Research article

EFFECTIVENESS OF DIFFERENT MEASURES TO CONTROL RED BLOOM IN CARP PONDS

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ABSTRACT

Red blooms caused by *Euglena sanguinea* (Ehrenberg, 1832) might cause severe depletion of dissolved oxygen significantly in the pond. An experiment was conducted to assess the effects of measures for controlling *E. sanguinea* on water quality, growth and yield of carp polyculture. The experiment included four treatments: without mitigation measure (T1), skimming using net skimmer (T2), fertilization with urea and diammonium phosphate (T3) and liming using agriculture lime (T4) with three replications. The experiment was carried out for 120 days. The results showed that abundance of *E. sanguinea* was significantly lower ($p < 0.05$) in urea and diammonium phosphate treated ponds (270 ± 10 cells L^{-1}) than control ponds (1650 ± 90 cells L^{-1}). Water quality parameter such as nitrite, total nitrogen and total phosphorus were significantly ($p < 0.05$) higher in control ponds (T1) than in treatment ponds. The net fish yield of rohu was significantly higher (0.19 ± 0.0 t ha^{-1}) in T3 ponds than T2 ponds (0.07 ± 0.0 t ha^{-1}). The present experiment effectively controlled abundance of *E. sanguinea* but admixture of urea and diammonium phosphate application appeared to be better control measures because dissolved oxygen content was at acceptable level in the ponds.

Keywords: *Euglena sanguinea,* phytoplankton, mitigation, fish production

INTRODUCTION

Red scum of *Euglena sanguinea* on water surface and often in carp polyculture ponds is a common occurrence (Mandal et al., 2016; 2018). *Euglena sanguinea* is a flagellated, unicellular algae found in high nutrient containing freshwaters (Ohio, 2013; Cimoli, 2014; Mandal et al., 2016, 2018). The occurrence of red colour is due to presence of carotenoid astaxanthin pigments in the cytoplasm (Grung & Liaaen-Jensen, 1993; Gerber & Häder, 1994; Frassanito et al., 2008; Laza-Martínez et al., 2019, Zheng et al., 2020). The reddening process includes the relocation of cytoplasmic lipid globules where astaxanthin accumulates from the center of the cell to peripheral locations when exposed to high light (Laza-Martínez et al., 2019)frequently carotenoids, under chronic stress is a response observed in diverse kinds of eukaryotic photoautotrophs. It is thought that red pigments protect the chlorophyll located underneath by a light-shielding mechanism. However, the synthesis or degradation of carotenoids is a slow process and this response is usually only observed when the stress is maintained over long periods of time. In contrast, rapid colour changes have been reported in the euglenophyte Euglena sanguinea. Here we study the ecophysiological process behind this phenomenon through chlorophyll fluorescence, and pigment, colour and ultrastructural analyses. Reddening in E. sanguinea was due to the presence of a large amount of free and esterified astaxanthin (representing 80% of the carotenoid pool. Red bloom by *E. sanguinea* is a threat to fish farming because dissolved oxygen (DO) content is lower in red bloom pond to affect fish growth (Mandal et al., 2016, 2018). Astaxanthin in the cytoplasm covers chlorophyll to reduce light absorption and consequently, photosynthesis and oxygen production during day time (Laza-Martínez et al., 2019) frequently carotenoids, under chronic stress is a response observed in diverse kinds of eukaryotic photoautotrophs. It is thought that red pigments protect the chlorophyll located underneath by a light-shielding mechanism. However, the synthesis or degradation of carotenoids is a slow process and this response is usually only observed when the stress is maintained over long periods of time. In contrast, rapid colour changes have been reported in the euglenophyte Euglena sanguinea. Here we study the ecophysiological process behind this phenomenon through chlorophyll fluorescence, and pigment, colour and ultrastructural analyses. Reddening in E. sanguinea was due to the presence of a large amount of free and esterified astaxanthin (representing 80% of the carotenoid pool which results in low DO in ponds. Red bloom inhibits photosynthesis, depletes DO, brings changes in phytoplankton communities, behavioral changes in fish and sometimes results in fish mortality (Zimba et al., 2004, 2010; Boyd & Tucker,

2014). Thus, the red bloom in fish ponds is an unnecessary and harmful occurrence. Hence, our purpose in the present study is to find out the ways to control the red bloom in fish ponds.

Quick lime (CaO) and duckweed (*Lemna minor*) have been tested to control euglenophytes in carp polyculture ponds so far (Rahman et al., 2012). Results were positive as both quick lime and duckweed significantly reduced population of euglenophytes. However, duckweed is least applicable in fish ponds (Cronk & Fennessy, 2001) because duckweed spreads rapidly in ponds to form thick floating mats over the water surface, inhibits light penetration beneath and decreases overall pond productivity. On the other hand quick lime is expensive and not easily available as agriculture lime, which limits its use in bulk to fish ponds in Nepal. Though there are herbicides found in the local agro-vets but these might be toxic to fish and needs verification tests for its safe dose to fish ponds. Therefore, this study aims to investigate use of skimmer, chemical fertilizers urea along with DAP and agriculture limestone ($CaCO_3$) for effective control of *E. sanguinea* bloom in carp ponds.

MATERIALS AND METHODS

Experimental design

The experiment was conducted in 12 ponds (150.9 ± 4.1 m^2) at the Aquaculture Farm of Fisheries Program, Faculty of Animal Science, Veterinary Science and Fisheries, Agricultural and Forestry University (AFU), Chitwan, Nepal for 120 days from 15 February to 14 June 2018. A completely randomized design (CRD) was used with four treatments and three replications of each treatment. Treatments included: (T1) without mitigation measure (control), (T2) skimming using net skimmer, (T3) fertilization with urea and DAP and (T4) liming using agriculture lime ($CaCO_3$).

Pond preparation

Prior to stocking, all ponds were completely drained and filled with canal water up to 1.2 m depth. After that ponds were fertilized with goat manure at the rate of 60 g m^{-2} on dry weight basis to enhance red algal bloom (Mandal et al., 2018).

Fish stocking and feeding

After two weeks of application of goat manure in each pond, six carp species were stocked at a rate of 1 fish m^{-2}. Silver carp (*Hypophthalmychthys molitrix* Valenciennes, 1844), common carp (*Cyprinus carpio* Linnaeus, 1758), rohu (*Labeo rohita* Hamilton, 1822), mrigal (*Cirrhinus mrigala* Hamilton, 1822), catla (*Catla catla* Hamilton, 1822) and grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) were stocked at 3.5: 2.5: 1.5: 1: 1: 0.5 ratio, respectively. Floating pellet containing 24 % CP was fed to fish at 3 % body weight per day. Feed proximate analysis showed that pellet contained 91.5 % dry matter, 24.0 % crude protein, 5.5 % crude fiber, 5.6 % ether extract, 5.3 % total ash and 51.0 % nitrogen free extract. Fish growth was assessed monthly by sampling 10 % population of each species to adjust feed ration.

Measures for controlling red bloom algae

A skimmer was made by a fine nylon net with mesh size of 0.5 mm for skimming red scum in skimming treatment. The skimmer was 25 meter long and 1.25 meter wide and it's each side was tied to two pieces of bamboo stick separately to stretch the net during operation. The skimmer was held by two persons and moved from one side of the pond to another side. On reaching next side, the skimmer was curved, pulled out from the pond and red scum attached to the net was removed by hand. The process was repeated two times. Urea and DAP was applied to ponds at rates of 9.4 g m^{-2} and 7 g m^{-2} respectively to maintain N:P ratio of 4:1 (Knud-Hansen et al., 1993) in fertilization treatment ponds while agriculture lime was applied at the rate of 12.5 g m^{-2} in liming treatment ponds (Rahman et al., 2012). Control measures began two weeks after fish stocking and was conducted in nine treatment ponds biweekly.

Water quality parameters

Water quality parameters such as temperature, DO, pH, conductivity, total dissolved solid (TDS) were analyzed in situ at 06:00 – 07:00 h using Hanna HI-98194 Multiparameter, Singapore. For further water quality parameter analysis, 3 L water sample from each pond was collected in a bucket using column sampler.

From collected sample, 250 mL of water was brought to Aquaculture lab for analysis of chlorophyll-a, total nitrogen (TN), total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate, nitrite and ammonia. Within an hour SRP, nitrate, nitrite and ammonia were analyzed using Hanna HI-83203-02 Multiparameter bench photometer, Singapore. Chlorophyll-a samples were obtained by filtering the pond water through Whatman GF⁄C glass fibber filters (≈1.2 µm pore size). Before extraction, chlorophyll-a samples were refrigerated in a sealed plastic container. Total phosphorus and total nitrogen were analyzed with standard methods (APHA, 2012). All these water quality parameters were analyzed on biweekly basis.

Phytoplankton

For plankton analysis, five liters of pond water samples were collected monthly at 06:00 – 07:00 h (from up to 50 cm depth) using column sampler and filtered to 30 mL in test tube using plankton net (mesh size 5 µm). Then the filtered sample was preserved in 5 % formaldehyde solution for phytoplankton analysis. Identification and enumeration of phytoplankton were done following Prescott (1951), Rai and Rai (2007), Guiry and Guiry (2016). Phytoplankton belonging to euglenophyceae was identified and presented to genus level and genus *Euglena* to species level while the rest phytoplankton was presented to class level. The number of phytoplankton was estimated using following formula.

$N = C \times 1000\ mm^3\ L^{-1}D^{-1}W^{-1}S^{-1}$

Where, N = Number of phytoplankton units, C = Number of organisms counted, L= Length of each stripe (mm), D = Depth of each stripe (mm), W = Width of each stripe (mm), S = Number of stripes.

Harvesting

Final harvesting of fish was done after 4 months of stocking by complete draining of each pond. Harvested fish were counted and weighed using electronic balance (0.1 g). Similarly, pond wise carps species were separated and their total batch weight taken.

During stocking and harvesting, fish were counted and weighed individually. Daily weight gains (DWG), total weight gain (TWG) and survival rate were used to compare fish growth performance.

DWG (g $fish^{-1}$ day^{-1}) = (Mean final wt. – Mean initial wt.) × 100/ Experimental period
TWG (kg ha^{-1}) = Final total wt. – Initial total wt.
Survival (%) = Number of fish harvested × 100/ Number of fish stocked
NFY (t ha^{-1} yr^{-1}) = Harvest weight (kg) – Stocked weight (kg) x 10 x 365/Culture area x Culture period
Apparent food conversion ratio (AFCR) = Quantity of feed supplied (kg)/Total weight gain (kg)

Data analysis

Data of carp production, water quality and phytoplankton were analyzed by one way ANOVA followed by Duncan Multiple Range Test (DMRT) using SPSS (version 16) and significance level was considered at 5 % ($p < 0.05$). All means were given with ± SE.

RESULTS

Growth and yield of carp

Species wise growth performance of six carps during 120 days is presented in Table 1 and combined net fish yield, survival and apparent food conversion ratio are presented in Table 2. Control measures did not affect combined net fish yield, survival and apparent FCR but affected growth and yields of silver carp, rohu, mrigal and grass carp. Final mean weight of silver carp was significantly higher ($p < 0.05$) in the control (T1) and fertilization ponds (T3) than in liming ponds (T4). Final total weight, total weight gain and net fish yield of rohu were significantly higher ($p < 0.05$) in fertilization ponds than in skimming ponds. Final total weight, total weight gain and net fish yield of mrigal were significantly higher ($p < 0.05$) in control ponds than in skimming and fertilization ponds but were not significantly different ($p > 0.05$) from liming ponds. Final mean weight, final total weight, growth rate, total weight gain and net fish yield of grass carp were significantly ($p < 0.05$) higher in fertilization ponds than in liming ponds but were not different from control and skimming ponds.

Table 1. Growth performances of carps in different treatments during 120 days (mean ± SE) (n = 12).

Parameter	Treatments			
	T1 (Control)	T2 (Skimming)	T3 (Fertilization)	T4 (Liming)
Silver carp				
Initial mean weight (g fish^{-1})	3.4 ± 0.1^{a}	3.9 ± 0.5^{a}	3.5 ± 0.0^{a}	3.5 ± 0.0^{a}
Initial total weight (kg ha^{-1})	11.3 ± 0.3^{a}	13.8 ± 1.9^{a}	12.8 ± 0.1^{a}	12.1 ± 0.1^{a}
Final mean weight (g fish^{-1})	146.1 ± 7.2^{a}	127.2 ± 25.1^{ab}	153.5 ± 11.1^{a}	91.6 ± 2.1^{b}
Final total weight (kg ha^{-1})	120.9 ± 46.4^{a}	229.3 ± 72.4^{a}	155.5 ± 30.8^{a}	69.9 ± 8.6^{a}
Daily weight gain (g fish^{-1} day^{-1})	1.2 ± 0.1^{a}	1.0 ± 0.2^{ab}	1.3 ± 0.1^{a}	0.7 ± 0.0^{b}
Total weight gain (kg ha^{-1})	109.1 ± 46.1^{a}	215.5 ± 46.1^{a}	143.1 ± 30.7^{a}	57.8 ± 8.5^{a}
Net fish yield (t ha^{-1} yr^{-1})	0.33 ± 0.1^{a}	0.65 ± 0.2^{a}	0.48 ± 0.1^{a}	0.17 ± 0.0^{a}
Survival (%)	23.4 ± 8.6^{a}	48.6 ± 12.6^{a}	29.8 ± 7.7^{a}	21.8 ± 2.4^{a}
Catla				
Initial mean weight (g fish^{-1})	8.4 ± 0.1^{a}	8.5 ± 0.0^{a}	8.5 ± 0.0^{a}	8.3 ± 0.1^{a}
Initial total weight (kg ha^{-1})	8.4 ± 0.1^{a}	8.5 ± 0.0^{a}	8.5 ± 0.0^{a}	8.3 ± 0.1^{a}
Final mean weight (g fish^{-1})	211.2 ± 68.4^{a}	113.2 ± 31.6^{a}	286.5 ± 76.6^{a}	155.5 ± 33.0^{a}
Final total weight (kg ha^{-1})	83.3 ± 17.7^{a}	59.8 ± 23.6^{a}	80.2 ± 12.2^{a}	67.6 ± 19.2^{a}
Daily weight gain (g fish^{-1} day^{-1})	1.7 ± 0.6^{a}	0.9 ± 0.3^{a}	2.3 ± 0.6^{a}	1.2 ± 0.3^{a}
Total weight gain (kg ha^{-1})	74.9 ± 13.8^{a}	51.3 ± 23.6^{a}	71.8 ± 12.2^{a}	59.2 ± 19.2^{a}
Net fish yield (t ha^{-1} yr^{-1})	0.22 ± 0.0^{a}	0.15 ± 0.1^{a}	0.22 ± 0.0^{a}	0.18 ± 0.0^{a}
Survival (%)	47.9 ± 16.3^{a}	48.3 ± 11.9^{a}	37.0 ± 17.7^{a}	41.7 ± 4.4^{a}
Common carp				
Initial mean weight (g fish^{-1})	18.7 ± 0.2^{a}	18.9 ± 0.0^{a}	18.8 ± 0.1^{a}	18.8 ± 0.1^{a}
Initial total weight (kg ha^{-1})	46.7 ± 0.4^{a}	47.2 ± 0.1^{a}	46.9 ± 0.2^{a}	46.1 ± 0.2^{a}
Final mean weight (g fish^{-1})	329.9 ± 51.8^{a}	346.8 ± 99.2^{a}	397.1 ± 32.9^{a}	312.6 ± 86.3^{a}
Final total weight (kg ha^{-1})	529.5 ± 48.0^{a}	472.4 ± 204.4^{a}	550.2 ± 133.3^{a}	471.0 ± 67.0^{a}
Daily weight gain (g fish^{-1} day^{-1})	2.6 ± 0.4^{a}	2.7 ± 0.8^{a}	3.2 ± 0.3^{a}	2.4 ± 0.7^{a}
Total weight gain (kg ha^{-1})	482.8 ± 48.1^{a}	425.2 ± 204.4^{a}	503.3 ± 133.5^{a}	423.9 ± 67.4^{a}
Net fish yield (t ha^{-1} yr^{-1})	1.45 ± 0.1^{a}	1.28 ± 0.6^{a}	1.51 ± 0.4^{a}	1.27 ± 0.2^{a}
Survival (%)	68.6 ± 15.8^{a}	47.5 ± 16.9^{a}	56.9 ± 16.6^{a}	68.7 ± 15.8^{a}
Rohu				
Initial mean weight (g fish^{-1})	4.2 ± 0.1^{a}	4.2 ± 0.1^{a}	4.2 ± 0.0^{a}	4.2 ± 0.0^{a}
Initial total weight (kg ha^{-1})	6.3 ± 0.1^{a}	6.3 ± 0.1^{a}	6.3 ± 0.1^{a}	6.3 ± 0.1^{a}
Final mean weight (g fish^{-1})	155.8 ± 58.0^{a}	135.0 ± 35.2^{a}	211.1 ± 31.1^{a}	213.3 ± 71.6^{a}
Final total weight (kg ha^{-1})	114.5 ± 53.2^{ab}	76.5 ± 15.8^{b}	193.6 ± 27.0^{a}	113.8 ± 24.1^{ab}
Daily weight gain (g fish^{-1} day^{-1})	1.3 ± 0.5^{a}	1.1 ± 0.3^{a}	1.7 ± 0.3^{a}	1.7 ± 0.6^{a}
Total weight gain (kg ha^{-1})	108.2 ± 53.3^{ab}	70.2 ± 15.7^{b}	187.4 ± 27.0^{a}	107.5 ± 24.2^{ab}
Net fish yield (t ha^{-1} yr^{-1})	0.32 ± 0.2^{ab}	0.21 ± 0.1^{b}	0.56 ± 0.1^{a}	0.32 ± 0.1^{ab}
Survival (%)	45.8 ± 4.5^{a}	44.8 ± 14.1^{a}	61.5 ± 2.8^{a}	49.5 ± 25.4^{a}
Mrigal				
Initial mean weight (g fish^{-1})	13.8 ± 0.1^{a}	13.9 ± 0.1^{a}	13.9 ± 0.1^{a}	13.9 ± 0.1^{a}
Initial total weight (kg ha^{-1})	13.9 ± 0.2^{a}	14.0 ± 0.0^{a}	14.0 ± 0.0^{a}	13.7 ± 0.2^{a}
Final mean weight (g fish^{-1})	198.4 ± 31.0^{a}	99.3 ± 24.4^{a}	127.1 ± 30.2^{a}	213.9 ± 63.1^{a}
Final total weight (kg ha^{-1})	198.4 ± 31.0^{a}	60.7 ± 28.6^{b}	96.8 ± 18.4^{b}	124.3 ± 36.2^{ab}

Parameter	Treatments			
	T1 (Control)	T2 (Skimming)	T3 (Fertilization)	T4 (Liming)
Daily weight gain (g fish^{-1} day^{-1})	1.5 ± 0.3^{a}	0.7 ± 0.2^{a}	0.9 ± 0.3^{a}	1.7 ± 0.5^{a}
Total weight gain (kg ha^{-1})	184.5 ± 31.2^{a}	46.7 ± 28.6^{b}	82.8 ± 18.5^{b}	110.6 ± 36.2^{ab}
Net fish yield (t ha^{-1} yr^{-1})	0.55 ± 0.1^{a}	0.14 ± 0.1^{b}	0.25 ± 0.1^{b}	0.33 ± 0.1^{ab}
Survival (%)	97.9 ± 2.1^{a}	70.8 ± 29.2^{a}	79.2 ± 11.8^{a}	61.7 ± 19.2^{a}
Grass carp				
Initial mean weight (g fish^{-1})	13.4 ± 0.1^{a}	13.5 ± 0.0^{a}	13.5 ± 0.1^{a}	13.4 ± 0.0^{a}
Initial total weight (kg ha^{-1})	6.7 ± 0.0^{a}	6.8 ± 0.0^{a}	6.7 ± 0.0^{a}	6.7 ± 0.0^{a}
Final mean weight (g fish^{-1})	293.4 ± 84.0^{ab}	285.7 ± 42.8^{ab}	460.5 ± 99.3^{a}	164.8 ± 49.2^{b}
Final total weight (kg ha^{-1})	84.0 ± 42.3^{ab}	90.3 ± 35.6^{ab}	172.3 ± 53.2^{a}	33.9 ± 8.3^{b}
Daily weight gain (g fish^{-1} day^{-1})	2.3 ± 0.7^{ab}	2.3 ± 0.4^{ab}	3.7 ± 0.8^{a}	1.3 ± 0.4^{b}
Total weight gain (kg ha^{-1})	77.3 ± 42.2^{ab}	83.6 ± 35.6^{ab}	165.6 ± 53.1^{a}	27.2 ± 8.3^{b}
Net fish yield (t ha^{-1} yr^{-1})	0.23 ± 0.1^{ab}	0.25 ± 0.1^{ab}	0.50 ± 0.2^{a}	0.08 ± 0.0^{b}
Survival (%)	55.6 ± 22.3^{a}	70.8 ± 29.2^{a}	75.2 ± 13.6^{a}	55.4 ± 24.2^{a}

Mean values with different superscript letters in the same row are significantly different ($p < 0.05$).

Table 2. Combined net fish yield, survival and AFCR in different treatments (mean ± SE) (n = 12).

Parameter	Treatments			
	T1 (Control)	T2 (Skimming)	T3 (Fertilization)	T4 (Liming)
Initial total weight (kg ha^{-1})	93.6 ± 0.9^{a}	96.4 ± 1.9^{a}	94.7 ± 0.1^{a}	94.4 ± 0.2^{a}
Final total weight (kg ha^{-1})	1130.5 ± 55.0^{a}	989.0 ± 228.9^{a}	1231.7 ± 150.3^{a}	880.5 ± 49.3^{a}
Total weight gain (kg ha^{-1})	1036.9 ± 54.2^{a}	892.6 ± 230.4^{a}	1137.0 ± 150.4^{a}	786.1 ± 49.2^{a}
Net fish yield (t ha^{-1} yr^{-1})	3.1 ± 0.2^{a}	2.7 ± 0.7^{a}	3.4 ± 0.5^{a}	2.4 ± 0.1^{a}
Survival (%)	49.6 ± 8.4^{a}	51.1 ± 11.4^{a}	49.2 ± 6.7^{a}	45.3 ± 10.7^{a}
AFCR	1.6 ± 0.2^{a}	2.4 ± 0.5^{a}	1.5 ± 0.2^{a}	2.1 ± 0.1^{a}

Mean values with different superscript letters in the same row are significantly different ($p< 0.05$).

Water quality

Water quality parameters such as pH, soluble reactive phosphorus, dissolved oxygen, nitrate, nitrite, chlorophyll-a, total dissolved solid, conductivity, total nitrogen and total phosphorus were significantly different ($p < 0.05$) among the treatments (Table 3). Dissolved oxygen and Chlorophyll-a were found highest in T3 and lowest in T1. Water pH was significantly higher ($p < 0.05$) in T3 ponds than control ponds but statistically similar ($p > 0.05$) with T2 and T4 ponds. NH_3-N, nitrite, TDS, conductivity, total nitrogen and total phosphorus were significantly higher ($p < 0.05$) in control ponds whereas nitrate was significantly higher ($P < 0.05$) in T3 ponds. SRP was significantly higher ($p < 0.05$) in T2 and T3 than T4 but statistically similar ($p > 0.05$) with T1.

Table 3. Water quality parameters in different treatments (mean ± SE) (n = 12).

Parameter	Treatments			
	T1 (Control)	T2 (Skimming)	T3 (Fertilization)	T4 (Liming)
Temperature (°C)	24.1 ± 0.1^{a}	24.1 ± 0.1^{a}	24.2 ± 0.2^{a}	24.2 ± 0.1^{a}
pH	7.5 ± 0.0^{a}	7.6 ± 0.1^{a}	7.7 ± 0.0^{a}	7.6 ± 0.0^{a}
DO (mg.L^{-1})	2.3 ± 0.1^{c}	2.7 ± 0.2^{bc}	4.0 ± 0.2^{a}	3.1 ± 0.3^{b}
SRP (mg.L^{-1})	0.08 ± 0.01^{ab}	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.05 ± 0.01^{b}
NH_3-N (mg.L^{-1})	0.81 ± 0.11^{a}	0.53 ± 0.27^{ab}	0.79 ± 0.08^{a}	0.22 ± 0.03^{b}
Nitrite (mg.L^{-1})	0.06 ± 0.01^{a}	0.04 ± 0.0^{b}	0.03 ± 0.01^{b}	0.03 ± 0.01^{b}
Nitrate (mg.L^{-1})	0.01 ± 0.0^{b}	0.04 ± 0.02^{b}	0.32 ± 0.04^{a}	0.18 ± 0.07^{a}
TDS (mg.L^{-1})	164.3 ± 9.6^{a}	146.4 ± 8.2^{ab}	127.5 ± 4.8^{b}	151.5 ± 11.2^{ab}
Conductivity (µS.cm^{-1})	329.8 ± 18.9^{a}	294.3 ± 12.6^{ab}	254.9 ± 9.7^{b}	298.8 ± 20.7^{ab}
TN (mg.L^{-1})	4.9 ± 0.3^{a}	3.0 ± 0.2^{b}	3.2 ± 0.2^{b}	2.9 ± 0.04^{b}
TP (mg.L^{-1})	0.9 ± 0.1^{a}	0.5 ± 0.1^{c}	0.7 ± 0.01^{b}	0.3 ± 0.04^{d}
Chlorophyll-a (mg.L^{-3})	13.1 ± 1.6^{c}	16.2 ± 0.9^{bc}	29.1 ± 2.4^{a}	19.4 ± 1.5^{b}

Mean values with different superscript letters in the same row are significantly different ($p < 0.05$).

Phytoplankton

Four classes of phytoplankton viz. bacillariophyceae, chlorophyceae, cyanophyceae and euglenophyceae and three genera of euglenophyceae such as *Euglena*, *Phacus* and *Trachelomonas* were identified and their densities are given in Table 4. It was found that density of *E. sanguinea*, *E. acus*, *Trachelomomnas* and *Phacus* was significantly lower ($p < 0.05$) in treatment ponds than in control ponds. Density of *E. sanguinea* was lowest (270 ± 10 cells L^{-1}) in fertilization and liming treatment ponds (300 ± 46 cells L^{-1}), intermediate in skimming ponds (530 ± 60 cells L^{-1}) and highest in control ponds (1650 ± 90 cells L^{-1}). Density of euglenophytes corresponded to the density of *E. sanguinea* and was lowest in fertilization (550 ± 40 cells L^{-1}) and liming ponds (570 ± 30 cells L^{-1}), intermediate in skimming ponds (830 ± 70 cells L^{-1}) and highest in the control ponds (2170 ± 116 cells L^{-1}). *Euglena sanguinea* shared more than 76 % of the total euglenophyte population in the control ponds.

Table 4. Abundance of phytoplankton (mean ± SE) (n = 12) in four different treatments

Phytoplankton (cells.L^{-1})	Treatments			
	T1 (Control)	T2 (Skimming)	T3 (Fertilization)	T4 (Liming)
Bacillariophyceae	810 ± 50^{a}	600 ± 30^{b}	760 ± 100^{ab}	570 ± 0^{b}
Chlorophyceae	1120 ± 40^{c}	1130 ± 150^{c}	2640 ± 40^{a}	1420 ± 40^{b}
Cyanophyceae	670 ± 40^{ab}	450 ± 60^{bc}	770 ± 112^{a}	420 ± 40^{c}
Euglenophyceae	2170 ± 116^{a}	830 ± 70^{b}	550 ± 40^{c}	570 ± 30^{c}
Euglena sanguinea	1650 ± 90^{a}	530 ± 60^{b}	270 ± 10^{c}	300 ± 46^{c}
Euglena acus	230 ± 10^{a}	130 ± 10^{b}	100 ± 10^{b}	110 ± 0^{b}
Trachelomonas	210 ± 20^{a}	100 ± 10^{b}	130 ± 20^{b}	120 ± 10^{b}
Phacus	80 ± 10^{a}	70 ± 10^{ab}	50 ± 0^{b}	50 ± 0^{b}
Total	**4770 ± 240^{a}**	**3010 ± 300^{b}**	**4720 ± 310^{a}**	**2990 ± 120^{b}**

Mean values with different superscript letters in the same row are significantly different ($p < 0.05$).

DISCUSSION

The present experiment was conducted to evaluate performance of three different methods: skimming by skimmer, fertilization with urea and DAP and liming with agriculture lime for controlling abundance of *E. sanguinea* without deteriorating water quality and fish yield. Combined net yield of carp did not differ among treatments indicated that measures and red bloom algae had no effect on overall carp production. However, effects of measures were found on species wise growth and yield of silver carp, rohu, mrigal and grass carp. Growth and net yield of rohu were higher in fertilized ponds which might be due to higher DO and abundant phytoplankton particularly of chlorophytes (Rai et al., 2010). Chlorophyte density was almost double in fertilized ponds (2640 cells L^{-1}). Final mean weight and growth rate of silver carp was also higher in the control and fertilizer used ponds where phytoplankton density was higher than skimmer used and lime used ponds. Both silver carp (Chen, 1990) and rohu are phytoplankton feeder (Chondar, 1999) and got benefitted from abundant phytoplankton in ponds. Higher individual growth and net yield of grass carp in fertilized ponds can be attributed to better water quality and eating more pellet. Since no grass and vegetables was given to grass carp, it might have utilized pellet efficiently for somatic growth. Probably silver carp and rohu shifted their food and feeding habit from pellet to natural food which were abundant in fertilized green ponds and left pellet for grass carp. On contrary, net yield of mrigal was higher in control ponds than treatment ponds which were probably due to relatively higher survival in control ponds (97.9 ± 2.1 %) than treatment ponds (61.7 ± 19.2 % in T4, 70.8 ± 29.2 % in T2 and 79.2 ± 11.8 % in T3).

Dissolved oxygen was lowest in control ponds due to the highest density of euglenophytes (Mandal et al., 2018; Rahman et al., 2007, 2012). During day time, carotenoid pigment in *E. sanguinea* migrates from center to periphery of the cell to cover chlorophyll which inhibits photosynthesis and oxygen production (Laza-Martínez et al., 2019)frequently carotenoids, under chronic stress is a response observed in diverse kinds of eukaryotic photoautotrophs. It is thought that red pigments protect the chlorophyll located underneath by a light-shielding mechanism. However, the synthesis or degradation of carotenoids is a slow process and this response is usually only observed when the stress is maintained over long periods of time. In contrast, rapid colour changes have been reported in the euglenophyte Euglena sanguinea. Here we study the ecophysiological process behind this phenomenon through chlorophyll fluorescence, and pigment, colour and ultrastructural analyses. Reddening in E. sanguinea was due to the presence of a large amount of free and esterified astaxanthin (representing 80% of the carotenoid pool. In addition, shading effects of scum also inhibits light penetration to lower level and diffusion of oxygen from air (Rehman, 1998; Zimba et al., 2004, 2017). Abundant phytoplankton (green and blue-green phytoplankton, and diatoms) and higher content of chlorophyll-a increased DO level in treatment ponds. DO was highest in fertilized ponds where phytoplankton excluding euglenophytes were enhanced by biweekly application of urea and DAP. In the control ponds, TP, TN, TDS and conductivity were found higher in red algal bloom ponds as reported by Rahman et al. (2007, 2012) and Mandal et al. (2018) .

Control measures had been effective to control both euglenophytes and *E. sanguinea* population. Chemical treatments were more effective than physical treatment to control their population. Urea and DAP reduced densities of euglenophytes and *E. sanguinea* by 3.9 and 6.1 times while lime reduced their densities by 3.8 and 5.5 times, respectively. Urea and DAP might inhibit euglenophytes because it was reported that increased concentration of both fertilizers decreased *E. gracilis* number (Azizullah et al., 2012) due to direct toxicity of fertilizers to algae. In comparison to DAP, *Euglena* can tolerate much higher concentrations of urea. Regarding lime treatment, limestone might have adsorbed phosphorus (Kavitha et al., 2016; Mortula et al., 2007) and made it unavailable for *E. sanguinea* (Fried et al., 2003; Ly et al., 2014) and overall phytoplankton. Hence, density of *E. sanguinea* and overall phytoplankton was lowest in lime treated ponds. Skimmer performed lower than chemical methods because *E. sanguinea* escaped from sides and bottom during skimming operation. Considering this, skimming was performed three times to increase its effectiveness. Skimming is relatively inexpensive method and does not have adverse effects to the ecosystem however; it is not applicable to large ponds.

CONCLUSION

Aquaculture is growing fast with annual growth rate 9.6 % in Nepal (DoFD, 2018). Semi-intensive carp polyculture is the main aquaculture practice in Nepal. The red bloom algae have been a common occurrence in carp ponds. All control measures used in the present experiment controlled red bloom algae by reducing population of euglenophytes and *E. sanguinea*. However, DAP and urea treated ponds appeared to be a better measure because dissolved oxygen was at acceptable level, natural food phytoplankton were abundant and growth and yield of silver carp, rohu and grass carp was also better in the ponds. Urea and DAP are easily available and it is easy to handle than lime hence more practical for farmers to use.

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