DIURNAL VARIATION OF EUGLENA BLOOM IN SOME SELECTED PONDS AND ITS BEHAVIORAL STUDY
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5.1 Introduction

Diurnal variation is the fluctuations in behavior that occurs each day. Eukaryotic algae are known to serve as a model organism for behavioral study. Circadian rhythms or daily rhythms are the endogenous biological programmes that time number of behavioral events occurring at the optimal phases of the daily cycle (Suzuki and Johnson, 2001). *Synechococcus, Chlamydomonas, Euglena* have been studied as model organisms for circadian studies (Mittag, 2001). Solar radiation plays a vital role in regulating the rate of inorganic nutrients uptake and in
the synthesis of organic matter in all phototrophic organisms, as well as in controlling the
distribution and abundance in an ecosystem (Larchar, 1995). It also controls the allocation,
behavior and metabolic activity of a variety of photosynthetic as well as non-photosynthetic
and Bohm 1994, Lindell and Edling, 1996). Exposure to solar radiation has been an essential
and integral part of the evolution of many species and communities of organisms. In aquatic
ecosystems, solar radiation sets up a pronounced vertical habitat gradient. The activity and
abundance of aquatic microorganisms vary depending on fluctuations in light spectrum and
intensity, nutrients and food supply, grazers, viruses, and other environmental factors (Bailey et
al., 1983, Riemann & Sandergaard, 1984, Suttle et al., 1993, Jeffrey et al., 1996, Sommaruga et
rhythms are known to persist under the constant conditions of light and temperature though
different types of pulses (light, darkness and temperature) can advance or delay to next
oscillation (Bourne and Danielli, 1977). Circadian rhythms are the product of evolution and the
circadian activities which are preprogrammed for an organism but not for the other organism has
an advantage over the other (Hoffman, 1976). Bunning (1963) pointed out that circadian clocks
operate with the photoperiodic time measurement mechanism.

Solar radiation is crucial to the synthesis of photosynthetic pigment in algae. Pigments
production in plant body is largely controlled by light. Chlorophyll a, b and carotenoids are the
major photosynthetic pigments found in Euglena. The green chlorophyll is the primary pigment
used for photosynthesis. Carotenoids, composed of carotenes and xanthophylls are secondary
pigments which are usually brown or yellow in colour providing Euglena colourful hues. A
mixture of carotenoid pigments and their derivatives is often referred to as hematohrome. It is
yellow, orange, or red biological pigment present in some algae, especially when exposed to
intense light. Carotenoids are thought to protect chlorophyll from the absorption of excess energy
which photobleach the chlorophyll (Krinsky, 1968). It is synthesized in nature by plant and also
by microorganism. Carotenoids are needed during photosynthesis and also offer protection
against destructive photooxidation (Britton et al., 1995).

Shade tolerant species, growing under light limiting conditions, generally invest more energy in
the formation of chlorophyll for the light harvesting system (Goodchild et al., 1972). It is
anticipated that additional chlorophyll in shade would be largely light harvesting pigment, so as
to provide a more efficient system of light capture in a light limiting environment (Goodchild et al., 1972). Blue light was found to be most effective as compared with yellow, green and red light (Sokawa and Hase, 1967).

“Behaviour” of a species is a direct response of an organism to the environment. Such response may be chemical or a physical movement. Bacteria, fungi, and plants are capable of “behaviour” but the most elaborate and extensive behaviours are limited to members of the animal kingdom. An animal shows different behaviour because of two causes, one is proximate causes and other is ultimate causes. Proximate causes relates to mechanism that enables the organism to exhibit the behaviour. But the ultimate cause – ‘how and why’ can only be explained in terms of evolution. Behaviour again can be classified into two types, one is innate behaviour and other is learned behaviour. Innate behaviour is developmentally fixed. Despite differing environments, all individuals can exhibit characteristic behaviour. Innate behaviours are triggered by a stimulus which closely related to the animal’s environment. Learned behaviours are modified by experience.

On the whole behaviours are governed by both genes and the environment. Genetic manifestation of behaviour differs from one behaviour to the other. All genes including those, whose expression underlies an innate behaviour, require an environment to be expressed. Simple, one-celled organisms are also capable of behaviour. The alga, Euglena tuba, for instance, will move in the direction of poor light, though it has no nervous or endocrine system. In fact, the photosynthetic and photosensitive flagellate Euglena tuba has been an intriguing subject for photo- biological studies. This flagellate dwells in natural shallow ponds, and uses sunlight as source of energy and information. The chloroplasts serve as energy-supplying devices, whereas the light detector is no less sophisticated. Two flagella are inserted in a subapical invagination of the cell termed the reservoir. The stigma is composed of red-orange pigment granules, lies in the adjacent cytoplasm. Only one flagellum emerges from the cell and consists of an axoneme, a paraxial rod running parallel to it, and a swelling known as paraflagellar body is placed near its base (Rosati et al., 1991).

Euglena tuba is a one-celled alga. Early biologists were often confronted with its status as a plant or an animal. It moves around like an animal, but photosynthesizes like a plant. Euglena tuba exhibits an interesting response to light. Hader et al. (1995) worked on gravitaxis behaviour of Euglena and observed that in the absence of other external stimuli the Euglena normally
shows a negative gravitaxis behavior ie. swims upward in the water column. Active physiological orientation mechanisms could be operative. On exposure to high light condition negative gravitaxis inverts to a positive one. Johnson and Thoedore (1942) mentioned the change of red pigment to green in *Euglena* was due to migration of the red granules to periphery or more uniform distribution of the same. The present investigation deals with the diurnal variation of the pigments of the *Euglena tuba* in its natural environment. The chapter describes the temporal variation of chlorophyll *a* and carotenoid pigments and how these pigments are associated with the colour appearance of the interesting organism. Some interesting behavioural pattern recorded in laboratory set up under different conditions are also incorporated in this Chapter.

5.2 Methodology
For study of diurnal variation of *Euglena tuba* bloom, four distant experimental sites were selected viz. Baskandi, Udarband, Silcoorie and Sonai. Baskandi is about 16 km East of Silchar town, Udarband is about 14km North-East of Silchar, Silcoorie is about 20km in South-West direction and Sonai is about 16 km South-East of Silchar. For observing the diurnal periodicity of the bloom four bright sunny days were selected. Experimental observation was started at 7 a.m. and concluded at 5 p.m. Eleven observations were made to estimate the diurnal variation. The samples were collected in centrifuge tubes at every one hour interval.

Observation on *Euglena tuba* was done both in its natural condition and also in laboratory set up under the microscope and behavioural changes were recorded. A systematic observation was done in laboratory condition. Observation of fresh *Euglena tuba* was done under the microscope and the changes in structure were recorded.-The *Euglena tuba* was placed in an environment where dim light is coming from one direction for 30 minutes and after that its changes were recorded. For shock reaction treatment, *Euglena tuba* samples were kept in a bottle with water and were shaken. Changes were noted after observing under the microscope.

5.3 Results and Discussion

5.3.1 Diurnal variation of *Euglena tuba*

The diurnal variation of *Euglena tuba* was observed and the mechanism responsible for this rhythm have been investigated which showed that the rate of photosynthesis is directly influenced by light intensity. Fig. 5.1 shows the action spectrum of *Euglena tuba* showing
chlorophyll \( a \) and carotenoid absorbance peaks. In Baskandi, highest concentration of chlorophyll \( a \) (6.04\( \mu \)g/ml) and carotenoid (6.56\( \mu \)g/ml) was recorded at 1 p.m. In morning 7 a.m. to 9 a.m. chlorophyll \( a \) concentration was higher than the carotenoid pigment which reversed beyond 10 a.m. Towards the evening a fall of both chlorophyll \( a \) and carotenoid pigments concentration were noticed and at 5 p.m. carotenoid concentration was found far lower than the chlorophyll \( a \) (Fig. 5.2). Similar pattern of chlorophyll and carotenoid synthesis were found in Udarband (Fig. 5.3), where highest value of chlorophyll and carotenoid were observed at 12 p.m. (12.03\( \mu \)g/ml and 12.98\( \mu \)g/ml). In this pond carotenoid pigment began to succeed over the chlorophyll a pigment from 9 a.m. and continued till 3 p.m. thereafter chlorophyll \( a \) succeeded over carotenoid. At the surface of aquatic ecosystems, these fluctuations are thought to be due to changes in various factors, including solar zenith angle, atmospheric aerosols, amount of ozone in the stratosphere, density of cloud cover and elevation above the sea level (Madronich 1992 and Kirk 1994). The concentration of chlorophyll and carotenoid synthesis in the cell of \textit{Euglena} follows a similar trend. In the morning when sun ray was soft the production of chlorophyll and carotenoid value was also less but with the increase of solar radiation the production of both the pigments starts increasing. In Silcoorie, (Fig. 5.4) high value of chlorophyll and carotenoids were recorded at 1 p.m. which were 9.63\( \mu \)g/ml and 9.13\( \mu \)g/ml, respectively. Here in this pond chlorophyll a pigment was found to be always higher than the carotenoid pigment. Both the pigments chlorophyll \( a \) (8.16 \( \mu \)g/ml) and carotenoids(7.15 \( \mu \)g/ml) had fall at 2 p.m. The synthesis of chlorophyll and carotenoid largely depend upon the exposer of solar radiation. Chlorophylls degrade at about the same rate as carotenoid pigments. In Sonai (Fig. 5.5) 11.03\( \mu \)g/ml concentration of chlorophyll \( a \) was recorded at 11 a.m. and at the same time high value of carotenoid was also recorded (11.14\( \mu \)g/ml). In most of the ponds in morning and evening hour chlorophyll \( a \) concentration was found higher as compared to carotenoids but with the increase of light intensity the concentration of carotenoids was more with less chlorophyll \( a \) value. The quality of chlorophyll \( a \) preservation provides useful indirect evidence about the quality of carotenoid pigments (Swain, 1985). Timing of increases and subsequent decreases in pigment concentrations are consistent. In the present study, it was found that the light-enhanced chlorophyll formation process but somewhat at a lower rate. The production of both the pigment was stimulated by the exposure to solar radiation, but after a certain period a gap , slowly more carotenoids are synthesized in the \textit{Euglena tuba} cells as compared with chlorophyll \( a \) pigment.
The alga shows a typical behavior of the shape and colour change. It exhibited variation in
colour, the colour of the cell remained green in the morning when the intensity of light is less but
during the day when the sun rays fell straight with strong intensity the colour of the cell become
red. Again in the evening when the sun rays fell oblique and soft the red colour of the cell turned
into green. The colour of the *Euglena* changed to green after disappearance of the red pigment
i.e. carotenoid pigment. Increasing light (higher intensities and longer days) in the early spring is
thought to be the principal factor which stimulates the spring outburst of phytoplankton
production since temperature often remains low during this period. It is likely that the decline in
its abundance is related to the slow regeneration of nutrients. As a motile form the cells are
possibly at an advantage during the nutrient deficient, less light and overcrowding condition
because they can seek out favorable positions in the water column in different time period of the
day and night conditions.

5.3.2 Behavioral study of *Euglena tuba* in laboratory condition

When the organism was kept in normal room condition for long time it became less active and
latter most of them changed the body to a round in shape. Normal movement of the cell was
slow. When the organism faces any stressful environmental condition it has shown quick and
rapid movement. Complete rotation of the cell was performed by spiral undulation of the whole
body (Plate 5.1 and 5.2). The highly organized flexible pellicle helps the cell in changing the
shape of *Euglena*.

Body more or less cylindrical or fusiform to ellipsoid rarely nearly spherical with anterior end
broadly rounded and the canal opening slightly towards one side giving it a lipped appearance;
posterior part ending in a sharp blunt point or with a very short tailor more often broadly
rounded; changing shape markedly by bulging in the middle or curving at anterior end or by
complete rounding up; (Plate-5.1 and 5.2). Pellicle with fine punctuate spiral striae;
chromatophores are 5-16 or more and usually in elongated broad bands or somewhat spindle-
shaped; in cysts and freshly liberated individuals often markedly curved with the free ends
almost touching each other; when inside the cyst or markedly crowded in free living individuals,
sometimes appears as spheroid or discoid.
The fresh *Euglena tuba* were collected from the field and observed under microscope, the organism shows its regular behavioral pattern in structure as well as in movement like twisting and turning continuously. The shape was seen as elongate to fusiform with continuous changes from elongate to triangular to oval and again become elongated. When *Euglena tuba* sample were exposed to dim light under laboratory conditions, it shows regular twisting and turning pattern. When the same samples were shaken vigorously all the cell become rounded in structure and their entire chloroplast arranged in compressed mass of body. The effects of artificial treatment on the orientation of the *Euglena* are stronger than those of solar radiation (Hader and Liu, 1990). UV radiation, particularly UV-B, imposes stress on a variety of aquatic organisms as suggested by Herndl et al., 1993, Holm- Hansen et al., 1993a, b, Cullen and Neale 1994, Williamson, 1995, Aas et al., 1996, Lesser, 1996, Wilhelm, et al., 1998.

5.4 Conclusion

*Euglena tuba* was found to have an interesting diurnal behavior. Temporal changes in chlorophyll a and carotenoid pigments were regulated by sunlight. High intensity of solar irradiation was found to have a significant effect on carotenoid synthesis by the cells which masked the chlorophyll a and consequently provided characteristic brick- red colour to the pond. *Euglena tuba* also exhibited interesting morphological changes under laboratory conditions when it was found that irritation had the marked effect on the frequent twisting and turning activity of the species.

![Fig. 5.1: UV-vis absorption spectrum of acetone extract of *Euglena tuba*](image-url)
Fig. 5.2: Diurnal changes in chlorophyll $a$ and carotenoid concentrations of *Euglena tuba* in Baskandi pond

Fig. 5.3: Diurnal changes in chlorophyll $a$ and carotenoid concentrations of *Euglena tuba* in Udarband pond
Fig. 5.4: Diurnal changes in chlorophyll $\alpha$ and carotenoid concentrations of *Euglena tuba* in Silcoorie pond

Fig. 5.5: Diurnal changes in chlorophyll $\alpha$ and carotenoid concentrations of *Euglena tuba* in Sonai pond
Plate 5.1: Optical micrograph showing behavioural patterns of *Euglena tuba*
Plate 5.2: Optical micrograph showing behavioural patterns of *Euglena tuba*