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Water quality influence the phytoplankton and bacteria abundance: a comparison between shallow freshwater and saltwater ponds

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ABSTRACT

Study for understanding the role of various water quality parameters on phytoplankton and bacteria density in shallow freshwater and saltwater ponds is lacking. Therefore, the present study was conducted to understand the difference in the influence of various water quality parameters on phytoplankton and bacteria density in freshwater and saltwater ponds. A series of water quality parameters, phytoplankton biomass [as indexed by chlorophyll-a (Chl-a)], the density of bacteria and trophic state (as indexed by TRIX index) were determined monthly in two freshwaters and two saltwater ponds located in Kuantan, Pahang, Malaysia. Apart from pond type and temporal effects, multivariate ordinations were performed using two different datasets: water quality, and phytoplankton and bacteria. Water temperature, salinity, and the concentration of total suspended solids (TSS) and ammonia was significantly ($p < 0.05$) higher in June–July than in August. This trend was not observed in the TRIX index and nitrite concentration, which were higher in August than in June–July. The Chl-a concentration differed significantly ($p < 0.05$) between ponds, with higher mean values in ponds with freshwater than in ponds with saltwater. The density of bacteria was consistent ($p > 0.05$) throughout the study period in both freshwater and saltwater ponds. All ponds under study are oligotrophic. In all ponds, the water quality dataset explained the overall variation in phytoplankton and bacteria abundance quite well. Phytoplankton biomass in freshwater ponds is greatly related to phosphate and slightly related to ammonia and water depth. In saltwater ponds, nitrogenous nutrients (nitrate and ammonia) were strong predictors of phytoplankton biomass and stronger than salinity, TSS and pH. Turbidity was the strongest predictor of bacteria density in freshwater and saltwater ponds. It had strong negative influences on bacteria density in freshwater ponds whereas, it had strong negative influences on bacteria density in saltwater ponds. The results of this study support and emphasize the importance of developing appropriate programs for the monitoring and conservation of various freshwater and saltwater ponds.

Keywords: Plankton biomass, Bacteria density, Index, Conservation, Monitoring

1. Introduction

Phytoplanktons uptake inorganic nutrients to produce energy and therefore, it is the first biological community that forms the foundation of the food web in the aquatic ecosystem. Bacteria mineralize organic matter and form the foundation of biogeochemical cycles. Therefore, phytoplankton and bacteria are very important components in all aquatic ecosystems, but they are sensitive to change in some water quality parameters [1]. However, it is important

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to understand their dynamics particularly changes in their density in aquatic ecosystems. Understanding their growth in aquatic ecosystems is difficult as they have complex relationships with many factors. Moreover, the nature of these relationships is not the same in all aquatic environments. For example, relationships among phytoplankton, bacteria and various water quality parameters may not be the same in freshwater and saltwater environments.

Many studies are conducted to understand the relationships between phytoplankton and water quality parameters. Most of these studies are focused only on the relationship between phytoplankton growth, and nitrogenous and phosphorous nutrients in lake ecosystems. For example, the effects of nitrogen and phosphorus on phytoplankton growth in Lake Taihu, China [2]. In another study, the effect of increased nitrogen load on phytoplankton in a phosphorus-limited lake [3]. According to a study, the factors other than N and P are important for the phytoplankton biomass [4]. However, study considering factors other than nitrogenous and phosphorous nutrients on phytoplankton growth is limited. To date, no study simultaneously considered relationships among phytoplankton, bacteria and various water quality parameters in freshwater and saltwater environments at the same geographical location. Such a study is important to improve our understanding of the role of various water quality parameters on phytoplankton and bacteria growths in both freshwater and saltwater environments.

The present study was conducted to understand the difference in the influence of various water quality parameters on phytoplankton and bacteria growth in freshwater and saltwater ponds. We selected ponds instead of lakes or large reservoirs as ponds are rarely considered for ecological study although they are more common than lakes or reservoirs throughout the world particularly in Asia. Moreover, the evolution of lake ecosystems is considerably different than pond ecosystems and therefore, knowledge about lake ecology is not useful to understand the pond ecology. In this study, in addition to the formal hypothesis testing between pond types (saltwater and freshwater ponds) and sampling months we were very interested to observe the overall relationships among all measured variables using a multivariate approach.

Multivariate techniques are often used for the analysis of aquatic ecosystem data [5,6]. Mostly an indirect gradient analysis such as principal component analysis is used, in which only one set of variables is used to calculate overall ordination without detecting explanatory (independent) and response (dependent) variables [7]. In this study, we detected explanatory and response variables and used a direct gradient analysis to explain the variation in one set of variables on a particular component of the ecosystem. In this way, direct relationships among sets of variables related to each of the ecosystem components were estimated. The objectives of this study were (1) to compare the mean difference in water quality parameters, phytoplankton abundance (Chl-a) and bacteria density between pond types and among sampling months, and (2) to assess the influence of various water quality parameters on phytoplankton and bacteria abundance in freshwater and saltwater ponds.

2. Materials and methods

2.1. Study site and experimental design

The study was conducted from June to August 2016 in two freshwaters and two saltwater ponds located in Kuantan, Pahang, Malaysia. All ponds were shallow (mean water depth: 0.9 ± 0.4 m), irregular shaped, with areas ranged from 0.5 to 3.5 h. The distance between two freshwater ponds is approximately 1 km and the distance between two saltwater ponds is approximately 100 m. The distance between freshwater and saltwater ponds is approximately 10 km. All ponds are well exposed to sunlight. None of the ponds are used for aquaculture.

2.2. Collection of water quality, chlorophyll-a, and bacteria data

Water samples for physico-chemical parameters and Chl-a analyses were collected monthly between 8:00 and 10:00 h. Physico-chemical data included temperature, dissolved oxygen (DO), pH, salinity, turbidity, water depth, total suspended solids (TSS), nitrite (NO_2–N), nitrate (NO_3–N), ammonia (total ammonia nitrogen: NO_3 + NO_4) and phosphate (PO_4–P). Temperature, salinity, turbidity, water depth, dissolved oxygen, and pH were determined in situ using portable Hydrolab equipment (Hydrolab Minisonde® Water Quality Multiprobes, Texas, USA). Water samples were collected by taking a 1 L sample from each of the three layers (surface, middle and bottom) with a Van Dorn water sampler. The composite 3 L samples were then used for nitrogenous and phosphorous nutrients, TSS, Chl-a and bacteria determination. Total ammonia nitrogen (Nessler reagent method) and PO_4–P were analyzed spectrophotometrically according to Stirling [8]. Nitrate (cadmium reduction method), was determined following APHA [22]. The total suspended solids were determined according to Stirling [8]. Chl-a was determined spectrophotometrically after acetone extraction according to Boyd [9]. It was determined instead of direct counting of phytoplankton as the relative concentration of Chl-a is indicative of phytoplankton biomass [10,11].

Water bacteria were cultured in both nutrient and marine agar. Aseptic technique was incorporated in all steps of the bacteriological works. To avoid problems regarding the overcrowding of bacterial colonies on the agar plates, a few trials were conducted to find the best dilution method. All colonies were counted and expressed in colony forming unit per ml of a water sample. The trophic state of all ponds was determined using the TRIX index, which was calculated using the equation, $\text{TRIX} = (\text{Log}_{10}((\text{Chl-a}) \times |\%\text{DOd}| \times \text{DIN} \times \text{SRP}) + K])/m)$.

Where, Chl-a indicates chlorophyll-a concentration (µg L^{-1}); |%DOd| indicates the absolute deviation from the DO percent saturation; DIN is the total dissolved inorganic nitrogen (nitrate + nitrite + total ammonia) (µg L^{-1}), and SRP indicates the soluble reactive phosphorus (µg P L^{-1}). The constants K = 1.5 and m = 12/10 = 1.2 are scale coefficients introduced to fix the lowest index value and define the extension of the related trophic scale, from 0 to 10 TRIX units.

2.3. Data analysis

All data were checked for normality using the Kolmogorov–Smirnov test and homogeneity of variance by Levene's test before analysis. Data were analyzed using a repeated-measures one-way analysis of variance to compare the mean difference in water quality parameters, Chl-a concentration, and bacteria density. Pond type was considered as the main factor and sampling time was considered as a sub-factor. If a factor was significant, differences between the means were analyzed using the Tukey test for unplanned multiple comparisons of means (at $P \leq 0.05$ level of significance). All the above statistical tests were performed using IBM SPSS statistics 20.

Two different datasets were used for multivariate ordinations. They were (1) water quality included temperature, DO, pH, salinity, turbidity, water depth, TSS, NO_2–N, NO_3–N, ammonia (total ammonia nitrogen: $NO_3 + NO_4$) and phosphate (PO_4–P); (2) phytoplankton and bacteria: Chl-a concentration and bacteria density. We calculated the canonical correlation index between two datasets to understand the highest direct explanatory power. The highest canonical correlation index indicates the highest direct explanatory power. We did not only calculate the explanatory power of physico-chemical parameters for Chl-a concentration and bacteria density but also the explanatory effect of Chl-a concentration and bacteria density on physico-chemical parameters. Based on the highest canonical correlation index, water quality datasets were used as explanatory variables (independent variables), and phytoplankton and bacteria data were used as response variables (dependent variables) in multivariate ordinations. Multivariate ordinations were performed with the computer program CANOCO 4 [12]. First, a detrended correspondence analysis was performed to understand the prevailing patterns of the response variables with the explanatory variable gradient. Ordination axes smaller than two standard deviations indicated monotonic responses. This suggested the redundancy analysis (RDA) as the best method for direct gradient analysis. RDA was run with variables centered and standardized by subtracting the mean and dividing by the standard deviation. The significance of the first ordination axis and the significance of the first four canonical axes together were evaluated with Monte Carlo-permutation tests with 1,000 permutations. RDA was used to directly explain the variation in the response variables from the variation in the explanatory variables.

3. Results

3.1. Effects of pond type, sampling time and their interaction on measured variables

The effects of pond type, sampling time and their interaction on water quality parameters, TRIX index, and concentrations of Chl-a and density of bacteria are presented in Table 1 and Fig. 1. All water quality parameters and TRIX index changed significantly ($p < 0.01$) overtime except DO, water depth, nitrite and phosphate. Water temperature, salinity, and the concentration of TSS and ammonia were significantly higher in June–July than in August. This trend was not observed in TRIX index and nitrite concentration, which were higher in August than in June–July. All water quality parameters in freshwater ponds were significantly different ($p > 0.05$) than in saltwater ponds except water depth, water turbidity, TRIX index, and concentrations of DO and phosphate. Water temperature, salinity, and concentrations

Table 1
Effects of pond type (PT), sampling month (Time) and their interaction (PT × Time) on various water quality parameters, and concentration of Chl-a and density of bacteria based on one-way repeated-measures ANOVA

	Significance (*P*-value)			Tukey test				
				Pond type		Time		
	PT	Time	PT × Time	Saltwater	Freshwater	June	July	August
Temperature (°C)	*	**	*	31.63 ± 0.20	30.68 ± 0.34	31.71^a ± 0.11	31.85^a ± 0.36	29.44^b ± 0.45
DO (mg/L)	ns	ns	ns	4.15 ± 0.85	3.37 ± 0.26	3.86 ± 0.31	3.56 ± 0.22	3.48 ± 0.93
pH range	–	–	–	7.12–8.40	7.34–9.24	7.54–9.24	7.89–8.45	7.12–8.11
Water depth (m)	ns	ns	ns	0.78 ± 0.07	0.99 ± 0.04	0.80 ± 0.11	0.83 ± 0.13	1.13 ± 0.27
Salinity (ppt)	**	**	ns	31.02 ± 2.65	0.13 ± 0.21	12.48^b ± 4.27	13.38^a ± 4.48	5.42^c ± 1.99
Turbidity (NTU)	*	ns	ns	7.86 ± 2.31	28.24 ± 3.69	16.01 ± 2.51	16.43 ± 1.86	31.89 ± 7.63
Nitrate (mg/L)	*	*	*	0.706 ± 0.106	0.467 ± 0.049	0.394^b ± 0.079	0.739^a ± 0107	0.506^{ab} ± 0.047
Nitrite (mg/L)	ns	*	ns	0.006 ± 0.001	0.004 ± 0.001	0.002^b ± 0.001	0.003^b ± 0.001	0.008^a ± 0.001
Ammonia (mg/L)	**	**	**	2.824 ± 0.363	0.135 ± 0.011	1.340^a ± 0.393	1.420^a ± 0.461	0.334^b ± 0.079
Phosphate (mg/L)	ns	ns	ns	0.475 ± 0.096	0.423 ± 0.069	0.343 ± 0.120	0.427 ± 0.060	0.553 ± 0.097
TSS (g/L)	*	**	*	0.085 ± 0.013	0.064 ± 0.007	0.090^a ± 0.011	0.076^a ± 0.014	0.048^b ± 0.004
TRIX index	ns	**	ns	2.75 ± 0.0.17	2.87 ± 0.08	2.64^b ± 0.11	2.57^b ± 0.11	3.28^a ± 0.10
Chl.-α (mg/L)	**	ns	ns	11.19 ± 1.483	50.11 ± 2.795	40.75 ± 5.604	39.22 ± 4.444	31.44 ± 6.302
Bacteria (CFU/ml)	ns	ns	ns	7,483 ± 815	9,667 ± 854	9,656 ± 1,178	9,239 ± 1,319	7,922 ± 796

Data are means ± Standard error. Mean values in the same row with no superscript in common differ significantly ($P < 0.05$). DO = dissolved oxygen; TSS = total suspended solid; Chl.-α = Chlorophyll-α. *$p \leq 0.05$; **$p < 0.01$; NS: not significant.

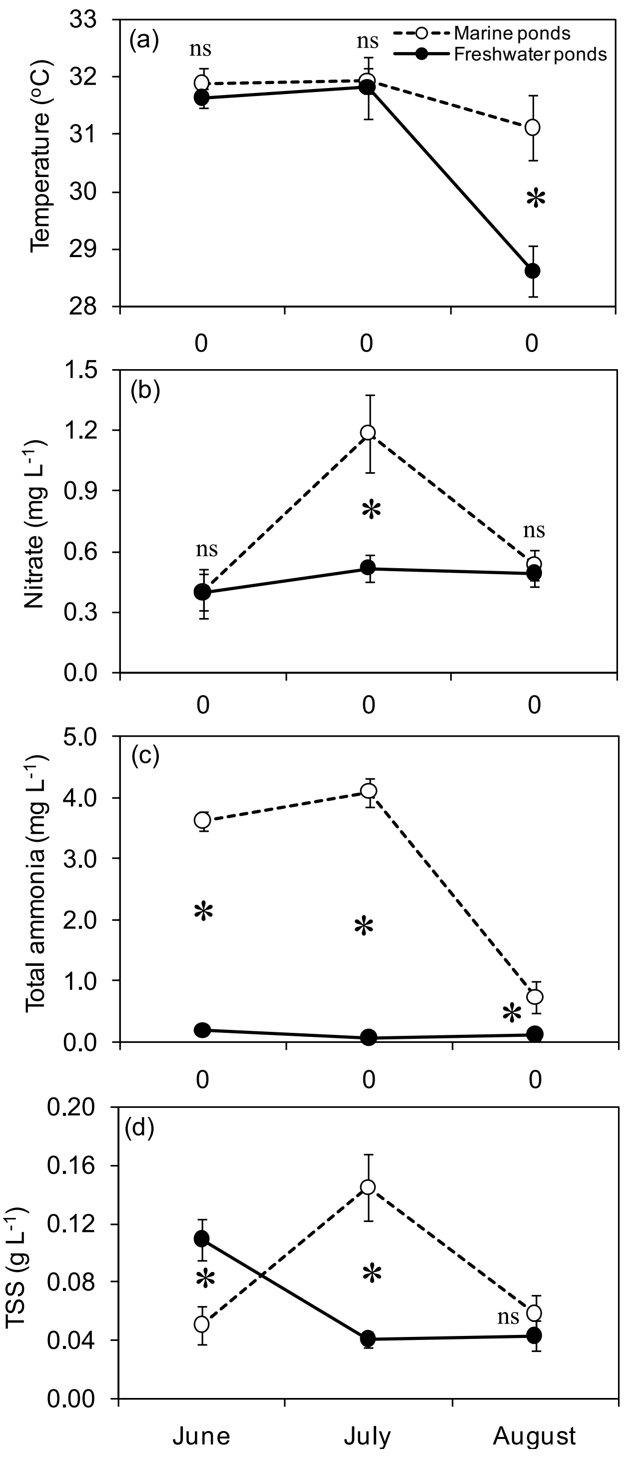

Fig. 1. Interaction effect of pond type and sampling months on water (a) temperature, (b) nitrate (NO_3–N), (c) total ammonia ($NH_4 + NH_3$) and (d) TSS (total suspended solids).
* and ns indicate significantly different and not significantly different between freshwater and marine ponds, respectively.

of nitrate, ammonia and TSS were higher in ponds with saltwater than in ponds with freshwater, whereas an opposite result was observed in case of the water turbidity. These results are different in different sampling months (except salinity and water turbidity), which can be explained by the interaction effects of sampling time and pond type (Table 1 and Fig. 1). The temperature was higher in saltwater ponds than in freshwater ponds only in August whereas, nitrate was higher in saltwater ponds than in freshwater ponds only in July. TSS concentration was higher in freshwater pond than in saltwater ponds in June whereas, an opposite result was observed in July. It was statistically the same in freshwater and saltwater ponds.

The Chl-a concentration differed significantly between ponds, with higher mean values in ponds with freshwater than in ponds with saltwater. The effects of time and its interaction with pond type on Chl-a concentration were not significant. The concentration of bacteria was consistent throughout the study period in both freshwater and saltwater ponds (Table 1).

3.2. Phytoplankton and bacteria density explained by water quality

The first canonical axis and the first four canonical axes combined were statistically significant ($p < 0.05$) for the redundancy analysis (RDA) using water quality parameters as explanatory variables and concentration of Chl-a and bacteria as response variables. The first two canonical axes explained 61.48% of the variance in phytoplankton and bacteria and 87.34% of the phytoplankton and bacteria–water quality parameters relationship in freshwater ponds and 72.42% and 93.08% of these relationships in saltwater ponds (Table 2). With the options used for RDA, a small angle between two variables is indicative of a high positive correlation between the variables, an angle of 90° indicates independence of variables, and an angle larger than 90° indicates a negative correlation.

In freshwater ponds, the density of bacteria scored high on the first RDA axis (Fig. 2), which may, therefore, be interpreted as a bacterial density axis. This axis is positively correlated with all water quality parameters except phosphate, nitrate, nitrite and water temperature. Turbidity, TSS, water depth, ammonia, DO and pH were positively correlated with the density of bacteria in water. The strongest correlation was observed between bacterial density and turbidity among all correlations between bacterial density and various water quality parameters. The concentration of nitrate is negatively correlated with the density of bacteria in the water. The concentration of Chl-a scored high on the second RDA axis (Fig. 2), which may, therefore, be interpreted as a phytoplankton density axis. This axis is positively correlated with concentrations of phosphate and ammonia, and water depth and negatively correlated with water temperature and the concentration of nitrite in the water. All other water quality variables have almost no effect on phytoplankton density in freshwater ponds. The strongest correlation was observed between phytoplankton density and phosphate concentration in the water among all correlations between phytoplankton density and various water quality parameters.

In saltwater ponds, the concentration of Chl-a scored high on the first RDA axis (Fig. 2), which may, therefore, be interpreted as a phytoplankton density axis. This axis is positively correlated with nitrate, ammonia, TSS, salinity and pH

Table 2
RDA of water quality explaining the abundance of phytoplankton (chl-a) and bacteria

Statistics	Axis1	Axis2	Axis3	Axis4
Freshwater ponds				
Eigenvalues	0.489	0.270	0.126	0.1167
Water quality-abundance of phytoplankton and bacteria correlation	0.811	0.3499	0.211	0.153
Cumulative % variance of an abundance of phytoplankton and bacteria	48.92	61.48	68.43	73.32
Cumulative % variance of water quality-abundance of phytoplankton and bacteria relation	79.58	87.34	92.34	96.41
Marine ponds				
Eigenvalues	0.662	0.269	0.052	0.017
Water quality-abundance of phytoplankton and bacteria correlation	0.881	0.414	0.171	0.115
Cumulative % variance of an abundance of phytoplankton and bacteria	57.33	72.42	76.57	78.38
Cumulative % variance of water quality-abundance of phytoplankton and bacteria relation	76.20	93.08	96.22	98.160

Total variance = 1.000; RDA was statistically significant at $P \leq 0.05$.

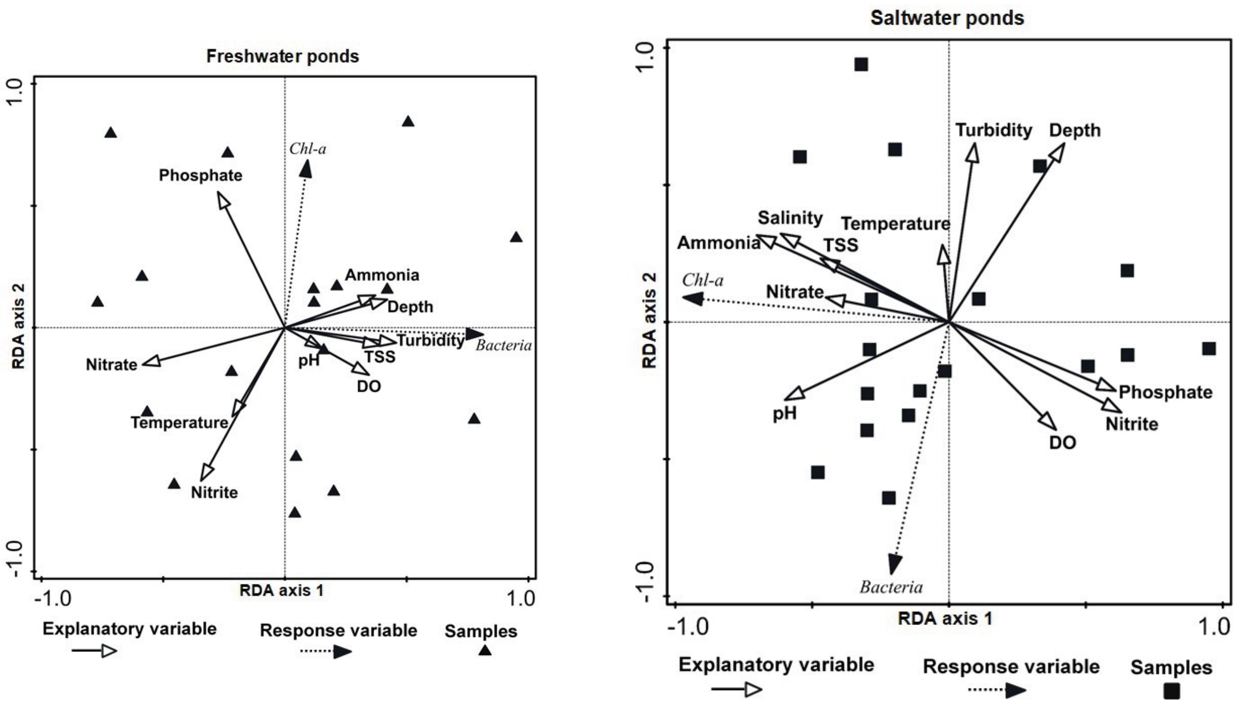

Fig. 2. RDA biplot (first two axes) of chlorophyll-a (phytoplankton) concentration and density of bacteria explained by lake water quality (Ammonia = total ammonia ($NH_4 + NH_3$), DO = dissolved oxygen and TSS = total suspended solids).

and negatively correlated with phosphate and nitrite concentration in water. All other variables have almost no influence on phytoplankton density in the water. The strongest correlation was observed between phytoplankton density and nitrate concentration in the water among all correlations between phytoplankton density and various water quality parameters. The density of bacteria scored high with the second RDA axis, which may, therefore, be interpreted as a bacterial density axis. This axis is positively correlated with pH and DO and negatively correlated with water turbidity, water depth and water temperature. All other measured variables have almost no effect on bacterial density in the water of saltwater ponds.

4. Discussion

This is the first study that simultaneously considered phytoplankton (as indexed by Chl-a), bacteria and various water quality parameters in freshwater and saltwater environments at the same geographical location. We observed that saltwater ponds had more inorganic nitrogen and less phytoplankton biomass compared to freshwater ponds although all ponds were oligotrophic as TRIX indexes were less than 4 in all months [13]. The observed TRIX indexes indicated that all ponds under study were low productive for aquatic organisms. The observed results on the tropic state cannot be compared to any other studies on these ponds as no such studies have been found.

Table 3
TRIX index and their related trophic state (Pettine et al. [13])

TRIX index	State water quality	Level of eutrophication	Trophic state
0< to ≤4	High-quality	Low	Oligotrophic
4< to ≤5	Good	Medium	Mesotrophic
5< to ≤6	Moderate	High	Eutrophic
6< to ≤10	Poor and degraded	Elevated	Hypertrophic

Multivariate analysis RDA shows the overall patterns of the influence of various water quality parameters on phytoplankton and bacteria abundance in freshwater and saltwater ponds. In both freshwater and saltwater ponds, the water quality dataset explained the overall variation in phytoplankton and bacteria abundance quite well (first two canonical axes explained 87.34% and 93.08% variances of the phytoplankton and bacteria abundance-water quality relation in freshwater ponds and saltwater ponds, respectively). Nutrients along with a few other factors enhance the phytoplankton biomass in all ponds.

In the case of phytoplankton biomass in freshwater ponds, the effect of phosphate on the ordination is stronger than the effect of ammonia and water depth. Therefore, phosphate was a strong predictor of phytoplankton biomass and stronger than ammonia and water depth in freshwater ponds. Our result indicated that phytoplankton abundance was more limed by phosphate than by ammonia in freshwater ponds during the study period. This result is in agreement with the findings in many other freshwater ecosystem studies, in which strong relationships were observed between total phosphorus and phytoplankton biomass [14]. According to Dzialowski et al. [10], phosphorus has been considered to be the primary nutrient limiting phytoplankton growth in freshwater ecosystems. Besides phosphate, the water temperature was a principal factor in the case of phytoplankton biomass in freshwater ponds. The water temperature had a negative relation with phytoplankton biomass, indicating high water temperature limited phytoplankton growth in freshwater ponds. However, the observed water temperature might be higher than the optimum temperature for the growth of freshwater phytoplankton species, which grew during the study period. According to a study, most of the freshwater phytoplankton species grow rapidly when the water temperature is between 18°C and 20°C. In the present study, water depth was a slightly weak predictor of phytoplankton biomass and weaker than ammonia [15,16]. However, both ammonia and water depth may be considered as co-limiting factors for phytoplankton production in freshwater ponds.

In the present study, the relationship between nutrients and phytoplankton was different in saltwater ponds compared to freshwater ponds. In saltwater ponds, nitrogenous nutrients (nitrate and ammonia) were strong predictors of phytoplankton biomass and stronger than salinity, TSS and pH. The observed positive relationship between phytoplankton and nitrogenous nutrients (total ammonia and nitrate) is consistent with some recent research in saltwater habitats [17–19].

In the case of bacteria density in freshwater ponds, the effect of water turbidity and TSS on the ordination is stronger than the effect of DO, ammonia and water depth. Turbidity is closely related to TSS, which includes organic and inorganic particles in the waterbody. However, turbidity was the strongest predictor of bacteria density. It had strong positive influences on bacteria density in freshwater ponds. This result concurs with the well-accepted view (TSS provides a medium for microbial growth) and many research findings (TSS has a strong positive relationship with the density of bacteria) [20]. Water turbidity was a strong predictor of bacteria density and stronger than water temperature, water depth, DO and pH in saltwater ponds. Later two are equally important for the bacteria density and both are positively influenced the density of bacteria in saltwater ponds. In the present study, turbidity had strong negative influences on bacteria density in saltwater ponds. In many ecosystems where turbidity is mostly caused by clay particles, the abundance of bacteria is negatively related to turbidity [21–30]. However, the relationship between turbidity and bacteria density depends on bacteria species. Unfortunately, detailed bacteria and phytoplankton species data were not collected from the ponds. To further evaluate species-specific relationships with various water quality parameters, research should focus on the responses of individual taxa to specific water quality parameters in both freshwater and saltwater ponds. In conclusion, the results of this study support and emphasize the importance of developing appropriate programs for the monitoring and conservation of various freshwater and saltwater ponds [31–51].

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