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Low water quality in tropical fishponds in southeastern Brazil

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ABSTRACT

Expansion of aquaculture around the world has heavily impacted the environment. Because fertilizers are needed to raise fish, one of the main impacts is eutrophication, which lowers water quality and increases the frequency of algal blooms, mostly cyanobacteria. To evaluate whether the water quality in 30 fishponds in southeastern Brazilian met the requirements of Brazilian legislation, we analyzed biotic and abiotic water conditions. We expected that the high nutrient levels due to fertilization would cause low water quality. We also analyzed cyanotoxins in seston and fish muscle in some systems where cyanobacteria were dominant. The fishponds ranged from eutrophic and hypereutrophic with high phytoplankton biomass. Although cyanobacteria were dominant in most of the systems, cyanotoxins occurred in low concentrations, possibly because only two of the 12 dominant species were potential producers of microcystins. The high phosphorus concentrations caused the low water quality by increasing cyanobacteria, chlorophyll-*a*, turbidity, and thermotolerant coliforms, and by depleting dissolved oxygen. We found that all the 30 systems were inappropriate for fish culture, according to Brazilian legislation, based on at least one of the parameters measured. Furthermore, there was not any single system in the water-quality thresholds, according to the Brazilian legislation, to grow fish. Our findings indicate the need for better management to minimize the impacts of eutrophication in fishponds, in addition to a rigorous control to guarantee good food.

Key words: cyanobacteria, cyanotoxins, eutrophication, fish culture.

INTRODUCTION

World aquaculture production has increased 39-fold from 1957 to 2008 and contributes significantly to global fish production for human consumption, now surpassing the supply of wild-caught fish (Samuel-Fitwir et al. 2012). At the same time, impacts on environmental conditions have also increased (Cao et al. 2007). Classical impacts include pathogens, introduction of genetically modified organisms, additives and drugs, antimicrobial resistance, spread of diseases, escapes, overexploitation of wild species, and nutrient enrichment (Pelletier et al. 2007). Recently, aquaculture ponds have also been identified as being a CO_2 sinks (Boyd et al. 2010) as well as an N_2O source to the atmosphere (Hu et al. 2012).

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Inorganic (nitrogen and phosphorus) fertilizers applied to fishponds are needed to grow fish by stimulating plankton growth and increasing production of high-protein fish biomass (Boyd and Queiroz 1997, Neori 2011). Organic fertilizers or manures from animal wastes or agricultural by-products are also used, which are either directly consumed by the fish (or by invertebrate fish-food organisms) or decompose slowly to release inorganic nutrients (Boyd and Queiroz 1997). However, only a portion of the nutrients from fertilizers is incorporated into the final product (Hargreaves and Tucker 2003). The remaining part is mineralized in the sediment, and then released into the water column or carried by the effluents to the watershed (Boyd and Queiroz 2001, Yokoyama 2003, Zhang et al. 2006). The movement of fish (bioturbation) also resuspends sediment, enhancing mineralization (Phan-Van et al. 2008).

The consequence of nutrient enrichment is an increase in eutrophication, one of the main impacts from aquaculture. This leads to, for example, the reduction of oxygen, outgassing of hydrogen sulfide, and phytoplankton blooms (Boyd 2006). Cyanobacteria is the main algal group forming blooms in enriched waters (Paerl and Huissman 2009), including species that are potentially toxic to humans and animals (Carmichael 1997, Paerl et al. 2011). Cyanobacteria is able to dominate in high biomass in conditions of high total phosphorus concentrations (Trimbee and Prepas 1987, Moss et al. 2011), low TN:TP ratios (Smith 1983), high temperature (Paerl and Huisman 2008, Kosten et al. 2012), low light (Smith 1986, Scheffer et al. 1997), and high pH/low CO_2 (Caraco and Miller 1998).

In spite of the importance of phytoplankton for the growth of fish in freshwater, few studies in Brazil have examined blooms and dominant algal groups in these systems. In these few studies, cyanobacteria have been reported as the most abundant algae (Sant'Anna et al. 2006, Minillo and Montagnolli 2006). They are potentially producers of toxins (e.g., hepatotoxins, neurotoxins) and compounds with an unpleasant taste and odor (e.g., geosmin) (Dzialowski et al. 2009, Paerl et al. 2011). Toxins can accumulate in fish muscle or viscera (Magalhães et al. 2001, Soares et al. 2004, Ibelings and Chorus 2007, Romo et al. 2012). In the state of São Paulo, Eler and Espíndola (2006) found microcystins in 46% of the 30 fishponds analyzed by them, of which two were at very high levels. However, as far as we know, there is no information about bioaccumulation in the muscle tissue of fish from commercial fishponds in Brazil.

To evaluate the water quality in 30 fishponds in southeastern Brazil, we analyzed biotic and abiotic water conditions and compared them to levels mandated by Brazilian legislation. We expected that the high nutrient levels resulting from fertilization would indicate low water quality. We also analyzed cyanotoxins in fish muscle and the seston fraction in some systems where cyanobacteria occurred in high abundance. We found low water quality in most of the fishponds.

MATERIALS AND METHODS

Study Sites

The 30 systems studied are located in southeastern Brazil, in the densely populated (366 inhabitants km^{-2}) state of Rio de Janeiro (Figure 1). The regional climate is tropical (Aw, Köppen classification) with a historical total annual precipitation of 1172 mm, and annual mean minimum temperature of 20.9°C and maximum of 27.2°C; with dry winters and wet summers (SIMERJ 2011). In most of the 30 fishponds, rotifers were dominant in richness and abundance, while cyclopoid copepods were in biomass (Loureiro et al. 2011).

Sample and Data Collections

The following variables were obtained from direct, structured and semi-structured interviews with the owners and employees during field work: type of

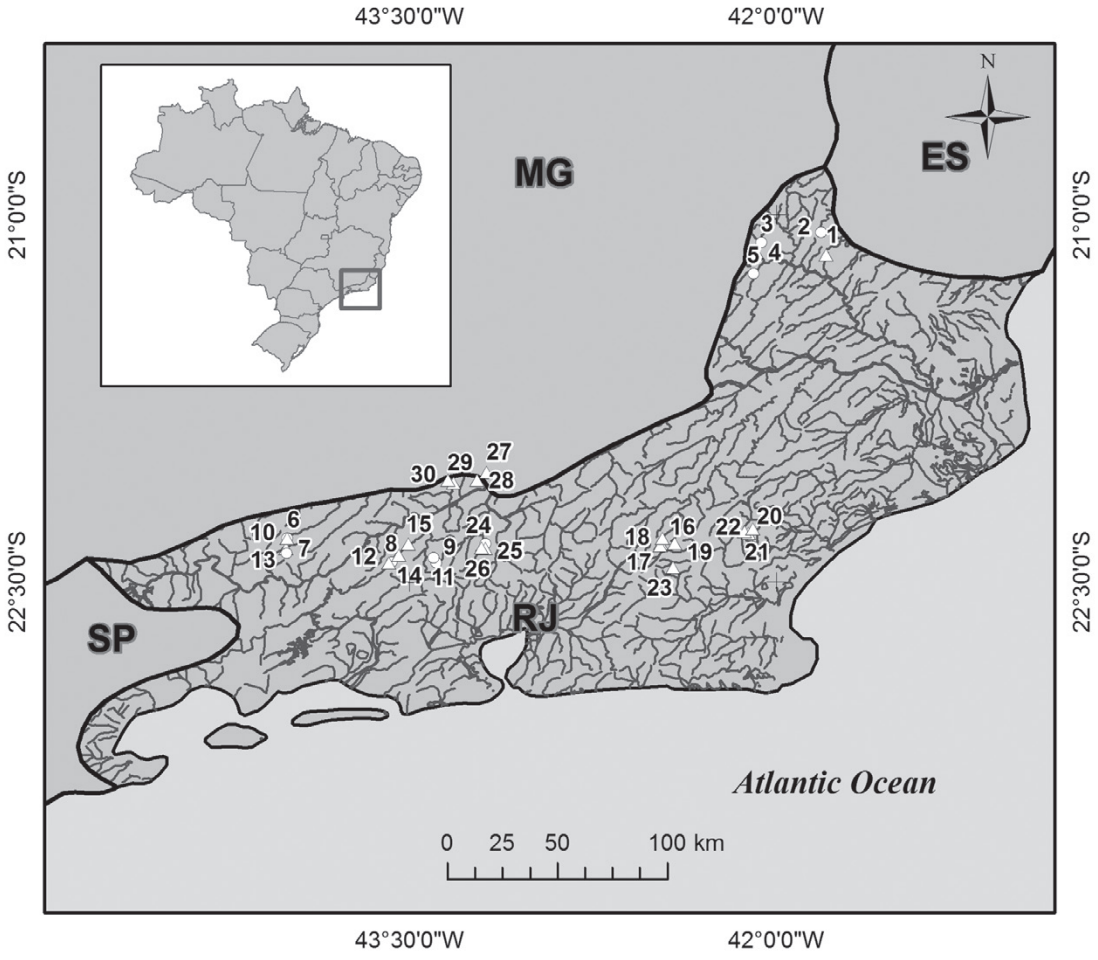

Figure 1 - Map of the state of Rio de Janeiro, showing the sampled fishponds. Circles = fee-fishing systems; triangles = fish-farming systems. MG = Minas Gerais, ES = Espírito Santo, RJ = Rio de Janeiro, SP = São Paulo.

activity (fee-fishing, fish-farming), water source (spring, stream), bottom (earthen, concrete), rearing system (multiple, monoculture), fertilizers (organic, inorganic), and fish stocking rates.

Water samples for nutrients, chlorophyll-*a*, and phytoplankton were taken once, using a van Dorn bottle at the subsurface (0.3 m) between November 2005 and January 2006, in the middle of each of the 30 fishponds. Thermotolerant coliforms were sampled directly from the surface water were sterile flasks. Water temperature and dissolved oxygen (YSI model 52), pH (Digimed), conductivity (Digimed), turbidity (Alfakit model AT), and water transparency (Secchi depth extinction) were measured *in situ*. Discharge inflow was measured by the volumetric method, which is based on the time taken for a given water flow to occupy a container of known volume. System area and volume were calculated from local measurements. Residence time was estimated as discharge inflow divided by the fishpond volume. Water samples for nutrients were divided for analysis of total (phosphorus, TP; nitrogen, TN) and dissolved nutrients (soluble reactive phosphorus, SRP; ammonium, $N.NH_4^+$; nitrate, $N.NO_3^-$; nitrite, $N.NO_2^-$). A fraction of the water sample for total nutrients was directly frozen at -18°C, and for

dissolved nutrients the water was filtered through Whatman GF/C filters and then frozen at -18°C until further processing. Phytoplankton samples were preserved with Lugol's Iodine solution.

Five of the 30 systems where cyanobacteria concentrations were above 20,000 cells mL^{-1} (ponds 12, 18, 20, 24, and 25) were selected for microcystin analysis (seston and fish muscle). Samples were taken in 2005 and repeated in 2008. To obtain the seston, 2 L of water were filtered on Whatman GF/C filters and then frozen at -18°C until microcystin analysis. Fish (Nile tilapia, *Oreochromis niloticus*) were collected in each system for further analysis of microcystins in muscle. Inflow volume was measured in systems where there was inflow.

Sample Analysis

Kjeldahl nitrogen, $N.NO_2^-$, $N.NO_3^-$, $N.NH_4^+$, TP and SRP were analyzed according to Mackereth et al. (1978) and Wetzel and Likens (1990). Phytoplankton population densities (cells mL^{-1}) were estimated using the settling technique (Utermöhl 1958) in an inverted microscope (Zeiss Oberkochen, Axiovert 10) under 400x magnification. Chlorophyll-*a* concentrations were estimated by the colorimetric method after extraction in 90% acetone (APHA 2005). Thermotolerant coliforms (MPN 100 mL^{-1}) were analyzed according to Standard Methods (APHA 2005).

For microcystin analysis in the seston, the filter was extracted three times with MeOH:TFA 0.1% for 1h, and the supernatant was combined and evaporated (dry extracts). The fish muscle for microcystin analysis was weighed and subsequently extracted three times with 100% MeOH for 1h; the extract was centrifuged at 3000 rpm for 15 min and the supernatant was evaporated, resuspended in 20 mL of Milli-Q water and passed through an activated HP-20 column, eluted with 10%, 20% and 30% methanol and MeOH: TFA 0.1%. The fraction MeOH:TFA 0.1% was collected and the extract was evaporated (dry extracts). The dry extracts from seston and muscle samples were resuspended in 2 mL of Milli-Q water, and then filtered on a cellulose acetate filter with 0.45 μm mesh. These solutions were analyzed by ELISA (Enzyme-Linked Immunosorbent Assay) using a microplate kit for MCYSTs (Beacon Analytical Systems Inc.) following the manufacturer´s protocol, with two replicates per sample.

Data Analysis

Theoretical residence time was estimated from the fishpond volume divided by inflow volume. TN was calculated from the sum of Kjeldahl nitrogen and $N.NO_3^-$. Dissolved inorganic nitrogen (DIN) was considered as the sum of $N.NO_2^-$, $N.NO_3^-$ and $N.NH_4^+$. TN:TP ratios were estimated on a molar basis.

Although fishponds are expected to be nutrient-enriched, the proportion of nutrients can become limiting to phytoplankton growth. To evaluate the differences in potential N-limitation to phytoplankton growth in the systems, we used the following indicators (Kosten et al. 2009): (i) TN:TP ratios in the pond water; ponds below 20 (molar based) were considered N-limited and above 38, P-limited (Sakamoto 1966); and (ii) DIN and SRP were compared to concentrations that have generally been considered to limit phytoplankton growth. P was considered limiting below ~10 μg P/L (Sas 1989) and N below ~100 μg N/ L (Reynolds 1997). Clearly this is only an approximation, as it depends on the affinities and storage capacities of the individual species (Reynolds 1999).

The trophic state of the fishponds was assessed by TP and chlorophyll-*a* concentrations according to Nürnberg (1996). To evaluate if the fertilizers used in the fishponds lowered water quality, we used as a criterion the Brazilian legislation, based on some selected variables (dissolved oxygen, turbidity, TP concentrations, chlorophyll-*a*, cyanobacteria abundance and thermotolerant coliforms). Class II water bodies may be used for aquaculture and fishing activities (CONAMA 357/2005).

The statistical differences in the variables among categorical groups were tested using a non-parametric Kruskal-Wallis test. To explore the relationships between phytoplankton abundance vs. environmental variables, stepwise multiple linear regression with forward selection and Spearman correlations (r_s) were used. All independent variables (except for pH) and phytoplankton abundance were log x transformed to attain normality. All statistical analyses were performed in Statview 5.0.

RESULTS

Main Features of the Fishponds

Of the 30 systems, 21 were fish farms dedicated only to fattening fish (15) or to both, breeding and fattening fish (6); nine were fee-fishing ponds. The areas of the aquaculture systems ranged from 350 to 6,000 m^2 and the maximum depths ranged from 0.8 to 2.0 m (Table I). Most fishponds used springs as the water source; 12 systems were closed with no inflow, and the others were open and high-flushing (Table II) with a median residence time of 1.9 days (0.1 to 19.2 days). Only two systems (fee-fishing) were made of concrete and the others were unlined earthen ponds. The most frequent fish species were the exotic tilapia (*Tilapia rendalii*) and Nile tilapia (*Oreochromis niloticus*), growing in monoculture or with other fish species (Table II). The stocking rates ranged from 1 to 4 fish m^{-2} in both the fee-fishing and fish-farming systems (Table II). Of the 30 ponds, 84% used organic, inorganic, or both types of fertilizers (Table II). Five fishponds, mostly fee-fishing systems, were not enriched by any type of fertilizer.

TABLE I
Range, median and mean values, and standard deviation (SD) of the limnological variables in 30 fishponds.

	Range	Median	Mean	SD
Area (m^2)	350-6000	2450	2962	2450
Maximum depth (m)	0.8-2.0	1.5	1.4	0.3
Water temperature (°C)	23.2-32.7	26.5	27.1	2.7
Dissolved oxygen (mg L^{-1})	1.2-12.8	4.8	5.7	2.6
Conductivity (µS cm^{-1})	24.0-610.0	56	86.8	104.6
pH	5.1-9.3	7.0	7.2	0.97
Secchi depth (m)	0.08-0.52	0.19	0.21	0.11
Turbidity (NTU)	9.9-262.9	51.0	65.2	52.1
$N\text{-}NH_4^+$ (µg L^{-1})	3.9-680.1	28.1	75.8	131.9
$N\text{-}NO_3^-$ (µg L^{-1})	2.0-1502.3	23.2	155.5	318.5
$N\text{-}NO_2^-$ (µg L^{-1})	0.5-19.4	3.0	5.1	4.9
Dissolved inorganic nitrogen (µg L^{-1})	14.4-1528.8	79.1	236.5	389
Soluble reactive phosphorus (µg L^{-1})	4.6-45.5	12.2	16.5	10.9
Total nitrogen (µg L^{-1})	112.0-4732.0	560	836.2	900.8
Total phosphorus (µg L^{-1})	33.4-669.5	173.2	213.3	171.4
Total nitrogen/total phosphorus (by atom)	0.7-171.3	9.4	18.9	32.34
Chlorophyll-*a* (µg L^{-1})	8.7-344.0	82.0	104.4	84.8
Cyanobacterial abundance (10^3 cells mL^{-1})	2.9-4758.0	480.7	637.0	1180.9
Thermotolerant coliforms (NMP 100 mL^{-1})	2-160000	1350.0	25705	50791

Water Conditions

There was limited variation in temperatures, but dissolved oxygen concentrations and conductivity varied over wide ranges (Table I). Dissolved oxygen levels were below 5 mg L^{-1} in 47% of the fishponds. The pH was neutral on average (median=7.0) but varied from slightly acidic to alkaline (Table I). Secchi depth was low and turbidity was higher than 100 NTU in 20% of the systems (Table I).

Total and dissolved nitrogen and phosphorus concentrations varied widely. Median values of TP concentrations were high (173 µg L^{-1}), but TN concentrations were not as high as expected (560 µg L^{-1}) (Table I). DIN and SRP concentrations were, on average, intermediate (median=79 and 12 µg L^{-1}, respectively). We observed a weak but significant relationship between total phosphorus and chlorophyll-*a* (r^2_{adj}=0.16, p=0.0157).

A trend for N limitation of phytoplankton growth was observed in most of the fishponds, if considered the median values of total N:P ratios (TN:TP = 9.4). This is consistent if the algal requirements, based on the half-saturation constants for most algal species, are taken into account (see Methods section); by this criterion, 60% of the systems were N-limited.

Therefore, on average, the fishponds were warm, with circumneutral water, low dissolved oxygen, and high turbidity. Total phosphorus concentrations were remarkably high, however, total nitrogen concentrations or dissolved inorganic nitrogen and phosphorus are not. Therefore, a trend of N limitation of phytoplankton growth was found.

Phytoplankton

Total phytoplankton abundance varied between 4.2 10^3 and 7.3 10^6 cells mL^{-1} in the fishponds. The most important group of the phytoplankton community in terms of abundance was cyanobacteria, which contributed, on average, 66% of the total phytoplankton abundance. Green algae were the second most abundant group, with 24% (Figure 2).

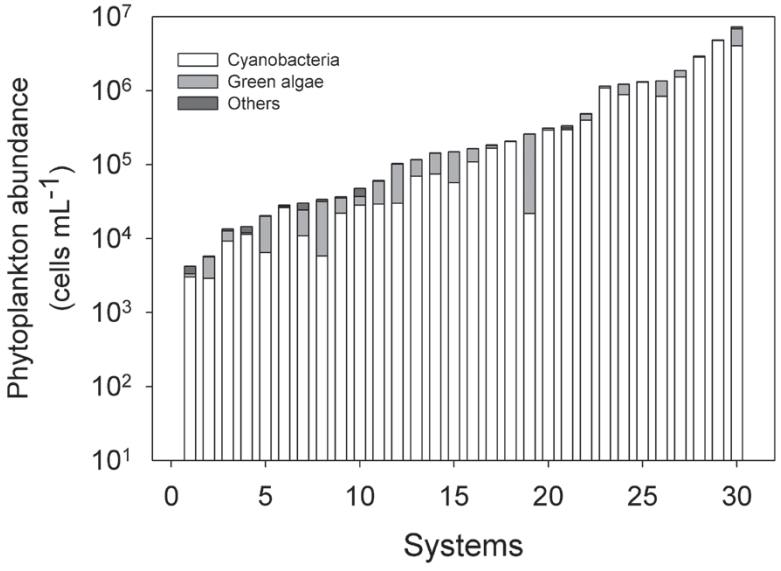

Figure 2 - Phytoplankton abundance (log scale) sorted by major taxonomic group, in 30 fishponds in southeastern Brazil.

TABLE II
Main features of the aquaculture systems. org . = organic, inorg. = inorganic, multiple = multiple species, mono = monoculture.

System number	Lat. (UTM)	Long. (UTM)	Type of activity	Water source	Pond bottom	Rearing system	Stocking rate (fish m^{-2})	Type of fertilizer
Closed systems								
3	21.113	43.062	fee-fishing	spring	earthen	multiple	4	none
9	22.423	43.391	fee-fishing	spring	concrete	multiple	1.5	none
10	22.322	44.000	fish-farming	unknown	earthen	mono	2	org.
11	22.402	43.403	fee-fishing	stream	concrete	multiple	unknown	none
15	22.345	43.506	fish-farming	spring	earthen	mono	2.5	org. + inorg.
17	22.351	42.474	fish-farming	spring	earthen	multiple	3	org. + inorg.
18	22.323	42.466	fish-farming	spring	earthen	multiple	1.5	org. + inorg.
19	22.342	42.415	fish-farming	unknown	earthen	multiple	2	org.
20	22.300	42.115	fish-farming	stream	earthen	mono	1	org.
21	22.295	42.131	fish-farming	stream	earthen	mono	1.5	org. + inorg.
22	22.282	42.095	fish-farming	spring	earthen	multiple	3	org. + inorg.
23	22.440	42.423	fish-farming	spring	earthen	multiple	1.5	org. + inorg.
24	22.344	43.191	fee-fishing	spring	earthen	multiple	unknown	none
25	22.364	43.191	fish-farming	spring	earthen	multiple	3.5	org. + inorg.
27	22.052	43.186	fish-farming	stream	earthen	multiple	2	org.
28	22.086	43.225	fish-farming	spring	earthen	mono	2	org. + inorg.
29	22.095	43.321	fish-farming	spring	earthen	multiple	2	org.
30	22.085	43.343	fish-farming	spring	earthen	multiple	2	org.
Open systems								
1	21.142	42.550	fee-fishing	spring	earthen	mono	1.5	org. + inorg.
2	21.050	42.571	fee-fishing	stream	earthen	multiple	unknown	none
4	21.152	42.082	fish-farming	spring	earthen	mono	2.5	inorg.
5	21.241	42.094	fee-fishing	spring	earthen	multiple	1.5	org.
6	22.322	44.001	fee-fishing	unknown	earthen	multiple	unknown	org.
7	22.364	44.005	fish-farming	spring	earthen	mono	2.5	org. + inorg.
8	22.421	43.575	fee-fishing	spring	earthen	multiple	3	org. + inorg.
12	22.422	43.586	fish-farming	spring	earthen	multiple	2	inorg.
13	22.384	44.004	fee-fishing	spring	earthen	multiple	1	org. + inorg.
14	22.390	43.546	fish-farming	spring	earthen	mono	2	org. + inorg.
16	22.343	42.472	fish-farming	spring	earthen	mono	1	org. + inorg.
26	22.362	43.205	fish-farming	spring	earthen	multiple	1	org. + inorg.

Systems with higher abundances of cyanobacteria (> 50,000 cells mL^{-1}) were those with higher TP concentrations (Figure 3a) and chlorophyll-*a*. In 23 fishponds, cyanobacteria contributed more than 50% of the total phytoplankton abundance, and green algae contributed more than 50% in only three ponds. The most abundant species of cyanobacteria were *Aphanocapsa delicatissima, A. incerta, A. elachista, Chrococcus* cf. *dispersus, C. minimus, Geitlerinema amphibium, Merismopedia tenuissima, Microcystis aeruginosa, Pannus mycrocystiformis, Planktolyngbya circumcreta,* and *Pseudanabaena* cf. *acicularis.* The most abundant green algae were *Desmodesmus communis, Dictyosphaerium pulchellum, Eudorina elegans, Kirchneriella dianae., Koliella longiseta* f. *tenuis, Scenedesmus ellipticus, Crucigenia tetrapedia, Scenedesmus ovalternus,* and *Tetrastrum* sp.

Of the 30 fishponds, 17 showed concentrations above 50,000 cells mL^{-1} of cyanobacteria and followed the gradient of chlorophyll-*a* and TP concentrations (Figure 3a). Chlorophyll-*a* concentrations ranged between 8.7 and 344.0 µg L^{-1} (median= 82.0 µg L^{-1}) and 90% of the systems showed levels higher than 30 µg L^{-1} (Table I).

Summarizing, cyanobacteria were highly abundant in most of our fishponds, and were the most important group, followed by green algae. Cyanobacteria abundance was positively related to TP concentrations, and they were more abundant in N-limited systems (Figure 3a, b).

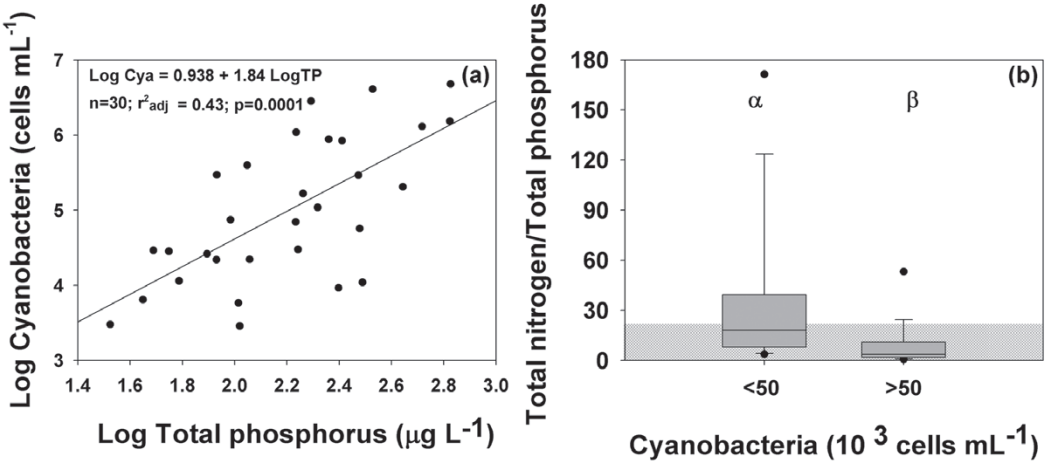

Figure 3 - (a) Relationship between Log Total phosphorus concentrations and Log Cyanobacterial abundance, showing the higher cyanobacterial abundance in higher TP concentrations; (b) Box plots of TN:TP ratios (by atom) in the fishponds where cyanobacteria abundances were higher and lower than 50,000 cells mL^{-1}. The gray area indicates N limitation. Significant differences ($p<0.05$) are indicated by different letters.

Thermotolerant Coliforms

The abundance of thermotolerant coliforms was highly variable (2 to 160,000 MPN 100 mL^{-1}) and numbers greater than 1,000 MPN 100 mL^{-1} were found in 50% of the fishponds. Total phytoplankton and cyanobacteria abundances were positively related ($p<0.05$) to the abundance of thermotolerant coliforms (r_S=0.18 and 0.26, respectively).

Cyanotoxins in the Seston Fraction and Fish Muscles

In 2005, we selected five systems from the fishponds, with total abundance greater than 100,000 cells mL^{-1} to analyze microcystins in the seston and in the Nile tilapia muscle. The same analyses were repeated in the same ponds in 2008. Microcystins varied from zero to 0.17 µg L^{-1} in the seston, and from zero to 0.05 ng g^{-1} in fish muscle (Table III).

In 2005, microcystins varied from zero (pond 25) to 0.11 μg L^{-1} (pond 18) in the seston and from 0.01 (pond 12) to 0.05 ng g^{-1} in fish muscle (ponds 20 and 25). In 2008, the variation of microcystins ranged from zero (ponds 24 and 25) to 0.16 μg L^{-1} (pond 12) in the seston, and from zero (pond 24) to 0.02 ng g^{-1} (pond 18) in fish muscle. The highest level in fish muscle was found in ponds 20 and 25 (0.05 ng g^{-1}) in 2005. Pond 25 showed the highest cyanobacteria abundance (1,295,751 cells mL^{-1}). Surprisingly, microcystins were detected in the seston of pond 20 but not in pond 25 (Table III).

TABLE III
Microcystins in the seston fraction and fish muscle, in five tropical fishponds in southeastern Brazil with high cyanobacterial abundance, in 2005 and 2008. (*Samples not analyzed).

Fishponds	Years	Microcystin-seston (μg L^{-1})	Microcystin-fish muscle (ng g^{-1})	Cyanobacteria (cells mL^{-1})	Main species
12	2005	0.02	0.01	30.038	*Planktolyngbya* sp.1
	2008	0.16	0.01	153.455	Planktolyngbya limnetica
					Aphanocapsa incerta
18	2005	0.11	*	204.108	*Synechocystis* sp.2
					Pannus microcystiformis
	2008	0.08	0.02	418.762	Aphanocapsa delicatissima
					A. incerta
20	2005	0.03	0.05	291.977	A. incerta
					A. delicatissima
	2008	0.01	*	756.553	Aphanocapsa holsatica
					A. incerta
24	2005	0.01	0.04	879.356	*Pseudanabaena* cf. *acicularis*
	2008	0.00	0.00	197.115	*A. delicatissima*
					A. incerta
25	2005	0.00	0.05	1.295.751	*Microcystis* cf. *aeruginosa*
					Synechocystis aquatilis
					A. incerta
	2008	0.17	0.01	931.972	*Microcystis* sp.
					A. holsatica
					Merismopedia tenuissima

The most important cyanobacteria species were *Pannus mycrocystiformis* (ponds 12 and 18); *Aphanocapsa incerta*, *A. delicatissima,* and *A. holsatica* (ponds 12, 18, 20, 24 and 25); *Planktolyngbya limnetica* (pond 12); *Synechocystis aquatilis* (ponds 18 and 25); *Microcystis aeruginosa* (pond 25); and *Pseudanabaena* sp. (pond 24).

Therefore, microcystin concentrations both in the seston fraction and in fish muscle were low, even though the systems showed high cyanobacterial abundance.

Established Parameters for Water Quality

About half of the fishponds indicated less than the allowed 5 mg L^{-1} of dissolved oxygen, and 80% showed turbidity lower than 100 NTU. In 90% of the systems, the total phosphorus content was higher than the threshold of 50 μg L^{-1} and chlorophyll-*a*

TABLE IV

Established parameters for Class II water quality, indicated by Brazilian legislation (CONAMA 357/2005) for aquaculture and fishing activities, and results for 30 fishponds in the state of Rio de Janeiro.(MPN = most probable number). In bold, parameters exceeding the mandated limits. * p<0.05; ** p<0.1; Max=maximum.

Parameters/ Systems	Dissolved oxygen (mg L^{-1})	Turbidity (NTU)	Total phosphorus (µgP L^{-1})	Chlorophyll-a (µg L^{-1})	Thermotolerant coliforms (10^3 MPN 100 mL^{-1})	Cyanobacterial abundance (10^3 cells mL^{-1})
Max. values allowed	**> 5**	**< 100**	**< 50**	**< 30**	**<1**	**< 50,000**
Closed systems						
3	10.2	12.9	**174.5**	**86.9**	**90**	30.0
9	**4.2**	34.0	**104.4**	**55.2**	**2.3**	2.9
10	6.4	**139.8**	**439.3**	**45.7**	**90.0**	**204.1**
11	12.8	21.0	**111.7**	**256.2**	**8.0**	**395.8**
15	**3.0**	45.9	**61.2**	**80.2**	**2.0**	11.4
17	**3.8**	36.7	**171.1**	**74.2**	**16.0**	**69.5**
18	6.5	43.2	**113.9**	**52.0**	**3.0**	22.0
19	7.0	50.2	**182.3**	**78.2**	**17.0**	**166.3**
20	**4.7**	51.7	**103.2**	28.0	0.1	5.8
21	5.9	**101.6**	**308.3**	**141.9**	0.2	10.9
22	**4.3**	**114**	**207.4**	**344**	0.1	**108.4**
23	7.8	33.9	**78.3**	**49.0**	23.0	26.1
24	7.1	13.0	48.8	**59.0**	0.3	29.1
25	5.3	**126.3**	**300.7**	**166.8**	0.7	**56.9**
27	**3.3**	86.8	**172**	**83.7**	0.1	**1088.6**
28	**3.5**	62.0	**96.5**	**103.2**	0.1	**74.5**
29	**4.4**	16.6	33.4	8.7	0.1	3.0
30	**3.9**	21.5	**85.0**	**69.2**	0.2	21.8
Mean	5.8	56.2	160.6 *	99.0	14.1 **	129.3 *
Open systems						
1	**4.1**	76.4	**85.2**	**50.3**	**17.0**	**294.4**
2	6.8	**262.9**	**249.7**	**91.7**	**160.0**	9.2
4	**3.4**	83.2	**195.8**	**288.1**	**8.0**	**2809.7**
5	9.3	56.7	**297,0**	**96.2**	**13.0**	**291.9**
6	**4.2**	42.5	**257.3**	**134.1**	**160.0**	**837.8**
7	10.2	97.9	**669.5**	**84.0**	**160.0**	**4758.2**
8	8.8	9.9	**55.9**	**41.2**	**22.0**	28.3
12	**1.2**	**101.6**	**665.4**	**88.0**	0.7	**1522.9**
13	**4.0**	95.3	**520.9**	**92.8**	0.2	**1295.8**
14	6.7	17.6	44.5	**64.3**	0.3	6.4
16	**2.8**	35.2	**229.3**	18.0	0.2	**879.3**
26	**4.8**	67.0	**337.0**	**300.0**	0.1	**4048.9**
Mean	5.5	78.9	300.6 *	112.4	45.1 **	1398.6 *

was higher than 30 μg L^{-1}. Cyanobacteria abundance was higher than 50,000 cells mL^{-1} in 57% of the fishponds and thermotolerant coliforms were higher than 1000 MPN 100 mL^{-1} in 56% of the ponds (Table IV). There was no single system where all variables were within the accepted range of water quality for fish culture.

DISCUSSION

In evaluating the water quality in 30 fishponds in southeastern Brazil, we found that they were highly phosphorus-enriched, due to fertilization, which lowered the water quality by increasing cyanobacteria, chlorophyll-*a*, turbidity, and thermotolerant coliforms, and by depleting dissolved oxygen.

As established in many previous studies, low water quality is expected in fish ponds (Boyd and Queiroz 2001, Mercante et al. 2004, Boyd 2006). The consequences of fertilization are related to the increase in nutrient availability, mainly phosphorus (Zhang and Fang 2006). Non-consumed fish ration remains in the system and leads to algal blooms, especially cyanobacteria, high chlorophyll-*a* concentrations (Mercante et al. 2004), high levels of turbidity and oxygen depletion (Simões et al. 2008). These effects, as well as high levels of thermotolerant coliforms, were found in the fishponds. Most of these variables are related to the eutrophication process.

According to criteria proposed by Nürnberg (1996), of the 30 fishponds, 21 were hypereutrophic and nine eutrophic, based on TP concentrations. A stronger trend was observed when trophic states were established based on chlorophyll-*a*: almost all the systems (28) were hypereutrophic. Although the TP concentrations were remarkably high, the TN concentrations did not follow proportionately high. Using TN as the indicator, 12 systems were eutrophic and hypereutrophic, 12 were mesotrophic, and 6 oligotrophic. This situation is commonly found in tropical waters (Brasil 2011, Rangel et al. 2012) probably because of the potential higher denitrification rates at warmer temperatures (Lewis 2000). For this reason, it has been argued that in tropical latitudes, N can be the most frequent limiting nutrient for phytoplankton growth (Lewis 2000). However, this finding has not been supported by the most recent studies (Huszar et al. 2006, Elser et al. 2007, Kosten et al. 2009), and there is no statistically significant relationship between latitude and denitrification rates in the warmest season (Piña-Ochoa and Álvarez-Cobelas 2006).

Independently of the causes of the low amount of nitrogen in our fishponds, the trend for N limitation of phytoplankton growth was clear, as shown by both the criteria of dissolved inorganic nitrogen concentrations and TN:TP ratios. The conditions of low TN:TP ratios (Smith 1983, Bulgakóv and Levich 1999), high total phosphorus concentrations (Moss et al. 2011), allied to the high temperature (Paerl and Huisman 2008, Lürling et al. 2013) might favor the dominance of cyanobacteria. In fact, the abundance of this algal group was positively related to TP concentrations, and cyanobacteria were more abundant in the N-limited fishponds.

Cyanobacteria dominance is potentially related to cyanotoxin production (Huisman et al. 2005). Despite the importance of information on bioaccumulation of cyanotoxins in fish muscle (Magalhães et al. 2003, Soares et al. 2004, Ibelings and Chorus 2007, Romo et al. 2012), knowledge of this factor in fishponds is still sparse. Our data revealed low levels of microcystins both in the seston and in fish muscle, in spite of the high cyanobacteria abundance in these systems. There are several possible explanations for this finding. First, among the most important cyanobacteria in the systems where cyanotoxins were analyzed, only three species were potential producers of toxic microcystins (*Planktolyngbya limnetica*, *Synechocystis aquatilis* and *Microcystis aeruginosa;* Sant'Anna et al. 2008). Second, the particular strains present may have been non-toxic. Third,

the period of exposure to the algae may have been insufficient for toxins to be accumulated by the omnivorous filter-feeding Nile tilapia, the main species reared in the fish farms. Cyanotoxins in fish muscle were below the limit of the Tolerable Daily Intake (TDI). According to Chorus and Bartram (1999), the TDI value of microcystins is 0.04 μg kg^{-1} body weight d^{-1}. Therefore, if an adult human weighing 60 kg ingests 300 g of fish muscle, the microcystin level of 0.05 ng g^{-1} in fish muscle in the estimated daily intake will be 0.00025 μg of microcystin per kilogram of human body weight. This value is 160-fold lower than the TDI suggested by Chorus and Bartram (1999) for this cyanotoxin. Further investigations should more thoroughly examine cyanotoxin levels in fish muscles and viscera, to better understand the bioaccumulation process.

Highly enriched fishponds with high concentrations of total phytoplankton and cyanobacteria also have high numbers of thermotolerant coliforms, indicating the decline in water quality. Organic fertilizers (bird and pig manure) are commonly used in these fishponds, and domestic animals are also present in the vicinity (unpublished data).

Based on our data, it was possible to evaluate the water quality of these fishponds with reference to the Brazilian legislation to classify inland waterbodies (CONAMA 357/2005). The classification is based on turbidity, total phosphorus, dissolved oxygen, chlorophyll-*a*, cyanobacteria abundance, and thermotolerant coliforms, among other variables. We found that all 30 systems were inappropriate for fish culture, for at least one of the parameters measured. Among the six parameters evaluated in this paper, 63% of the systems exceeded the regulated limits for at least four of the items of which phosphorus and chlorophyll-*a* were the most common. Open systems supported significantly higher TP concentrations and cyanobacterial abundance ($p<0.05$) than closed systems. Thermotolerant coliforms were also found in higher concentrations (marginally significant, $p=0.07$) in open fishponds than in closed ones. Because the samples were taken during the rainy season, the open systems seemed to be more vulnerable to nutrient input from the watershed. Therefore, in these systems, in addition to the input from fertilizers and the internal loading, external loading could also have contributed to the eutrophication process. As a consequence, cyanobacterial abundance also increased, reducing the water quality. For example, the fish-farming ponds with the highest levels of TP and highest cyanobacterial abundances were open systems (7 and 12). The interaction of the management practices with land uses in the watersheds, modulated by regional climate, could accelerate the eutrophication process in the fishponds. Similar conditions have been found in other aquaculture systems in the Upper Tietê River and Mogi-Guaçu River basins, state of São Paulo (Sant'Anna et al. 2006, Eler and Espíndola 2006) (Table V). However, although

TABLE V
Comparison of the variables used to evaluate water quality in this study, with other similar studies in Brazil. * Sant´Anna et al. 2006, ** Eler and Espíndola 2006. n.i.=not informed.

	Upper Tietê River*			Mogi-Guaçu River**			This study		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Dissolved oxygen (mg L^{-1})	4.4-13.5	7.8	n.i.	0.31-11.6	6.9	2.2	1.2-12.8	5.7	2.6
Turbidity (NTU)	14.0-235	45.9	n.i.	2.0-239.0	32.7	45.4	9.9-262.9	65.2	52.1
Total phosphorus (μg L^{-1})	35.0-315.0	115	n.i.	3.7-335.5	130.8	88.3	33.4-669.5	213.3	171.4
Chlorophyll-*a* (μg L^{-1})	0.0-300.0	50	n.i.	254.0-327.4	n.i.	n.i.	8.7-344.0	104.4	84.8

turbidity, total phosphorus and chlorophyll-*a* concentrations were in the same range, higher mean values were found in the fishponds in the state of Rio de Janeiro.

In synthesis, high nutrient concentrations, mainly phosphorus, cause low water quality in these fishponds by increasing cyanobacteria, chlorophyll-*a*, turbidity, and thermotolerant coliforms, and by depleting dissolved oxygen. Our findings indicate the need for better management practices to minimize the impacts of the eutrophication process, in addition to rigorous control policies for these systems, in order to guarantee food quality.

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RESUMO

A expansão da aquicultura no mundo tem causado fortes impactos ambientais. Um dos principais impactos é a eutrofização, por causa do necessário uso de fertilizantes para o crescimento de peixes, o que resulta em baixa qualidade da água e promove florações de algas, sobretudo de cianobactérias. Para avaliar se a qualidade da água em 30 sistemas de pisciculturas no sudeste do Brasil atinge os requerimentos da legislação brasileira, foram analisadas as condições bióticas e abióticas da água. Espera-se que os altos níveis de nutrientes ocasionados pela fertilização promovam uma redução na qualidade da água. Nós também analisamos cianotoxinas no seston e no músculo dos peixes em alguns sistemas onde cianobactérias foram dominantes. Os sistemas de pisciculturas variaram de eutróficos a hipereutróficos com altas biomassas de cianobactérias. Apesar das cianobactérias serem dominantes na maioria dos sistemas, cianotoxinas ocorreram em baixas concentrações provavelmente porque somente duas das 12 espécies dominantes foram potencialmente produtoras de microcistinas. As altas concentrações de fósforo promoveram baixa qualidade da água, com aumento de cianobactérias, clorofila-*a*, turbidez e de coliformes termotolerantes e com depleção do oxigênio dissolvido. De acordo com a legislação brasileira, todos os 30 sistemas foram considerados inapropriados para o cultivo de peixes, em pelo menos um dos parâmetros medidos. Além disso, nenhum dos sistemas apresentou todas as variáveis de qualidade da água analisadas dentro dos limites aceitos para sistemas destinados ao cultivo de peixes, de acordo com a legislação brasileira. Nossos resultados indicam a necessidade de um melhor manejo e um rigoroso controle dos sistemas de aquicultura para minimizar os impactos da eutrofização e garantir uma boa qualidade do alimento produzido.

Palavras-chave: cianobactéria, cianotoxinas, eutrofização, pisciculturas.

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