Research Article

EFFECTS OF SUNLIGHT ON THE ABUNDANCE OF EUGLENOPHYCEAE IN EARTHEN PONDS

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ABSTRACT

Algal red bloom in carp ponds has been a serious concern to farmers due its scum covering the entire pond surface area during the day and disappearing in the evening. Thus it is important to examine the effects of sunlight on dynamics of red bloom algae in ponds. An experiment was done with the use of three treatments, i) non-red bloom pond with sunlight, ii), red bloom pond with sunlight, and iii) red bloom pond without sunlight; each treatment was replicated thrice. Density of Euglenophyceae was assessed from two different water depths (10 cm and 50 cm) at three different times: morning, afternoon, and evening. Results showed that *Euglena sanguinea* Ehrenberg, 1832 was dominant among euglenophytes and it showed vertical and temporal migration with sunlight intensity. Density of *E. sanguinea* was significantly higher ($p<0.05$) at 10 cm and lower ($p<0.05$) at 50 cm in the afternoon. Preventing sunlight to the red bloom pond decreased density of Euglenophyceae and *E. sanguinea* by 69% and 80%, respectively. Maximum red bloom was observed during 12.00 to 13.00 hours, when light intensity was highest (1928 Lux to 1988 Lux). Appearance and disappearance of red bloom in the pond was due to vertical migration of *E. sanguinea* with sunlight intensity.

Key words: Red bloom, *Euglena sanguinea,* dynamics, light intensity, density

INTRODUCTION

Red bloom in fish ponds is caused by euglenophytes dominated by *Euglena sanguinea* (Mandal et al., 2016). Red bloom has been a threat to fish farming because it affects the growth and yield of fish by hindering respiration (Xavier et al., 1991; Rahman et al., 2012) because scum formed on the surface shades the lower waters, inhibits photosynthesis and depletes dissolved oxygen beneath. In addition, scum gives an unpleasant look and brings behavioral changes in fish (Zimba et al., 2010; Boyd & Tucker, 2014). *Euglena* spp. including *E. sanguinea* inhibits the growth of other beneficial algal groups such as chlorophytes and bacillariophytes (Xavier et al., 1991; Rahman et al., 2007, 2012; Mandal et al., 2018) which are preferred natural food of silver carp and rohu (Siddiquee et al., 2012).

Water colour in the pond changes with time; it is green at the dawn and at the dusk when the sun rays are oblique and light intensity is low and is red during mid-day when sunlight is intense (Rehman, 1998; Rahman et al., 2007; Costa, 2014). Red colouration in *E. sanguinea* is due to red carotenoid pigment that contains main component of haematochrome which turns water into a red colour when there is a bloom of *E. sanguinea* (WoRMS, 2011; Zimba et al., 2017; Heidt, 1934). Haematochrome migration (Gerber & Hader, 1994; Xavier et al., 1991) in the cell is influenced by sunlight intensity which together with positive phototaxis in *E. sanguinea* (Gerber & Hader, 1994) could be the reasons for colour change in red bloom pond water. Phototaxis of *E. sanguinea* could bring spatial and temporal variation in its population which might be the cause for red colouration. Moreover, it is also important to identify the colour change pattern of red bloom algae in pond water during daytime. Therefore, an experiment was done to understand the phenomenon of red bloom of algae in earthen ponds in relation to sunlight.

MATERIAL AND METHODS

The experiment was done in earthen ponds of the Aquaculture and Fisheries Department of Agriculture and Forestry University (AFU) during November 20 to 25, 2016. The experiment was done by using complete factorial ($3 \times 2 \times 3$) design including three factors: factor 1: pond types i) non-red bloom pond with sunlight (control), ii) red bloom pond with sunlight and iii) red bloom pond without sunlight (covered with black plastic sheet); factor 2: pond depths i) 10 cm and ii) 50 cm; and factor 3: sampling time i) morning, ii) afternoon and iii) evening. Each treatment (pond types) had three replicates whereas sampling days were taken as replicates. Prior to the experiment, all ponds were completely netted with drag net of 0.5 cm mesh size to remove all fish from ponds and filled with canal water to 1.2 m depth. Water sampling for phytoplankton analysis was done for six days (20 to 25 November 2016) whereas water colour change pattern was observed for three days (20 to 22 November 2016) in the red bloom pond. Density of phytoplankton in non-red bloom ponds for six days were averaged and mean values were compared with red bloom ponds with and without sunlight. In the early morning of 23 November, 2016 the entire surface of the red bloom pond was covered by black plastic sheet to check sunlight to the pond.

Water colour in the red bloom pond was observed for 12 hours from early morning 6.00 am to evening at 6.00 pm through video camera (HXR – MC2500) for three days. Light intensity was monitored for three days at every 15 minutes of time interval by Lux meter (LX-100) which was placed at one meter above the pond dike with a sensor facing towards the light source. Both the video camera and Lux meter were placed on the east bank of the red bloom pond. Colour change pattern of red bloom algae in 12 hours was determined by visual observation and video recording from a video camera which was observed later in the laptop. Water sample was taken using column sampler at three time intervals - morning (6:00 to 7:00 hour) when pond water looked green in colour, afternoon (13:00 to 14:00 hour) when pond water showed intense red bloom, and evening (17:00 to 18:00 hour) when water again returned to green colour.

Phytoplankton were identified using the keys of Prescott (1951) and Rai and Rai (2007) and classified by following the method suggested by Guiry and Guiry (2016). Phytoplankton were counted using Sedgwick-Rafter (S-R) cells and quantified by following APHA (2012). Data were analyzed by using two-way ANOVA followed by Duncan's multiple range test to compare the means. Significant level was set at 5% (p=0.05).

RESULTS

Abundance of phytoplankton in different treatments

The status of abundance of phytoplankton in three treatments is presented in Table (1). A total of four classes of phytoplankton: Euglenophyceae, Chlorophyceae, Bacillariophyceae and Cyanophyceae were identified in three treatment ponds. Among these, Euglenophyceae was significantly higher ($p<0.05$) in the red bloom treatment whereas Chlorophyceae, Bacillariophyceae and Cyanophyceae were significantly higher ($p<0.05$) in the non-red bloom treatment. Euglenophyceae was significantly higher ($p<0.05$) in the red bloom treatment, intermediate ($p<0.05$) in red bloom without sunlight, and lower ($p<0.05$) in non-red bloom treatment. Chlorophyceae dominated rest groups in non-red bloom (48.7%) and red bloom without sunlight (40.8%) treatments while Euglenophyceae (61.5%) dominated rest groups in the red bloom treatment. Total phytoplankton density in red bloom treatment was 40% and 45% higher ($p<0.05$) than in non-red and red bloom without sunlight treatments, respectively (Table 1).

Table 1. Abundance ($\times 10^3$ cells/L) of phytoplankton in different treatments (Mean±SE)

	Non-red bloom	Red bloom	Red bloom without light
Euglenophyceae	0.15 ± 0.02^{C}	0.80 ± 0.08^{A}	0.25 ± 0.02^{B}
Chlorophyceae	0.38 ± 0.04^{A}	0.30 ± 0.02^{B}	0.29 ± 0.01^{B}
Bacillariophyceae	0.14 ± 0.02^{A}	0.12 ± 0.01^{A}	0.09 ± 0.01^{B}
Cyanophyceae	0.11 ± 0.01^{A}	0.08 ± 0.01^{B}	0.08 ± 0.01^{B}
Total	0.78 ± 0.01^{B}	1.3 ± 0.01^{A}	0.71 ± 0.01^{B}

Note: Similar uppercase superscripts for values in a row indicate no significant difference ($p>0.05$) among the values.

Abundance of euglenophytes in different treatments

Abundance of different euglenophytes in different treatments (pond factor) is shown in Table (2). Four genera, viz. *E. sanguinea*, *E.acus*, *Trachelomonas,* and *Phacus* were found in three treatments during the experimental period. Among these, *E. sanguinea* dominated the rest three euglenophytes in all cases. *Euglena sanguinea* was significantly higher ($p<0.05$) in the red bloom treatment than the rest whereas *Euglena acus* and *Trachelomonas* were significantly higher ($p<0.05$) in red bloom with and without sunlight than non-red bloom treatment.

Table 2. Abundance ($\times 10^3$ cells/L) of Euglenophyceae in different treatments (Mean±SE)

	Non-red bloom	Red bloom	Red bloom without light
Euglena sanguinea	0.11 ± 0.01^{B}	0.66 ± 0.00^{A}	0.13 ± 0.01^{B}
Euglena acus	0.02 ± 0.01^{B}	0.06 ± 0.00^{A}	0.05 ± 0.01^{A}
Trachelomonas sp.	0.01 ± 0.00^{B}	0.05 ± 0.01^{A}	0.04 ± 0.0^{A}
Phacus sp.	0.01 ± 0.00^{A}	0.02 ± 0.00^{A}	0.02 ± 0.01^{A}
Euglenophyceae	0.15 ± 0.02^{C}	0.80 ± 0.08^{A}	0.25 ± 0.02^{B}

Note: Similar uppercase superscripts for values in a row indicate no significant difference ($p>0.05$) among the values.

Depth wise and time wise abundance of euglenophytes in different treatments

Abundance of *E. sanguinea*, *E. acus*, *Trachelomonas,* and *Phacus* in different factors of pond types: non-red bloom, red bloom with sunlight and red bloom without sunlight; depth: 10 cm and 50 cm depth, and three sampling time periods: in the morning, afternoon, and in the evening has been presented in Table (3). *Euglena sanguinea* was significantly higher ($p<0.05$) in red bloom treatment than non-red and red bloom without sunlight at both depths and three time periods. Similarly, density of *E. sanguinea* was significantly higher ($p<0.05$) in the afternoon than in the morning at 10 cm. Similarly, its density was significantly higher ($p<0.05$) in the evening than in the afternoon at 50 cm pond depth, indicating its vertical migration with sunlight intensity. Density of *Euglena acus*, *Trachelomonas* and *Phacus* sp. were significantly higher ($p<0.05$) at both depths, and time periods in the red bloom with and without sunlight than non-red bloom. Mean density of *E. sanguinea* and *Phacus* sp. was significantly higher ($p<0.05$) at 10 cm than 50 cm in red bloom indicating spatial variation.

Table 3. Abundance ($\times 10^3$ cells/L) of Euglenophyceae in different treatments at two depths and three time periods (Mean±SE)

Euglenophyceae	Time	Non-red bloom	Red bloom	Red bloom without light
Euglena sanguinea	Morning	0.14 ± 0.04^{aB}	0.47 ± 0.01^{bA}	0.14 ± 0.03^{aB}
10 cm	Afternoon	0.11 ± 0.05^{aB}	1.19 ± 0.03^{aA}	0.19 ± 0.03^{aB}
	Evening	0.09 ± 0.01^{aC}	0.65 ± 0.0^{abA}	0.10 ± 0.01^{aB}
	Mean	**0.11 ± 0.02^{aB}**	**0.77 ± 0.01^{aA}**	**0.14 ± 0.0^{aB}**
50 cm	Morning	0.14 ± 0.04^{aB}	0.51 ± 0.15^{abA}	0.13 ± 0.06^{aB}
	Afternoon	0.14 ± 0.03^{aB}	0.33 ± 0.07^{bA}	0.16 ± 0.04^{aB}
	Evening	0.07 ± 0.02^{bC}	0.80 ± 0.04^{aA}	0.07 ± 0.02^{aB}
	Mean	**0.12 ± 0.02^{aB}**	**0.55 ± 0.05^{bA}**	**0.12 ± 0.03^{aB}**
Euglena acus	Morning	0.01 ± 0.01^{aB}	0.07 ± 0.01^{aA}	0.08 ± 0.01^{aA}
10 cm	Afternoon	0.03 ± 0.01^{aB}	0.09 ± 0.0^{aA}	0.06 ± 0.01^{aA}
	Evening	0.02 ± 0.01^{aB}	0.06 ± 0.01^{aA}	0.05 ± 0.0^{aA}
	Mean	**0.02 ± 0.0^{aB}**	**0.07 ± 0.01^{aA}**	**0.06 ± 0.01^{aA}**
50 cm	Morning	0.01 ± 0.01^{aB}	0.06 ± 0.02^{aA}	0.06 ± 0.02^{aA}
	Afternoon	0.05 ± 0.03^{aA}	0.05 ± 0.01^{aA}	0.05 ± 0.02^{aA}
	Evening	0.01 ± 0.01^{aC}	0.06 ± 0.01^{aA}	0.05 ± 0.01^{aA}
	Mean	**0.02 ± 0.02^{aB}**	**0.06 ± 0.01^{aA}**	**0.06 ± 0.02^{aA}**
Trachelomonas	Morning	0.00 ± 0.0^{bB}	0.06 ± 0.02^{aA}	0.05 ± 0.01^{aA}
10 cm	Afternoon	0.01 ± 0.01^{aB}	0.07 ± 0.02^{aA}	0.06 ± 0.02^{aA}
	Evening	0.00 ± 0.0^{bC}	0.08 ± 0.02^{aA}	0.03 ± 0.0^{aB}
	Mean	**0.01 ± 0.0^{aB}**	**0.07 ± 0.02^{aA}**	**0.05 ± 0.01^{aA}**
50 cm	Morning	0.00 ± 0.00^{bB}	0.03 ± 0.01^{aA}	0.03 ± 0.01^{aA}
	Afternoon	0.03 ± 0.01^{aB}	0.03 ± 0.01^{aA}	0.03 ± 0.01^{aA}
	Evening	0.00 ± 0.00^{bB}	0.05 ± 0.02^{aA}	0.03 ± 0.01^{aA}
	Mean	**0.01 ± 0.01^{aB}**	**0.04 ± 0.01^{aA}**	**0.03 ± 0.01^{aA}**
Phacus sp.	Morning	0.01 ± 0.01^{aB}	0.03 ± 0.01^{abA}	0.03 ± 0.01^{aA}
10 cm	Afternoon	0.00 ± 0.00^{aB}	0.05 ± 0.00^{aA}	0.04 ± 0.01^{aA}
	Evening	0.01 ± 0.01^{aB}	0.03 ± 0.01^{abA}	0.02 ± 0.01^{aA}
	Mean	**0.01 ± 0.01^{aB}**	**0.04 ± 0.01^{aA}**	**0.03 ± 0.01^{aA}**
50 cm	Morning	0.01 ± 0.01^{aA}	0.00 ± 0.00^{bB}	0.01 ± 0.01^{aA}
	Afternoon	0.01 ± 0.01^{aA}	0.00 ± 0.00^{bB}	0.00 ± 0.00^{aB}
	Evening	0.01 ± 0.01^{bA}	0.01 ± 0.01^{aA}	0.01 ± 0.01^{aA}
	Mean	**0.01 ± 0.01^{aA}**	**0.00 ± 0.00^{bB}**	**0.01 ± 0.00^{aA}**

Note: Similar lowercase superscripts for values in a column and similar uppercase superscripts for values in a row indicate no significant difference ($p>0.05$) among the values.

Colour change pattern of water in the red bloom pond

Variation of water colour with different light intensity is shown in (Table 4) and Figure (1). Light influenced pond water colour in the red bloom pond. Clear reddening began from 10 am with 890 Lux, and reached maximum during 12.00 to 13.00 hours when light intensity was maximum of 1928 to 1988 Lux. Reddening of water colour again gradually began to disappear in the afternoon from 16.00 hours with 905 Lux.

Table 4. Water colour change in red bloom pond in different time periods with light intensity.

Time	Light intensity (Lux)	Water colour
6.00	0.01	Reddish green
7.00	117.66	Mixed red
8.00	387.33	Mixed red
9.00	616.00	Light red
10.00	890.00	Red
11.00	1455.00	Red
12.00	1928.00	Brick red
13.00	1988.00	Brick red
14.00	1156.00	Red
15.00	904.67	Red
16.00	582.00	Mixed red
17.00	78.00	Mixed red
18.00	0.00	Reddish green

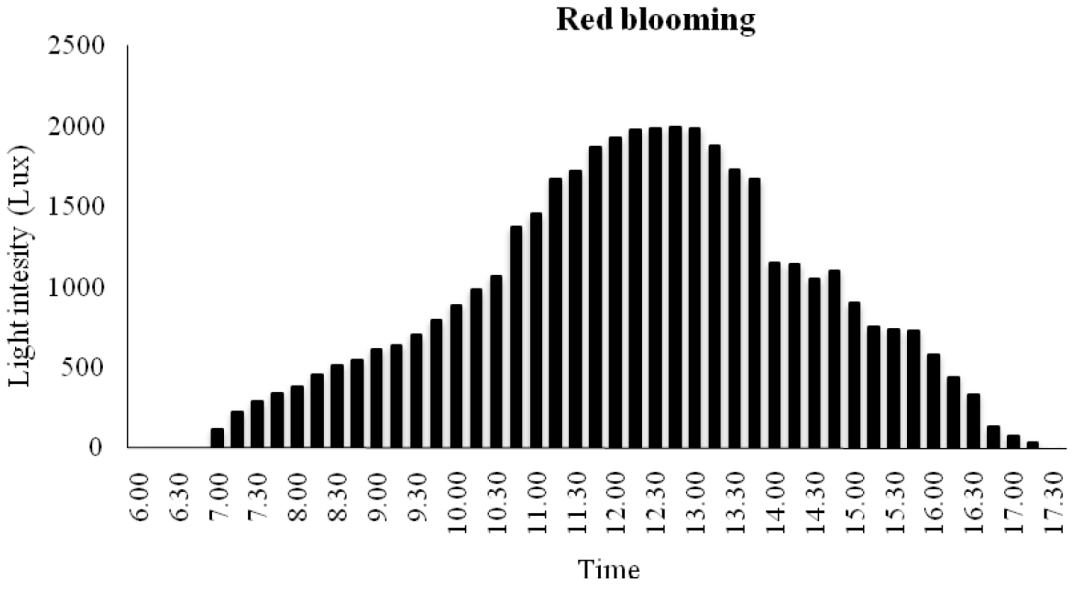

Figure 1. Relation between light intensity and reddening in the red bloom pond

DISCUSSION

An experiment was conducted to understand dynamics of red bloom algae and the influence of sunlight on the colour change pattern of red bloom algae in ponds. Red bloom pond was covered by black plastic sheet to check sunlight so that the effect of sunlight on red bloom could be assessed. A total of four classes of phytoplankton, viz. Euglenophyceae, Chlorophyceae, Bacillariophyceae and Cyanophyceae were identified, and among them Euglenophyceae were dominant in red bloom treatment (Mandal et al., 2016, 2018). Euglenophytes contributed 61.5% of the total phytoplankton population in the treatment. Preventing sunlight decreased density of Euglenophyceae by 31% in the red bloom without sunlight treatment which indicated light influenced their population and eventually red scum in the pond. Chlorophyceae, Bacillariophyceae and Cyanophyceae were dominant in the control pond indicating abundance of Euglenophyceae that could affect phytoplankton communities. Phytoplankton communities were found altered in the red bloom pond which could be due to abundant Euglenophyceae (Xavier

et a1., 1991, Rahman et al., 2007; Rahman et al*.,* 2012) and it could affect fish growth and production. Similar density of Chlorophyceae and Cyanophyceae in red bloom with and without sunlight treatments indicated low effect of sunlight on them. This might be because experimental duration was short and their population was also comparatively lower for visible effect of sunlight prevention.

Euglena sanguinea dominated rest species *Euglena acus*, *Trachelomonas* and *Phacus* in all treatments (Mandal et al., 2016). *Euglena sanguinea* shared 82.5%, 73.3% and 33.3% population of euglenophytes in red bloom, non-red bloom, and red bloom without sunlight treatments, respectively. Density of *E. sanguinea* was 6 and 5 times higher in red bloom treatment than the control and red bloom without sunlight treatments indicating it is the major species for reddening. Preventing sunlight to enter the pond decreased density of *E. sanguinea* by five times indicated sunlight is essential for survival of this species (Azizullah et al., 2012). Blocking the sunlight decreased mean population of euglenophytes including *E. sanguinea* and bacillariophytes (Hader et al., 2015).

Among euglenophytes, only *E. sanguinea* showed spatial and temporal variation in abundance in red bloom ponds with sunlight. *Euglena sanguinea* was present in different abundances at 10 cm and 50 cm pond depths at different time periods which was probably due to their vertical movement in response to the light source. Significantly higher population (153%) in the afternoon than in the morning, at 10 cm and lower population (59%) in the afternoon than in the evening at 50 cm indicated that *E. sanguinea* exhibited phototaxis (Gerber & Hader, 1994). Upward migration of *E. sanguinea* with increased light intensity increased population at the surface, but decreased its population at 50 cm in the afternoon in the red bloom pond. *Euglena sanguinea* did not show vertical migration in the light blocked red bloom pond. *Euglena* possesses an eye like photoreceptive organ which helps to orient toward the light source (Kim et al., 1998). Development of red bloom algae including *E. sanguinea* depends on the combination of a set of factors such as sunlight, temperature and nutrient concentrations, and light intensity, as reported by Deb (2016). *Euglena sanguinea* changes its colour from green to red colour with light intensity when carotenoid pigment migrates from center to peripheral cells to cover chlorophyll (Laza-Martinez et al., 2019), and after sunset, it changes its colour from red to green (Heidt, 1934). In a bloom situation, cells may occupy the upper water surface and then migrate downward by late afternoon and evening (Lackey, 1968; Laza-Martinez et al., 2019). Hence vertical migration of *E. sanguinea* along with biochemical activity inside the cell probably caused red blooming in the fish pond.

Red colour began to appear from 7.00 hour when light intensity was 117.66 Lux and intense red bloom of brick-red colour was observed at 12.00 to 13.00 hour when light intensity reached maximum ranged from 1928 to 1988 Lux. Reddening began to disappear gradually from 16.00 hour with decreased light intensity. This showed that reddening of pond water increased with light intensity. Higher population of *E. sanguinea* in the afternoon due to surface migration and intense red colour during 12.00 and 13.00 hour sufficiently explained the role and relation of intensity of sunlight with bloom of *E. sanguinea* and colour change mechanism in ponds.

CONCLUSION

Euglenophyceae is the dominant phytoplankton group in red bloom ponds. Colour change from green in the morning to intense red in the afternoon in the red bloom pond was due to abundant *E. sanguinea* and its surface migration with increased sunlight intensity. Reddening of the pond reached its peak when the light intensity was maximum of 1928 to 1988 Lux in the afternoon 12.00 to 13.00 hours. Bloom of Euglenophyceae was found to alter phytoplankton communities, including favourite chlorophytes and bacillariophytes for carps to affect their growth. Since this experiment was conducted in fish-less ponds for relatively short duration, verification trial in fish containing ponds for longer period is recommended.

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