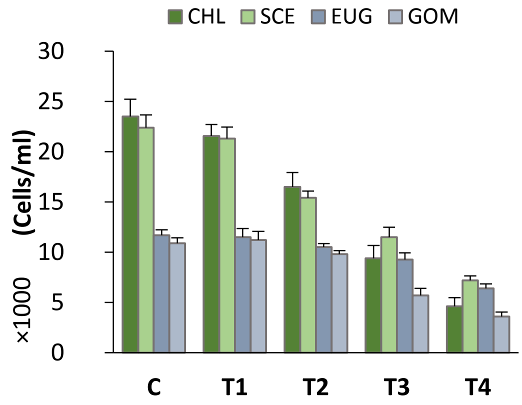

Figure 2. Average cell density of the four taxa.

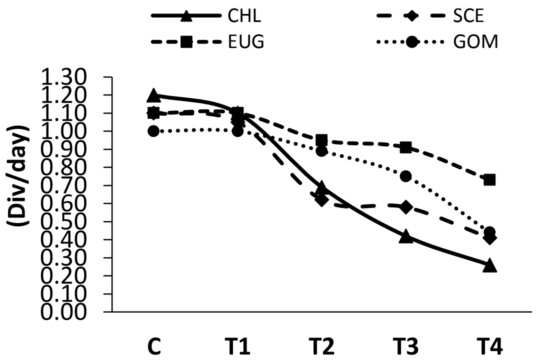

Figure 3. Changes of growth rates of the four taxa.

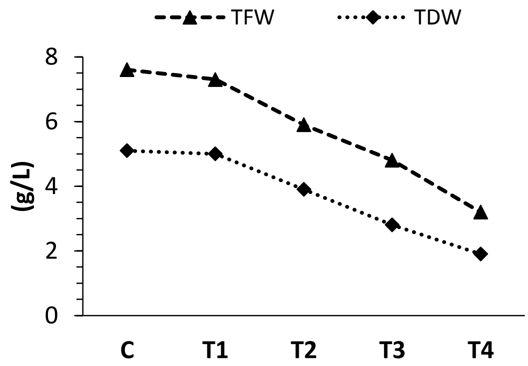

Figure 4. Changes in total biomass of the taxa.

DISCUSSION

From the study it was clear that, lowered pH has significant impacts on phytoplankton in respect of their community composition, growth and cell morphologies. Although this study included only four phytoplankton taxa, but they represented the chlorophytes and diatom species as the most dominating phytoplankton taxa in many freshwater ecosystem in Barishal region of Bangladesh (15,16). The pH 6.5 did not show any significant changes in any examined parameters that mean the pH is tolerable by these studied species. This small scale change in water pH creates negligible impacts on phytoplankton in freshwater ecosystem. When pH was changed to pH 6.0, there was found significant decreased in growth rates of the two chlorophytes, but the both diatoms were remains almost same of initial, and thus the community was started to diatoms dominating and the total biomass reduced in significant level. When the environment was more acidic (pH 5.5), *C. vulgaris* was highly affected but the *S. quadricauda* and *E. granulata* showed they were able to cope up with this environment. As a result, the individual cell density was different than the previous environments and the community structure was *S. quadricauda* and *E. granulata* dominated, and the biomass was almost half of the initial environments means overall growth was affected than pH 6.5 and 6.0. However, when the fresh water touches pH 5.0, it created a huge change in phyto-plankton community. For example, this environment affected all the taxa significantly, although com-paratively the response will not same by all species. The environments observed very slower in growth rate and the biomass more reduced than the other pH concentrations. That means, the pH 6.5 is tolerable for the all species, pH 6.0 is tolerable by both diatoms, pH 5.5 is tolerable for only *E. granulata*, and pH 5.0 is not tolerable by any one of the species. In case of cell morphologies, it was clear that the pH range 6.5 to 5.0 has no effect on the cell shape of the examined phytoplankton. When pH lowered to 5.0, the *S. quadricauda* cell possessed reduced in length while *E. granulata* increased. The diatoms are more tolerant to the conditions comparatively than the chlorophytes. Some previous reports also have shown altered species composition in response to lowered pH, while other studies have shown very limited effects on species composition and community production (17). The overall growth and biomass reduction following the lowering pH values meant that the acidic environments in the freshwater ecosystem just could alter the community composition but their cell morphologies responded differently.

Since phytoplankton occupy an important position in the structure and function of freshwater ecosystems, any environmental fluctuations associated with this tiny organisms may directly affect the function of aquatic ecosystems (18,19). Therefore, freshwater ecosystem with pH 5.0 may cause serious problems in certain natural phytoplankton species as impacts of climate change.

CONCLUSION

As group consideration, the chlorophytes were more susceptible than the experimented diatoms in these studied lowered pH environments. Very lowered pH can affects the cell size of *S. quadricauda* and *E. granulata*. To monitor the changes more accurately, a long term research is needed to be carried out including numerous phytoplankton species.

CONFLICT OF INTEREST

The authors declared that they have no any known interest with others about the article.

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Author's contributions
Shaswati Chakraborty performed the experiment and Riyad Hossen prepared the manuscript. Mahin Afroz participated in data analysis and manuscript preparation.

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