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Research Article

Blue-green algae and nutrient concentrations in two *Tor tambroides* aquaculture ponds differing in construction

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ABSTRACT. Two mahseer fish *(Tor tambroides)* aquaculture ponds located in Serian district, Sarawak were investigated for blue-green algae composition and nutrient dynamics in a six month period (January to June 2007). A total of 35 blue-green algae species belonging to 11 genera and four families were recorded. Genera *Chroococcus*, *Nostoc*, *Oscillatoria*, *Pleurocapsa* and *Synechocystis* were found in both ponds. Potential toxin producer genera, *Microcystis* and *Anabaena* formed a visible brown bloom on the surface of the earth pond. The highest blue-green algal cell density was recorded in June for the earth pond (1,009,000 cells $m\ell^{-1}$) and in January for the HDPE pond (521,000 cells $m\ell^{-1}$). The highest chlorophyll *a* concentration was documented in June for both ponds (10.2377 $\mu g\ell^{-1}$ in HDPE pond and 172.1160 $\mu g\ell^{-1}$ in the earth pond). Nutrient concentration, namely soluble reactive phosphorus (SRP) (0.01-2.02 $mg\ell^{-1}$ in HDPE pond, and 0.01-0.29 $mg\ell^{-1}$ in earth pond), ammonia-nitrogen (0.01-0.90 $mg\ell^{-1}$ in HDPE pond and 0.01-0.45 $mg\ell^{-1}$ in earth pond) and nitrate (0.02-0.08 $mg\ell^{-1}$ in HDPE pond and 0.01-0.05 $mg\ell^{-1}$ in earth pond) were also recorded. This finding suggested that the earth pond is prone to experience algae bloom and at the same time, could support greater population of blue-green algae. Results also showed that SRP and nitrate are not the only factors that influence blue-green algae composition in aquaculture ponds but rather a combination of other multiple environmental factors.

Keywords: Blue-green algae composition, HDPE pond, earth pond, nutrients, *Tor tambroides*

INTRODUCTION

Cyprinid Fish, genus *Tor,* commonly known as mahseer, is distributed throughout Southeast Asian regions (Roberts, 1999). One of the members of the genus, *Tor tambroides,* is an indigenous fish of Sarawak (de Silva *et al.*, 2004). Locals call this fish "empurau." In natural rivers in Sarawak, *T. tambroides* plays an important role in ecology as they feed on wild fruits, reflecting their status as the primary consumer in a complex riverine food web. In addition, *T. tambroides* is associated with mountainous streams and rivers, preferring clear, swift flowing waters with stony, pebbly and rocky bottoms (Inger & Chin, 1990), therefore becoming one of the bioindicators of a non-polluted river.

In South-east Asian regions, particularly in Sarawak, *empurau* has economic importance as it could fetch RM400.00 to RM600.00 per kilogramme depending on size, grade and whether the fish comes from wild or semi-wild stock. It is common in top-notch river fish specialty restaurants in Sarawak to

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have a menu of *empurau* costing RM1,300.00 per dish, reflecting the high status of this fish. Due to its overwhelming demand, there are reports saying that wild *empurau* is now experiencing 'overfishing.' The Sarawak State Government has many conservation programmes in place to combat the declining population of *empurau*. One of the programmes is the *T. tambroides* artificial propagation research, established at the Indigenous Fisheries Research and Production Centre (IFRPC), Tarat, Serian, with the aim of increasing the aquaculture production of this species as well as producing fries to carry out systematic re-stocking at selected rivers.

In IFRPC, two types of pond constructions are commonly used in the cultivation of *T. tambroides*. Earth ponds are constructed without using any bottom-lining material, while high density polyethylene (HDPE) ponds are lined with high density polyethylene plastic. Observation and communication with IFRPC staff during field trips indicate that it is common to have algae bloom in selected aquaculture ponds. Although there were reports on mass mortality of fish in certain ponds at certain times of the year, there is no conclusive and strong evidence yet on causes of such incidents. Laiping & Hassan (2010) had reported that ponds at the IFRPC Serian supported many species of blue-green algae (cyanobacteria), and some were confirmed as toxin-producer species using the Polymerase Chain Reaction (PCR)-based technique. It is well known that in mineral-rich eutrophic systems, for example shallow ponds in tropical regions, blue-green algae produce high cell densities and sometimes they produce toxins that could endanger animals and human health (Codd *et al.*, 2005).

This paper describes our early work on assessment of blue-green algae assemblages in two types of ponds (earth pond and HDPE pond) at IFRPC, documentation on concentration of selected nutrients in those ponds as well as discussions on the relationships between them.

MATERIALS AND METHODS

The IFRPC Tarat, situated in Serian district, was selected for the blue-green algae study. Two types of *Tor tambroides* aquaculture ponds, namely pond AP22 and P12, were selected based on the recommendation from the IFRPC. Pond AP22 is an earth pond layered with black HDPE (High Density Polyethylene) which was stocked with 120 individuals of F1 *empurau* fries (approximately 0.07 g weight per individual fish). P12 is an earth pond stocked with 70 individuals of F1 *empurau* juveniles (approximately 0.07 g weight per individual fish). The stocking was done by the IFRPC staff. The number of fries and juveniles released in each pond were based on availability of stocks and the management plan of the IFRPC. The mean depth of both ponds was approximately 0.4 m. The surface areas of Pond AP22 and P12 are 495m^2 (33 × 15m) and 648m^2 (36 × 18m), respectively. Sampling was conducted monthly from January 2007 to June 2007 (stocking stage until rearing stage). In this paper, AP22 will be referred to as HDPE pond while Pond P12 will be referred to as an earth pond.

The sampling of cyanobacteria was adapted from blue-green algae and algae sampling procedures by Chorus & Cavalieri (2000). Blue-green algae samples from the water subsurface were collected using the 2ℓ Wildco® water sampler (SN: 4109) and sieved through 20 µm mesh net size of a custom-made sieve. Specimens retained in the sieve were kept in separate Kortell® polyethylene bottles, preserved with Lugol's solution and transported back to the laboratory for species identification and enumeration. During sampling, selected ambient physico-chemical parameters such as temperature, pH, dissolved oxygen (DO) and turbidity were measured *in situ* using Horiba's Multiprobe W-22XD Series. The values were recorded in triplicates.

Subsurface water samples were also taken for chlorophyll *a* determination and nutrients analysis. Water samples were placed in Kortell® polyethylene bottles, labeled and

stored in an ice chest filled with crushed ice and transported back to the UNIMAS laboratory for further analysis.

For preserved samples, observation was carried out using the Inverted Light Microscope Olympus (Olympus, Tokyo, Japan, SN:3LO9174) attached with a cool CCD camera. Species identification was based on keys according to Anagnostidis & Komarek (1985; 1986; 1988; 1989; 1991), Bold & Wynne (1985), Hoek *et al.* (1995), Prescott (1954), Aishah (1996) and Sze (1998). Enumerations were carried out using a hemacytometer (improved Neubeuer) following standard methods by Lawton *et al.* (1999). Results are expressed in cells per millilitre and were recorded in triplicates.

Prior to chlorophyll *a* extraction, water samples were homogenized and sieved through a glass microfibre filter GF/C 47 mm in diameter Whatman® Schleicher & Schuell. The sieved samples were then subjected to chilled acetone extraction (Kowalewska *et al.*, 2004). The extracts were analyzed using a spectrophotometer Hach© DR2800 according to the APHA method (APHA, 1998). Wavelengths used were 630nm, 647nm, 664nm and 750nm.

Selected nutrients, namely ammonia-nitrogen, nitrate and soluble reactive phosphorus (SRP), were also analyzed using the Hach© DR2010 standard method (Table 1). The number of blue-green algae cells was expressed as cells per millilitre of water. The physico-chemical parameters were obtained from the triplicate samples at each station (n=3). The Microsoft Excel 2003 and SPSS Ver 17.0 (Software Statistical Package 17.0 for Windows) were used to perform analysis of physical and chemical variables in samples. Mean values of triplicates with a vertical line of standard deviation were presented, while the bivariate correlation was used to assess the relationships between blue-green algal density, chlorophyll *a*, physico-chemical parameters and nutrient concentrations as suggested by Fowler *et al.* (2000).

RESULTS AND DISCUSSION

Thirty five taxa belonging to 11 genera and four subsections of blue-green algae were recorded (Table 2). *Synechocystis* spp., *Oscillatoria* spp., *Chroococcus* spp., *Nostoc* spp. and *Pleurocapsa* spp. were common in both ponds. In the earth pond, a brownish bloom of *Anabaena* spp. and *Microcystis* spp. were observed on the surface water. Altogether, 21 taxa from 10 genera were recorded from the earth pond and 14 taxa of six genera in the HDPE pond. In the earth pond, the maximum cell density observed during the study period was 1,009 × 10^3 cells/mℓ attained in June (Figure 1b). In the HDPE pond, the maximum algal density observed during the study period occurred in January (521 × 10^3 cells/mℓ) (Figure 1a). Overall, the cell density in an earth

Table 1. Analyses of physical and chemical variables in samples taken from two ponds in IRFPC, Tarat.

PARAMETERS	METHOD OF ANALYSES	REFERENCE
Water temperature, dissolved oxygen, pH, DO & turbidity	*In situ*, using a multiparameter device	Horiba, Ltd.
Nitrate	Spectrophotometry	Hach Kit DR2010 Manual
Nitrite	Spectrophotometry	Hach Kit DR2010 Manual
Ammonia-Nitrogen	Spectrophotometry	Hach Kit DR2010 Manual
Soluble Reactive Phosphorus (SRP)	Spectrophotometry with acetone extraction	APHA (1998)
Photosynthetic Pigments (Chlorophyll *a*)		

Table 2. Blue-green algae taxa identified from different collection sites.

Study Area	Blue-green algae diversity
HDPE Pond	**Family: CHROOCOCCACEAE** *Synechocystis aquatilis* *Chroococcus* sp.(1) *Chroococcus* sp.(2) *Gloeocapsa* sp.(1) *Gloeocapsa* sp.(2) *Synechocystis* sp.(1) **Family: OSCILLATORIACEAE** *Oscillatoria quasiperforata* *Oscillatoria nigra* *Oscillatotia* sp.(1) *Oscillatoria* sp.(2) **Family: NOSTOCACEAE** *Nostoc* sp.(1) *Nostoc* sp.(2) **Family: PLEUROCAPSACEAE** *Pleurocapsa* sp.(1) *Pleurocapsa* sp.(2)
Earth Pond	**Family: CHROOCOCCACEAE** *Microcystis aeroginosa* *Microcystis flos-aquae* *Microcystis* sp.(1) *Chroococcus limneticus* *Chroococcus* sp.(3) *Chroococcus* sp.(4) *Synecchocystis* sp.(1) **Family: OSCILLATORIACEAE** *Oscillatoria princeps* *Spirulina* sp.(1) *Spirulina* sp.(2) *Oscillatoria* sp.(3) *Lyngbya birgei* *Lyngbya* sp.(1) *Lyngbya* sp.(2) **Family: NOSTOCACEAE** *Anabaena circinalis* *Anabaena* sp.(1) *Anabaena* sp.(2) *Anabaenopsis* sp. *Nostoc* sp.(3) *Nostoc* sp.(4) **Family: PLEUROCAPSACEAE** *Pleurocapsa* sp.(3)

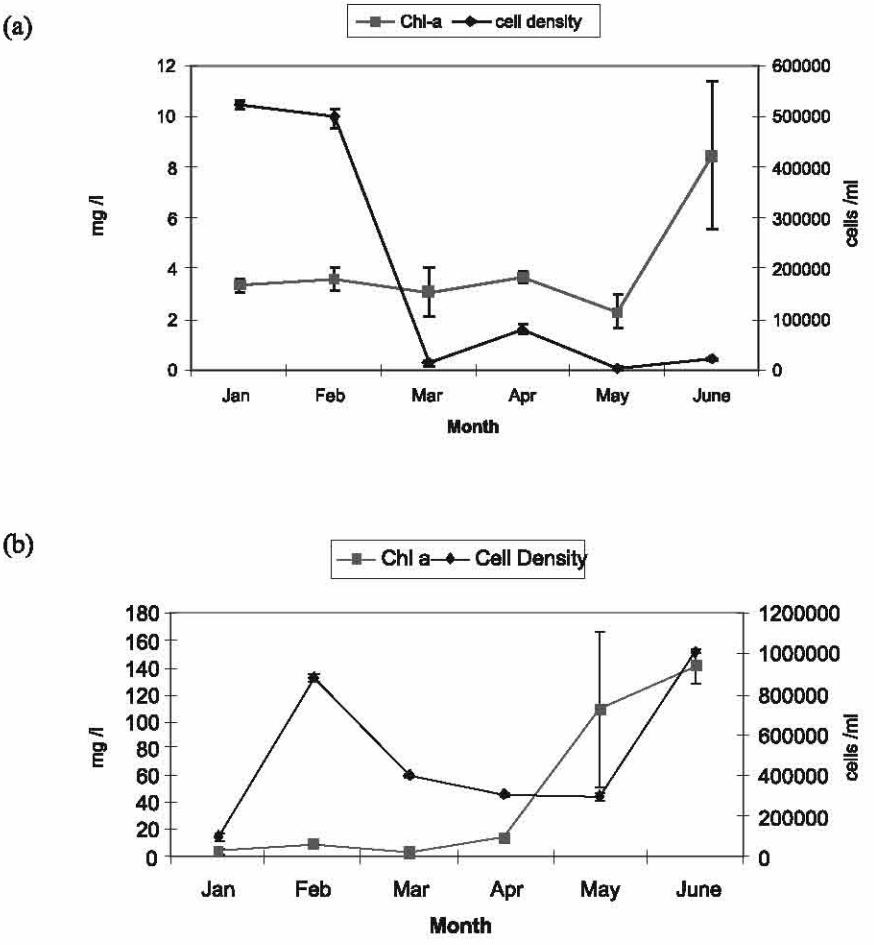

Figure 1. Changes in blue-green algae density (cells/ml) and variation in chlorophyll *a* biomass (μg/ℓ) in High Density Polyethylene (HDPE) pond (A) and earth pond (B) during January until June 2007. Points are means of three replicates with vertical bar showing standard deviation of the mean.

pond was relatively higher compared to the HDPE pond throughout this study, thus suggesting that the earth pond is a better habitat for blue-green algae. This situation is most likely due to the influx of nutrients from sediments of an earth pond that are favourable for the growth of blue-green algae compared to HDPE lined structure of the HDPE ponds that prevents accumulation of sediments (Mc Larney & William, 1987).

The chlorophyll *a* measurement in each sampling site is given in Figure 1 which showed that there were slight fluctuations in chlorophyll *a* concentration in the HDPE pond (Figure 1a), while for the earth pond, the chlorophyll *a* concentration showed an increasing trend (Figure 1b). The chlorophyll *a* concentration in the HDPE pond was the highest in June in both ponds (10.2377 μg/ℓ in HDPE pond and 172.1160 μg/ℓ in earth pond).

This variation in pattern reflects the ecological success of blue-green algae, which may very much depend on their ability to compete with other types of phytoplankton with the combination of several factors (Boyd & Tucker, 1998). The brownish bloom that formed at the subsurface of the earth pond during this study was largely composed of *Microcystis aeruginosa*, *M. flos-aquae*, *Microcystis*. sp., *Anabaena circinalis* and *Anabaena*. sp.

Microcystis spp. are often the dominant genera in eutrophic water bodies containing high concentrations of inorganic nutrients. Frankelin (1972) reported that *Microcystis* is one of dominant blue-green algae in persistent bloom in tropical freshwaters, and are exposed to constant sunshine, warmth and nutrients such as phosphate, silicate, nitrate, carbon dioxide and lime. The buoyant nature of many planktonic blue-green algae contributes to the formation of blooms in freshwater bodies (Chorus & Cavalieri, 2000).

In the HDPE pond, the number of blue-green algae cells was low eventhough the chlorophyll *a* concentration was relatively high, suggesting the occurrence of other phytoplankton (Figure 1A). This finding is in accordance with Hariyadi *et al.* (1994) who stated that most of the blue-green algae species encountered in freshwater aquaculture occur as minor components of the plankton community. Only a few species such as *Anabaena* and *Microcystis* (recorded at the earth pond) and *Oscillatoria* (recorded at the HDPE pond) might produce bloom which contributed to the chlorophyll *a* concentration (Boyd & Tucker, 1998). Cell density and chlorophyll *a* from the earth pond were recorded as the highest in June, 1,009,000 cells/mℓ and 172.1160 μg/ℓ respectively. Moreover, in any ecosystem, all organisms including blue-green algae cannot grow independently and indefinitely because all the species are interlinked and has cyclic transformation of nutrients (Muthukumar, 2007). The physico-chemical changes in the environment may effect particular species and induce the growth and abundance of other species which leads to the succession of several species in temporal and spatial aspects (Pingale & Deshmukh, 2005). In this study, the utilization of the HDPE lining system in the HDPE pond maintains the concentration of dissolved oxygen and sustains the water quality which are not suitable for the blue-green algae, compared to an earth pond that is highly exposed to the bottom substrate "oxygen sink" and input of bottom soil nutrients (Mc Larney & William, 1987).

The high chlorophyll *a* but low blue-green algae cell density was also due to the relatively low growth rates of blue-green algae (Boyd & Tucker, 1998). Although they are slow growing, they can compete well for limited sources-termed "K-selected" organisms by ecologists which include common blue-green algae observed in warm water aquaculture ponds (Kilham & Hecky, 1988). K-selected organisms are poor oxygenators of the water on a per unit biomass basis compared to most eukaryotic species of phytoplankton. In addition, under extreme low-light conditions for photosynthesis and low rates of water column turbulent mixing, persistent positive buoyancy of bloom-forming blue-green algae may result in the formation of surface scums (Boyd & Tucker, 1998) which were observed in the earth pond during this study.

The correlation analysis of the HDPE pond showed that temperature ($r = -0.594$, $P<0.01$), pH ($r = -0.642$, $P<0.01$), dissolved oxygen ($r = -0.550$, $P<0.05$) and turbidity ($r = 0.861$, $P<0.01$) had significant effects on blue-green algae cell density and chlorophyll *a* concentration. Low levels of dissolved oxygen, especially in the HDPE pond may have effected the blue-green algae cell density. Similar results were also reported by Subha & Chandra (2005) and Rani *et al.* (2005).

The pH from both ponds ranged from 7.59 to 9.94. This condition is optimal for freshwater aquaculture (Boyd, 1990) and particularly, favourable to the growth of blue-green algae (Bold & Wynne, 1985).

Turbidity was positively correlated with chlorophyll *a* in both ponds (r = 0.861 in the HDPE pond and r = 0.849 in an earth pond, P<0.01) reflecting the presence of phytoplankton and zooplankton in large numbers (Boyd, 1998). Water turbidity is important as it determines the amount of light penetration that occurs in the water column of the pond. This in turn will have an effect on the temperature of the water and the amount of vegetation and algae that will grow in the pond itself (Walker, 1994). The HDPE structure in the HDPE pond will prevent soil particle input into the water thus reducing turbidity that is not favourable for plankton, particularly blue-green algae (Mc Larney & William, 1987).

Overall, the nitrate concentration for both ponds was in the acceptable concentration ranges for dissolved inorganic substances in aquaculture pond water (Boyd, 1998), ranging from 0.01 mg/ℓ to 0.05 mg/ℓ. Although the concentration of nitrate is within range, it is considerably low as the range of acceptable concentration was 0.2 mg/ℓ to 10 mg/ℓ (Boyd, 1998). This suggests that there was minimal input of nitrate (food pellet) into the aquaculture pond.

In both ponds, nutrient concentrations did not have any significant relationship with cell density of blue-green algae (Table 3). However in the earth pond, chlorophyll *a* was positively correlated with ammonia-nitrogen (r =0.735, P<0.01). It is possible that ammonia-nitrogen is the limiting factor for the phytoplankton (Prommana *et al.* 2006).

According to Boyd (1998), phosphorus is important to aquaculture systems in promoting healthy plankton growth, necessary to provide food for fish. Nutrients levels can be increased by adding inorganic or organic fertilizers in measured doses. However, increased levels of nutrients may be harmful as they can cause blooms of toxic blue-green algae blooms and oxygen depletion (Falconer, 2001). However, the present study showed no significant correlation between SRP and blue-green algae cell density (Table 2). This result may be due to the minimal input of phosphorus resources in the form of food pellets into the pond. In addition, this situation may also be due to the tendency of blue-green algae consuming the usable form of nitrogen such as nitrate and ammonium rather than in phosphorus form (Sze, 1998).

The amount of ammonia present in the pond can be estimated by recording the total ammonia-nitrogen which is one of the nutrient analyses performed in this study (Walker,

Table 3. Pearson Correlation coefficients between the analysed physical, chemical variables, blue-green algae density and chlorophyll a in both ponds * represents the correlation was significant at the 0.05 level and ** represents the correlation was significant at the 0.01 level.

	HDPE Pond		Earth Pond	
	Cell Density	Chl *a*	Cell Density	Chl *a*
Temp	-0.594**	0.114	0.422	-0.014
pH	-0.642**	-0.345	0.467	0.562*
DO	0.550*	-0.422	0.209	0.196
Turbidity	-0.260	0.861**	0.348	0.849**
NO_3^--N	0.349	-0.333	-0.363	0.312
NO_2^--N	0.366	0.131	0.245	0.185
NH_3-N	-0.282	0.584	0.301	0.735**
PO_4^{3-}	0.398	-0.094	-0.248	-0.197
Chl *a*	-0.192	1	0.413	1
Cell Density	1	-0.192	1	0.413

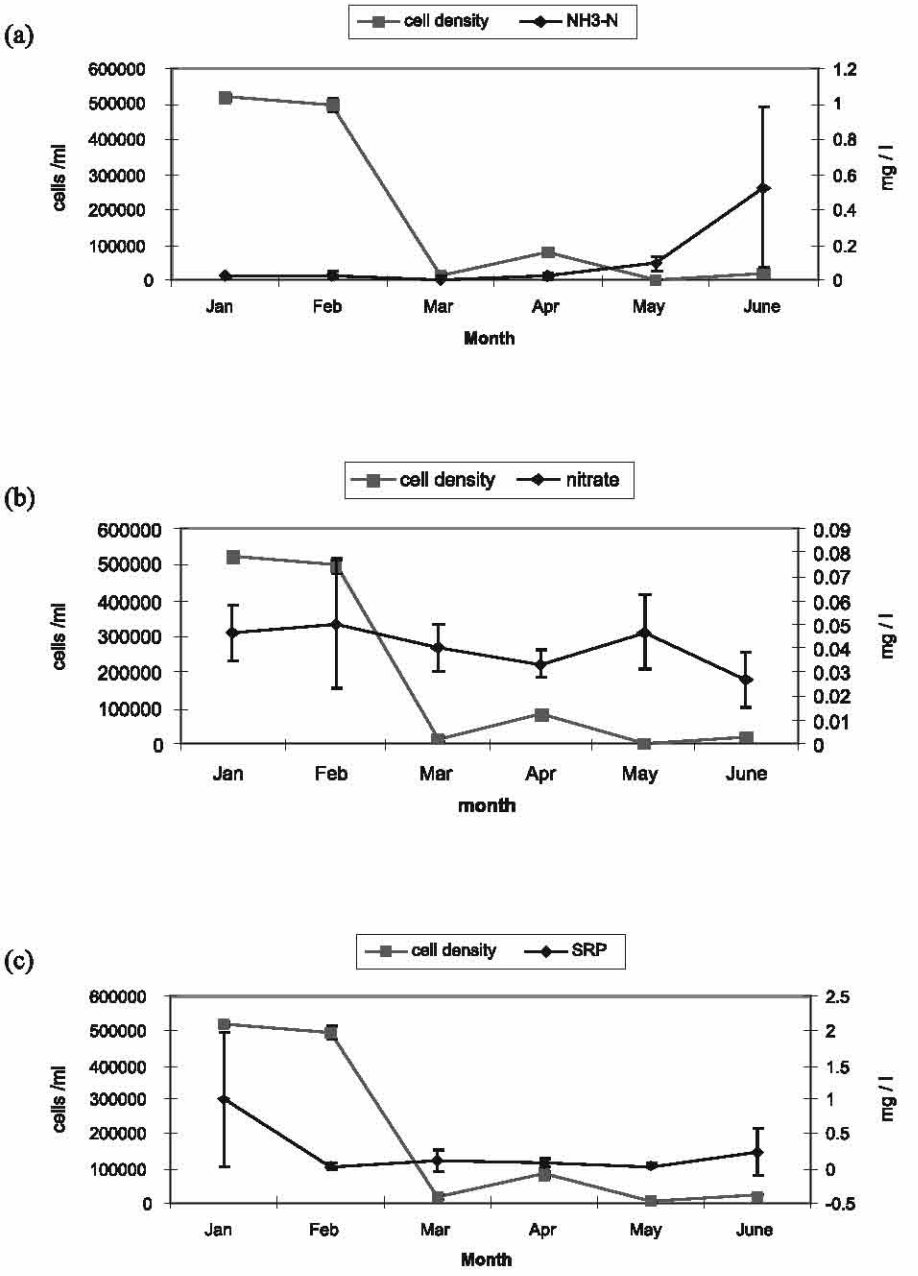

Figure 2. Changes in blue-green algae cell density (cells/ml) in High Density Polyethylene (HDPE) pond *versus* (a) ammonia-nitrogen (mg/ℓ), (b) nitrate (mg/ℓ) and (c) soluble reactive phosphorus (mg/ℓ). Points are means of three replicates with vertical bar showing standard deviation of the mean.

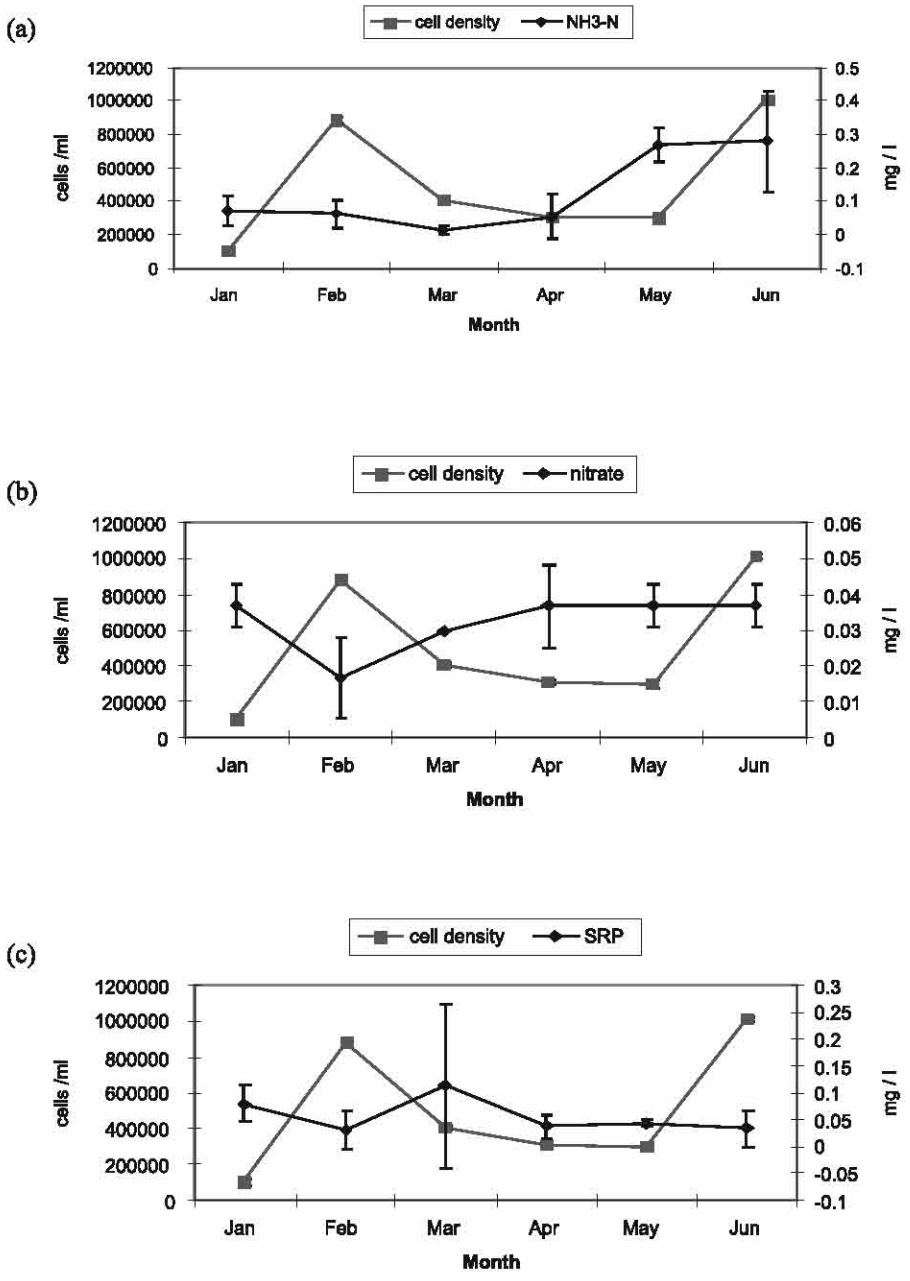

Figure 3. Changes in blue-green algae cell density (cells/ml) in earth pond *versus* (a) ammonia-nitrogen (mg/ℓ), (b) nitrate (mg/ℓ) and (c) soluble reactive phosphorus (mg/ℓ). Points are means of three replicates with vertical bar showing standard deviation of the mean.

1994). The concentration of ammonia-nitrogen in both ponds increased during April to June (Figure 2a & 3a) as a result of the decomposition of algae, plants, uneaten fish food (Ingram *et al.* 1997) and allochthonous materials (Payne, 1986). Ammonia is also produced by fish as an excretory product and the amount of this excretion increases as the fish becomes bigger in size.

CONCLUSION

A total of 35 blue-green algae species belonging to 11 genera and four families were recorded, among them were the potentially toxic species *Microcystis* spp., *Anabaena* spp. and *Oscillatoria* spp. Lower blue-green algae densities with lower number of species were documented in the HDPE pond hence it can be considered as an unsuitable habitat for the development of blue-green algae compared to an earth pond. Chlorophyll *a* concentration showed strong positive correlation with turbidity and ammonia-nitrogen, suggesting soluble reactive phosphate (SRP) and nitrate are not the only factors that influence blue-green algae composition in aquaculture ponds but rather a combination of complex environmental factors such as turbidity, light, other groups of phytoplankton and other nutrients such as ammonia-nitrogen. Future work should include cyanotoxin (blue-green algae toxin) research in order to develop risk assessment and management of potential cyanotoxin contamination in water, and to evaluate its bioaccumulation potential in *T. tambroides.*

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