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RESEARCH ARTICLE

Impacts of pH and salinity on community composition, growth and cell morphology of three freshwater phytoplankton

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ABSTRACT

Impacts of climate change on phytoplankton species are very focusing issues nowadays. This research explored the probable impacts of different pH (pH 5.90 to 9.10) and salinity (0.60 to 3.0 ppt) concentrations on freshwater phytoplankton *Chlorella vulgaris*, *Euglena granulata* and *Scenedesmus quadricauda*. The initial community composition was 4:2:1 for the three taxa, which changed to 6:3:1, 6:2:1, 6:3.5:1 and 9:4:1 in pH 5.90, pH 9.10 and salinity 2.20 and 3.0 ppt respectively. *E. granulata* showed more tolerance in a wide range of pH (pH 5.90 to 8.30) based on growth rate studies. The other two species showed growth rates reduction gradually in changes with pH and salinity concentrations. Conspicuous changes of total biomass were seen in pH 5.90 and 9.10, and salinity 2.20 and 3.0 ppt conditions. Moreover, significant changes in cell morphology were found in pH 9.10 and 3.0 ppt salinity. The authors concluded that as group, the Chlorophytes were more susceptible than the diatom in these variable pH and salinity conditions, while *S. quadricauda* was comparatively more vulnerable as a single species.

Introduction

Climate change as an act of nature might create tremendous impacts on biodiversity which has been focused of extensive research nowadays. It generally occurs by a complex amalgam of stressors including alterations in pH, salinity, temperature and so on (1, 2). As a part of biodiversity, freshwater ecosystem is more vulnerable to climate changes than marine and terrestrial realms, because many species within these ecosystems have limited abilities to cope up the changes (3). Although they occupy less than 1% of the earth's surface, 10% of all animals, one-third of all vertebrates and 40% of all fishes are found in freshwater ecosystems (4-6). Most climate change studies to date have focused on individuals or species rather than the higher levels of organization to assess the created impacts on the organisms living in the respective ecosystem.

Phytoplankton are considered as the most important primary producers in freshwater ecosystems which contributed a major share of oxygen for other organisms living there. They serve as food, fertilizers and considered as an effective energy source for first tier of tropic levels (7). Besides, they play important roles in material circulation by controlling the growth, productivity and population characteristics of aquatic biota in the ecosystems. They are extremely responsive to environmental changes and are being used for environmental impact studies as much. It is expected that changing climate would modify aquatic ecosystems over the next century which would alter the phytoplankton standing stock and primary productivity (8, 9). Moreover, these changes will modify their community structure, individual growth and morphology which will directly impact the food web dynamics as well as elemental cycling (10, 11), although model predictions on phytoplankton community composition are in their infancy (12). Thereby, the changes can make serious ecological impacts within freshwater ecosystem in future.

Variations in pH value within a freshwater ecosystem can change the availability of trace metals and essential nutrients, which have direct physiological effects on phytoplankton, thus could change the community composition, biomass and cell

morphology. According to the reports, the effects of lowered pH on growth and productivity of phytoplankton are few (13, 14), although it would alter the species composition (15, 16). However, studies related pH impacts on cell morphology of freshwater phytoplankton are sparse (17). Salinity fluctuation in freshwaters is another climate changing factor that would have deleterious effects on phytoplankton species (18). It was reported that some phytoplankton species are able to cope up with over a wide range of salinity fluctuations (19, 20). However, different levels of salinity were reported to impact on biomass and cell morphology of some marine phytoplankton in laboratory based experiments (21-25), while for freshwater phytoplankton these types of experiments are very rare (26).

Freshwater ecosystems are home to numerous living organisms and provide provisioning support of well being to billions people (27). Despite their immense importance, they are considered as some of the most heavily vulnerable ecosystems on earth due to climate change (28). Any change in phytoplankton communities in freshwater ecosystem can alter the overall biodiversity. Therefore, the study aimed to know the prospective changes in community composition, growth rate and biomass and cell morphologies of some phytoplankton species in freshwater ecosystem under different pH and salinity concentrations.

Materials and Methods

Isolation and pre-cultures

Three phytoplankton taxa from two major groups of algae were included as model species in this experiment (Table 1), which were isolated from water samples of different freshwater reservoirs of Barishal City in Bangladesh and isolation was done following standard laboratory protocols. Later on, isolated taxa were stored in Central laboratory of Botany Department at the University of Barishal. A pre-culture was prepared before starting experiment and the pre-culture took the taxa and their initial community composition randomly but based on natural availability of the taxa in this region (29, 30). As the Chlorophytes are dominating in this region, pre-culture followed the 4:2:1 ratio of *Chlorella vulgaris* Beyerinck, *Scenedesmus quadricauda* (Turpin) Brebbison and *Euglena granulata* (Klebs) Schmitz. Isolation of single cells was done with the use of a drawn-out Pasteur pipette and the cells were washed by transferring them through 3 drops of fresh medium using an inverted microscope from the each previously stored isolated species and inoculated in Erlenmeyer flask (100 ml). The pre-culture was considered as a stock culture for this experiment, which was maintained in pH 7.5 and 0.60 ppt salinity with Bold Basal nutrient medium (Almost neutral pH and normal saline condition of freshwater reservoirs) (31). The pre-culture was kept in 16±1 °C temperature, 45 to 60 µmol photons m^{-2} s^{-1} light intensity and 14:10 hr of light:dark cycle.

Table 1. Phytoplankton species used in the study with their isolation and cell description

Phytoplankton group Name of taxon	Place of collection	Isolation Date	Cell dimension (µm)	Cell density (cell/ml)
Chlorophytes				
Chlorella vulgaris	N Islam Sarak Pond	07.09.20	4.0×4.5	40000±700
Scenedesmus quadricauda	Rupatali Lake	16.09.20	10.0×3.5	38850±625
Diatom				
Euglena granulata	College Road Pond	11.09.20	10.0×4.0	36500±880

Experimental design

The study was completed through two different set of treatments based on various pH and salinity concentrations. The treatments for pH concentrations were denoted as 'P' and for salinity it was 'S'. The pH treatments were designed from the base freshwater pH 7.50 which regarded as control culture (C). The pH treatments were set with a regulation interval of 0.80 pH units, where two treatments were lower (P1= pH 6.70; P2= pH 5.90) and two treatments were higher (P3= pH 8.30; P4= pH 9.10) than the control culture pH concentrations, consecutively. 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. This corresponded to ±0.05 pH units around the set pH value. In terms of salinity treatments, base salinity was taken 0.60 ppt as a control culture (C). The regular 0.80 ppt interval was maintained in all treatments viz., 1.40 ppt (S1), 2.20 ppt (S2) and 3.0 ppt (S3) and steady salinity was maintained by NaCl monitoring daily. Salinity 0.60 ppt was fixed in pH treatments, while pH 7.5 was fixed in salinity treatments. The cultures of both set treatments 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 hr. Bold's Basal medium were used as a basic nutrients requirement and bottles were placed following randomized block design. 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 7.00 am, 12.00 am and 5.00 pm. The experiment was conducted between October to December, 2020 and both set of treatments were established with triplicate cultures.

Sampling and measurements

For cell counting and cell morphologies observation, 2 ml of samples from each bottle were taken once after each 5 days and immediately fixed with Lugol's iodine and stored at –20 ^{0}C for further examination. Mixing was done before sampling to ensure homogeneity of cells. Instantly the same amount stock culture was added to each bottle to maintained steady culture volume. Counting was done by a Burker haemocytometer using a light microscope at 100X magnification equipped with modern photography devices. It was attempted to record at least 100 cells of each taxon to achieve 95%

confidence limits. Then community composition, average cell density (cells/ml) was measured based on the cell number counted in final week and average growth rate (div/day) was measured based on the changes in produced cell number of each 5 days counted. To measure cell dimension (length and width) and cell shape, measuring tool of Adobe Photoshop CS3 were used in the recorded micrographs. For fresh weight as biomass measurement (gm/L), algal samples were harvested by flocculant $Al_2(SO_4)_3$ and collected through filtration and finally measured by digital scaling.

Data analysis

All data were processed in MS Excel 10 version. Community composition was expressed as ratio of the experimented phytoplankton species. Significance test of the treatments was carried out by ANOVA (STATISTICS 10). LSD (Least Significant Difference) between the calculated results at 5% probability was done by the JMP software program.

Results

Changes in community composition

In pH treatments, the average cell density of *C. vulgaris* and *E. granulata* found highest in control culture 23580±1715 and 10258±1254 cells/ml respectively and they were almost same in P1 and P3 treatment. *S. quadricauda* showed slightly higher density in P3 (5500±690 cells/ml) than the control and P1 treatment culture. As a result, the calculated community ratio was found 4:2:1 in C, P1 and P3. When the environment was highest acidic (P2= pH 5.90) in this study, the average cell density was almost unchanged for *E. granulata* (9110±695 cells/ml), while the significant reduction seen in *C. vulgaris* and *S. quadricauda* (16575±1742 and 2850±355 cells/ml respectively) and thus the ratio was changed to 6:3:1. On the other hand, when pH increased to 9.10 the highest basic condition (P4), the significant changed was observed in cell density for the three phytoplankton taxa, 15700±867, 4760±450 and 2540±450 cells/ml respectively; therefore, overall community composition shifted to 6:2:1 (Fig. 1). In salinity treatment, the counted final average cell density thus phytoplankton community composition was same (4:2:1) in C and S1 treatment. In S2 treatment, the significant reduction in cell density was observed for *C. vulgaris* (15170±1036 cells/ml) and *S. quadricauda* (2580± 421 cells/ml), despite the change in cell density of *E. granulata* remain non-significant statistically. Consequently, the observed species composition was 6:3.5:1 approximately. When they cultured in S3 salinity condition, the changes was drastic for the all three taxa (Fig. 2). For example, *C. vulgaris* and *E. granulata* decreased to almost half of the control culture, and surprisingly *S. quadricauda* faced one-fifth reduction of the control environment. Finally, the ratio was counted as 9:4:1 approximately in S3 treatment.

Changes in growth rate and total biomass

In pH treatment, growth rate of *C. vulgaris* was almost similar in C, P1 and P3 which was about 1.2 div/day, while it slowed down to 0.69 and 0.72 div/day respectively in P2 and P4 culture. *S. quadricauda* also followed the same changing pattern (Fig. 3). Growth rate of *E. granulata* changed significantly only in P4 cultures (0.70 div/day). The highest total biomass in pH treatment was determined 7.6 gm/L^{-1} in control culture, following 7.4 gm/L^{-1} in P3 and 7.1 gm/L^{-1} in P1 treatment (Fig. 5). The surprising changes were found in P2 treatment (5.8 gm/L^{-1}) and P4 treatment (4.8 gm/L^{-1}). In terms of salinity, *C. vulgaris* showed almost gradual decrease in growth rate due to increase in salinity viz., 1.3, 1.0,

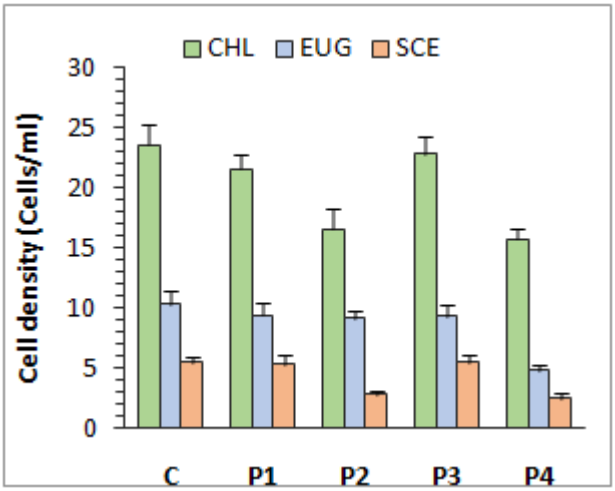

Fig 1. Cell density of the taxa in pH variation.

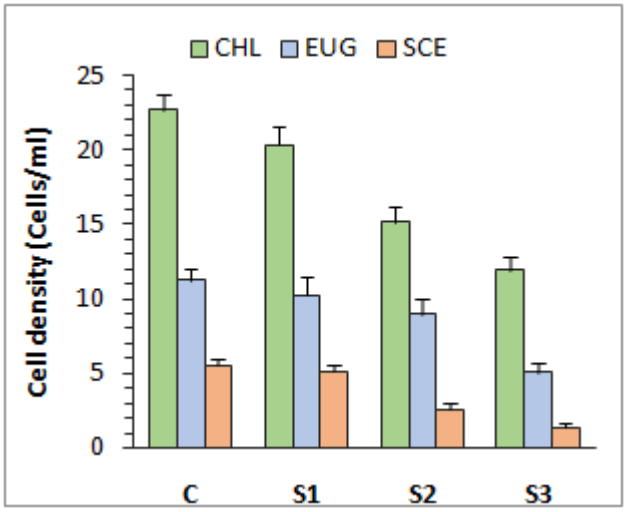

Fig 2. Cell density of the taxa in salinity variation.

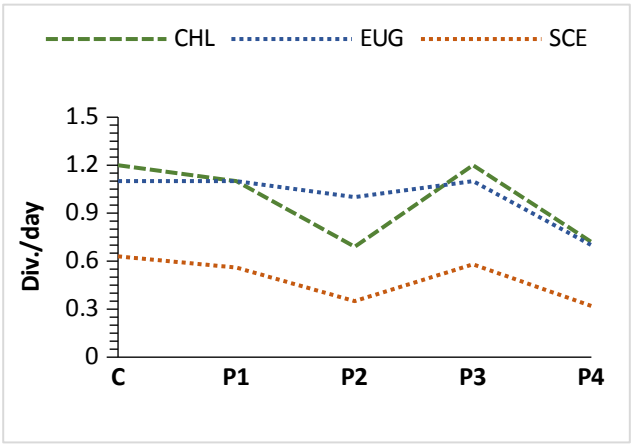

Fig 3. Growth rates of the taxa in pH variation.

0.79, 0.50 div/day respectively in C, S1, S2 and S3 salinity treatments. Growth rate of *E. granulata* was steady in C (1.1 div/day), S1 (1.0 div/day) and S2 (0.89 div/day), but almost restricted in S3 (0.45 div/day).

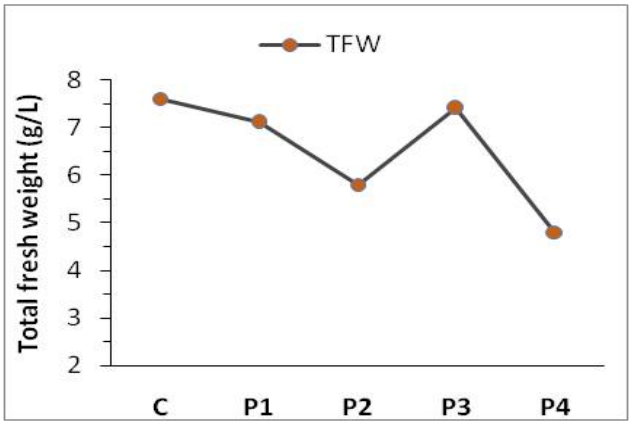

Fig. 5. Changes in total biomass in pH variation.

The growth rate of *S. quadricauda* was almost equal in C (0.62 div/day) and S1 (0.58 div/day), while it was 0.36 div/day in S2 and 0.21 div/day in S3 treatment. The highest biomass in salinity treatment was (7.9 gm/L^{-1}) in control culture (Fig. 6). Almost same amount produced in S1 ((7.5 gm/L^{-1}), while it was 5.6 gm/L^{-1} in S2 and 4.1 gm/L^{-1} in S3 treatment culture.

Changes in cell morphology

Measured cell dimension of *C. vulgaris* slightly changed in pH treatments (4.0×4.0 µm to 4.5×4.5 µm); however, the changes were statistically non-significant and the shape was almost rounded during the entire experiment. *E. granulata* possessed lowest

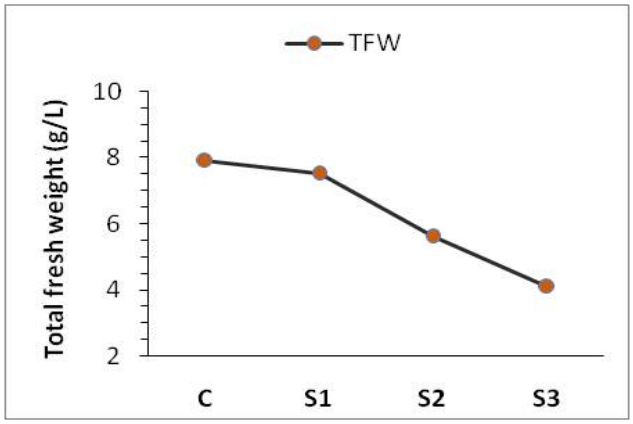

Fig 6. Changes in total biomass in salinity variation.

dimension 9.0×3.5 µm in P2, which reached 10.75×3.5 µm in P4 treatment culture. *S. quadricauda* showed opposite pattern of *E. granulata in* cell dimension (Table 2). Cell shape changes only found in P4 treatment for *E. granulata* (Lanceolate shaped) and *S. quadricauda* (shortened ellipsoidal). Salinity treatment showed significant reduction in cell dimension in S3 treatments for *E. granulata* (8×4.0 µm) and *S. quadricauda* (9×4.0 µm), while *C. vulgaris* increased in cell size (5.5×5 µm). The observed shape found rounded for *C. vulgaris* throughout the research, and the only change occurred in *E. granulata* (widely ovate) and *S. quadricauda* (shortened ellipsoidal) in S3 treatment culture (Fig. 7).

Discussion

This study included only three phytoplankton taxa which represented Chlorophytes and diatoms as the most dominating phytoplankton groups in many freshwater ecosystem from where they were collected (32, 33). Moreover, the study used pH 7.5 in stock culture as well as control culture to make them adapted in normal or pH neutral water conditions in order to assess pH fluctuations impacts on the phytoplankton species from the neutral to acidic or basic water condition. It was clear from the results that variation in pH concentration in freshwater ecosystem has significant impacts on the phytoplankton species. When pH was fluctuated 0.80 units from the pH 7.5, the three taxa were able to cope up with the change and they did not showed any significant changes in their community composition, growth rates, total fresh weight and cell morphology. Moreover, when pH fluctuate over 1.60 units there was found significant impacts, particularly when the environment was acidic (pH 5.90), it can restrict the growth rates of both Chlorophytes, thus the acidic pH was responsible for altering the community composition. Some previous reports also have shown altered species composition for some phytoplankton species in response to acidic pH (15, 16). However, that acidic environment was not responsible for altering their cell morphologies and the diatom was more tolerant to the condition comparatively than the Chlorophytes. On the other hand, when the environment was basic (pH 9.10), it lead the drastic changes in the studied phytoplankton taxa altering overall community composition, growth rates, biomass and cell morphologies, except the cell morphology of *C. vulgaris.* Even the acute reduction in growth rate of *S. quadricauda* meant the environment was comparatively more polluted than the acidic conditions (34). Therefore, the pH treatment indicated that any freshwater ecosystem with 1.60 unit or more pH fluctuations would create many harsh effects on phytoplankton communities living there.

In salinity variations, no harsh effects on the experimented phytoplankton in 0.60 ppt to 1.40 ppt saline conditions were recorded, and noted that some phytoplankton can tolerate over a wide salinity range (20). However, the Chlorophytes faced reduction in cell density and growth rates when the environment increased to 2.20 and 3.0 ppt salinity, while *E. granulata* only restricted by growth rates and cell productivity in 3.0 ppt salinity. That means these two saline environments are responsible for community composition in this study. Besides, comparatively the highest growth rate of *E. granulata* indicated the future dominance of diatom in the provided 3.0 ppt saline condition (Fig. 4). Overall biomass also decreased due to increasing salinity and before 3.0 ppt salinity there was not found any impacts on cell morphologies of the three phytoplankton species. Among them, *S. quadricauda* showed very high sensitivity with increasing salinity that meant the species will face serious problems in increasing salinity. It was reported only the height of phytoplankton was reduced at increased salinity, but the diameter

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

T	Cell dimension (µm)			Cell shape		
pH	**CHL**	**EUG**	**SCE**	**CHL**	**EUG**	**SCE**
C	4.5×4.0	9.5×3.50	10.25×3.5	Round	Spindle	Ellipsoidal
P1	4.2×4.0	9.5×3.50	10.75×3.5	Round	Spindle	Ellipsoidal
P2	4.5×4.5	9.0×3.50	10×3.25	Round	Spindle	Ellipsoidal
P3	4.5×4.5	10.25×3.5	9.75×3.5	Round	Spindle	Ellipsoidal
P4	4.0×4.0	10.75×3.5	9.0×4.0	Round	Lanceolate	S. Ellipsoidal
Sal						
C	4.5×4.0	9.5×3.50	10.25×3.5	Round	Spindle	Ellipsoidal
S1	5.0×4.5	9.0×3.25	9.75×3.5	Round	Spindle	Ellipsoidal
S2	5.2×5.0	8.5×3.0	9.5×3.25	Round	Spindle	Ellipsoidal
S3	5.5×5.0	8.0×4.0	9.0×4.0	Round	W. Ovate	S. Ellipsoidal

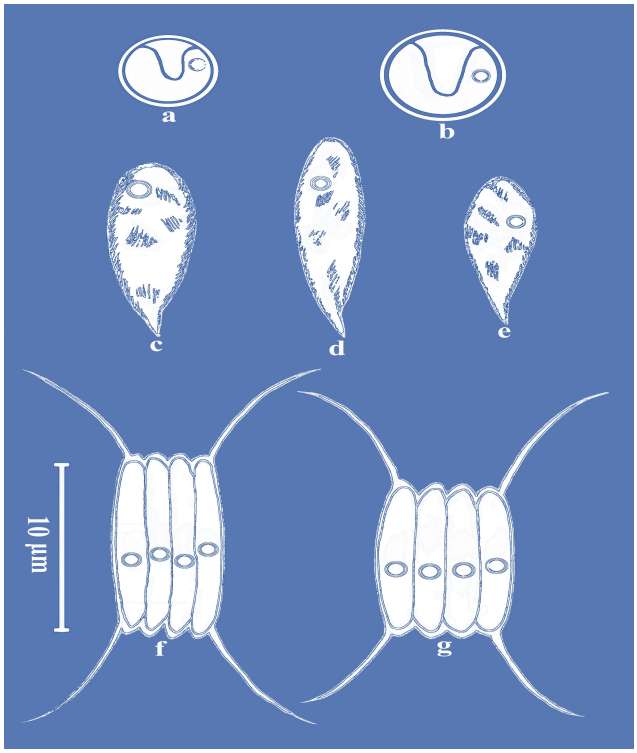

Fig. 7. Observed significant cell morphology in this study. Here, a. *C. vulgaris* in control culture, b. *C. vulgaris* in S3: 3.0 ppt, c. *E. granulata* in control culture, d. *E. granulata* in P4: pH 9.10, e. *E. granulata* in S3: 3.0 ppt, f. *S. quadricauda* in control culture, g. *S. quadricauda* in S3:3.0 ppt and P4: pH 9.10. Bar = 10 µm.

remained unchanged (26). Moreover, other scientists also said salinity changes could restrict the growth and cell height of some photosynthetic organisms (35-38). However, this experiment also observed almost same results in cell height. According to studies, some phytoplankton species exhibit macro-evolutionary decrease in mean cell size due to pH, salt and nutrient ions level changes over geological timescales (39, 40). 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 (41, 42). Therefore, 1.60 units or more pH or salinity change may cause serious problems in certain natural phytoplankton communities in freshwater ecosystem. The authors suggested that as it was laboratory based attempt, there need to be more extensive and long term research including more natural environmental parameters to draw conclusive attention.

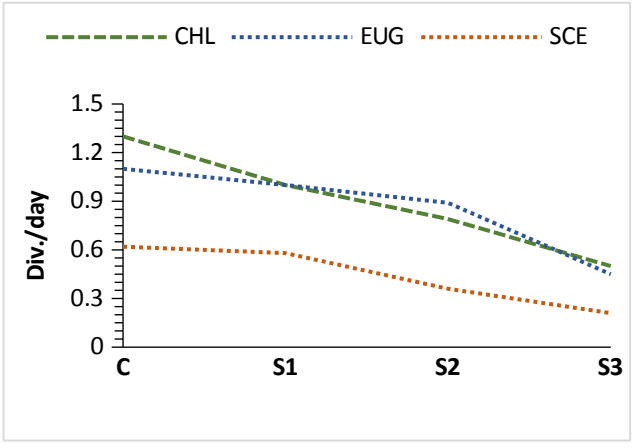

Fig. 4. Growth rate of the taxa in salinity variation.

Conclusion

E. granulata showed more tolerance in a wide range of pH and salinity variations in freshwater ecosystem than *C. vulgaris* and *S. quadricauda*. Salinity 3.0 ppt as well as pH 9.10 had impacts on cell morphologies of the three phytoplankton species. However, to monitor the such changes more accurately in a freshwater ecosystem, a long term research including numerous phytoplankton species from each respective group would be needed to carry out.

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Authors' contributions

SC and RH planned and designed the experiment. DK, MAR, SCB and SKD participated during the experiment and all authors equally contributed to the data analysis. Finally, the manuscript was prepared by SC and edited by RH.

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