First record and ecologic aspects of *Isthmochloron neustonica* Zalocar & Pizzarro (Tribophyceae) in São Paulo State, Brazil[1]

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ABSTRACT - (First record and ecologic aspects of *Isthmochloron neustonica* Zalocar & Pizzarro (Tribophyceae) in São Paulo State, Brazil). We present the morphometric characteristics of *Isthmochloron neustonica* populations as well as their abundance and the relationship with some environmental characteristics in a fishpond. This is the first record of the species in São Paulo State and second citation to Brazil. *Isthmochloron neustonica* was described in neustonic habit; in this study, with sampling for phytoplankton analysis, it was found in high density and short duration blooming (time interval up to 15 days). It represented 59% of total phytoplankton density on May 26, 2010, forming a delicate and bright film on the water surface, in an environment with high concentration of nutrients and associated with drought and low temperature. The species has a restricted range in South America, probably due to their biological characteristics, such as small cell size and neustonic habit. Few records in literature can also be one of the reasons that hampers the identification of the species in subtropical environments.
Keywords: Ecology, freshwater, taxonomy

RESUMO - (Primeiro registro e aspectos ecológicos de *Isthmochloron neustonica* Zalocar and Pizzarro (Tribophyceae) no Estado de São Paulo, Brasil). Apresentamos as características morfológicas de populações de *Isthmochloron neustonica* bem como, sua abundância e a relação com algumas características ambientais em tanque de piscicultura. Trata-se do primeiro registro da espécie para o Estado de São Paulo e segunda citação para o Brasil. *Isthmochloron neustonica* foi descrita com hábito neustônico e no presente estudo, com amostragem para análise do fitoplâncton, foi registrada com elevada densidade, caracterizando floração de curta duração (intervalo de tempo de 15 dias), representando 59% da densidade total fitoplanctônica em 26 de maio de 2010, formando sobre a superfície da água uma película delicada e brilhante, em ambiente com alta concentração de nutrientes e no período seco e baixa temperatura. A espécie apresenta distribuição geográfica restrita a América do Sul, provavelmente em função de suas características biológicas, como células de pequeno tamanho e hábito neustônico, os poucos registros na literatura, pode dificultar a identificação e o registro da espécie em ambientes subtropicais.
Palavras-chave: Água doce, ecologia, taxonomia

Introduction

The genus *Isthmochloron* (Nägeli) Skuja 1948, described in Ettl (1978) and characterized by unicellular organisms, free life, strongly flattened quadrangular cells, in front view, with four concave sides, two of which more sharply constricted than the others and that divide the cell into two identical halves. The poles are salient and sometimes ending in short spines or may be extended in furcate projections, with only a plan developed, thereby differentiating it from *Pseudostaurastrum*. Presents several chromoplasts, parietal and discoid, without pyrenoid. Sexual reproduction is not described.

The name Tribophyceae that has succeeded Xanthophyceae, mostly have planktic habit, epiphytic and some representatives in the soil microflora and a few that have adapted to neuston life (Zalocar & Pizarro 1993). *Isthmochloron neustonica* was described in 1993 by Zalocar & Pizarro, and the organisms were collected in a shallow artificial lake with concrete sides that worked as a landscape at the

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Centro de Ecologia Aplicada Del Litoral, Corrientes (Argentina), on December 22, 1989. They were described as belonging to epineuston community.

In Brazil the organisms belonging to Tribophyceae are registered, especially the freshwater algal flora (Sant'Anna *et al.* 1989, Nogueira & Leandro-Rodrigues 1999, Ferragut *et al.* 2005, Tucci *et al.* 2006, Biolo & Rodrigues 2010). Specifically on the class, Bovo-Scomparin *et al.* (2005) and Bicudo *et al.* (2006) discussed the group taxonomy, with descriptions and illustrations of species recorded in Brazilian aquatic systems, floodplain of the Paraná River Basin and shallow reservoirs like Parque Estadual das Fontes do Ipiranga in São Paulo State, respectively. In Brazil, 28 genus, 72 species (two endemic) and four varieties were recorded (Menezes & Bicudo 2010; Menezes *et al.* 2015). However, little is known about the ecology and dynamics of the species on this class.

Currently *Isthmochloron neustonica* have occurrences restricted to South America, recorded in two countries. The first was in Argentina (Corrientes) by Zalocar & Pizarro (1993), which is the work type of the species developed performed on a bloom on a sampling date and containing only a brief physical and chemical descriptions of water, and Brazil is on the list of the algae from upper Parana River floodplain (Paraná State) by Bovo-Scomparin *et al.* (2005), where the environmental characteristics are not determined. That fact makes the species biology practically unknown. The initial aim of this study was to evaluate the phytoplankton community and, therefore, sampling was designed for this purpose. However, the species was recorded in abundance between plankton. In this study, we presented the morphometric characteristics of *Isthmochloron neustonica* populations as well as its density and the relationship with some environmental characteristics, documenting the first record of the species in the São Paulo State and the second record of the species in Brazil.

Material and methods

This study was carried out in a semi-intensive system, in production stage grow-out with monosex male of Nile tilapia (*Oreochromis niloticus*), stocked at a density of three fish m^{-2}, for research purposes, located in Crustacean Sector, Aquaculture Center, Universidade Estadual Paulista (UNESP), Jaboticabal, São Paulo, Brazil (21º15'S and 48º18'W). The dimensions of the fishpond presented an area of 0.02 ha, average depth of 1.08 m and volume of 220 m³. The water flow values were determined by the volume meter method based on the time spent by a determined water flow to fill a recipient of a known volume. The estimated residence time of the water was four days. The water renewal of the fishpond was constant and without mechanical aeration.

The local climate is warm temperate, with a dry winter and a hot summer (Cwa), according to Köppen classification. The rainfall during the culture period ranged from 183 mm in March to 7.8 mm in June. The average monthly air temperature was higher in the first half of the culture period, 24.6 ± 5.7 °C in March and 22.2 ± 6.1 °C in April, declining to the end of the culture period, with 19.5 ± 6.5 °C in May and 18.5 ± 7.7 °C in June (CIIAGRO 2012).

The samples referred to analyses of environmental variables and phytoplankton were obtained using bottle collected fortnightly of the March 3 to June 24, 2010 (n = 9) in the sub-surface of the water column. Temperature of water, dissolved oxygen, electrical conductivity, pH and turbidity were registered in situ using field sensors. Total suspended solids (TSS) according to APHA (1998). Total Kjeldahl nitrogen (TKN), dissolved Kjeldahl nitrogen (DKN), nitrite-N and nitrate-N concentrations were determined according to Mackereth *et al.* (1978) and ammoniacal-N by the method proposed by Koroleff (1976). Total inorganic nitrogen concentration (TIN) was calculated on the sum of nitrite-N, nitrate-N and ammoniacal-N; Total phosphorus (TP), dissolved phosphorus (DP) and orthophosphate (PO_4-P) concentrations were determined according to Golterman *et al.* (1978).

Taxonomic analyses were obtained of phytoplankton samples using a 20 µm mesh plankton net (n = 9) and preserved in a 4% formaldehyde solution. The taxa were identified under standard light microscopy, Zeiss Axioplan 2 microscope, with the aid of specialized bibliography. For the morphometric analysis of the *Isthmochloron neustonica* (n = 30), the program AxionVision LE® was used. After identification, the material was deposited in the Scientific Herbarium "Maria Eneyda P. Kauffmann Fidalgo" (SP), in the Instituto de Botânica de São Paulo. The samples for quantitative analysis of phytoplankton were fixed in 1% Lugol's solutions. Phytoplankton populations (ind mL^{-1}) were counted in random fields using the settling technique (Utermöhl 1958) using an inverted microscope Zeiss Axiovert 25 (630 ×). The count limit was given when dominant taxa achieved 100 individuals (Bicudo 1990). The dominant species were those which occurrence exceeded 50% of the total number of individuals of the sample and the abundant species were those which occurrence was higher than the mean of the total number of individuals of the sample (Lobo & Leighton 1986).

Isthmochloron neustonica biovolume (n = 30) were estimated using geometric shapes similar to the cell format, as follows: four triangular prisms and a cuboid. The value obtained in $\mu m^3\ mL^{-1}$ was converted to $mm^3\ L^{-1}$ by dividing this value by 10^6 (Hillebrand *et al.* 1999).

The canonical correspondence analysis (CCA) was used to correlate environmental variables (*Isthmochloron neustonica* and phytoplankton groups), using PC-ORD 6.0 for Windows (McCune & Mefford 2011). Data were converted applying [log (x + 1)].

Results and Discussion

Taxonomic aspects of *Isthmochloron neustonica*

Class Tribophyceae
  Order Mischococcales
    Family Pleurochloridaceae
      Genus *Isthmochloron* (Nägeli) Skuja 1948

Isthmochloron neustonica Zalocar & Pizarro, Cryptogamie Algologie 14(4): 200 and 201-202 figures. 1993.
Figure 1 a-k

They are free single-celled organisms, cross-shaped, quadrangular, strongly flattened with four processes arranged in the same view; 12.4 µm diameter (with processes), rounded angles. Thin and smooth cell wall without ornamentation. Numerous discoid parietal chloroplasts without pyrenoid. Presence of central vacuole and oil droplets.

Individuals with linear dimensions 12.38 ± 1.93 µm (n = 30), were considered cells adult, and showed no morphological variability. Zalocar & Pizarro (1993) observed cells between 7.5 and 14 µm, and just 5-10% of the population was polymorphs cells. Individuals with larger dimensions were observed by Bovo-Scoparin *et al.* (2005) cells between 13 and 18 µm, these authors did not observe morphological variation. Individuals surrounded by mucilage were registered (figure 1 c) as described by Zalocar & Pizarro (1993), it is possibly resistance cells. Cells taken as young individuals for their dimensions (dimensions: 5.95 ± 1.23 µm) (figure 1 d, e), possibly arising from cell division. These cells are smaller registered by Zalocar & Pizarro (1993) and Bovo-Scoparin *et al.* (2005). Large amount of bacteria were observed between the cells (figure 1 h), as observed by Zalocar & Pizarro (1993), these authors reported that the number of bacteria was 150 to 200 times more to the algae. The side view is generally biconvex (figure 1 d, f) second Zalocar & Pizarro (1993) top view is higher than the bottom view and stay in touch with air, while the bottom view in contact with water.

Examined material: BRAZIL: São Paulo: Jaboticabal, 1, 26-V-2010, *J.A.S. Osti s.n.* (SP427370); Jaboticabal, 9-VI-2010, *J.A.S. Osti s.n.* (SP427371); Jaboticabal, 24-VI-2010, *J.A.S. Osti s.n.* (SP427372).

Geographic distribution: restricted to South America: Corrientes, Argentina (Zalocar & Pizarro 1993) and Paraná State, Brazil (Bovo-Scomparin *et al.* 2005). This is the first citation of the taxon in the São Paulo State.

Ecological Considerations

Isthmochloron neustonica is not a well-known species and so, its geographical distribution is not known, thus making its biology basically unknown. Was originally described as the first species of the Tribophyceae adapted to the epineuston life, however, more detailed studies based on morphological and physiological characteristics should be carried out to confirm this way of life. The neuston habit part of the original description of the species, and, as there are records species commonly periphytic habit on plankton, the same can have occurred in samples obtained, in relation to the neuston on plankton.

Isthmochloron neustonica was recorded in a shallow artificial system with concrete walls and soil bottom during tilapia grow-out period. Zalocar & Pizarro (1993) originally collected in a shallow artificial system, at the Centro de Ecologia Aplicada Del Litoral, Corrientes (Argentina), on December 22, 1989.

Isthmochloron neustonica and others one hundred and seven taxa were identified and distributed in nine phytoplankton groups. The canonical correspondence analysis (CCA) was used to evaluate the contribution of *I. neustonica* to the structure of the phytoplankton community front of some environmental variables during grow-out period of tilapia (figure 2; table 1).

The CCA utilizing nine phytoplankton groups, besides of *I. neustonica* and seven environmental variables, explained 81.7% of the variability data of the first two components, was statistically significant ($p < 0.01$) to eigenvalues for the axis 1($\lambda1 = 0.092$) and axis 2 ($\lambda2 = 0.050$) according Monte Carlo test. The species-environment correlations were significant ($p < 0.01$) for axis 1 ($r = 1.000$) and axis 2 ($r = 0.973$) indicating a strong relationship between the distribution of *I. neustonica*, phytoplankton groups and environmental variables.

The first axis was the most important to explain the distribution of *I. neustonica*, phytoplankton groups and environmental variables during tilapia culture. Intra-set correlation and canonical coefficients indicated that dissolved Kjeldahl nitrogen (DKN) ($r = 0.69$), total Kjeldahl nitrogen (TKN) ($r = 0.66$), total inorganic nitrogen (TIN) ($r = 0.53$), electrical conductivity (Cond) ($r = -0.53$) and water temperature (Temp) ($r = -0.74$) were the most important environmental variables. *Isthmochloron neustonica* had the highest contribution to the axis ($r = 0.99$), followed by Chrysophyceae ($r = 0.87$), Cryptophyceae ($r = 0.82$) and Dinophyceae ($r = 0.70$), these were related to lower values of water temperature and electrical conductivity and with higher nitrogen concentrations. On the other hand, Chlamydophyceae ($r = -0.74$) were inversely related to these values.

The density and relative density of phytoplankton groups and *I. neustonica* during grow-out period of tilapia are showed in figure 3. Phytoplankton groups that presented the highest species richness (Chlorophyceae, Bacillariophyceae and Cyanobacteria), together with Chlamydophyceae, also had the highest densities. Chlamydophyceae and Chlorophyceae together contributed with more than 50% of the entire density in the first five samples when it was registered high water temperatures. On May 12, 2010 and June 09, 2010 samples they lost the position to Cyanobacteria and to Bacillariophyceae (figure 3) during the last sampling.

The first record of the species is dated April 14, 2010 in a period of water temperature decrease

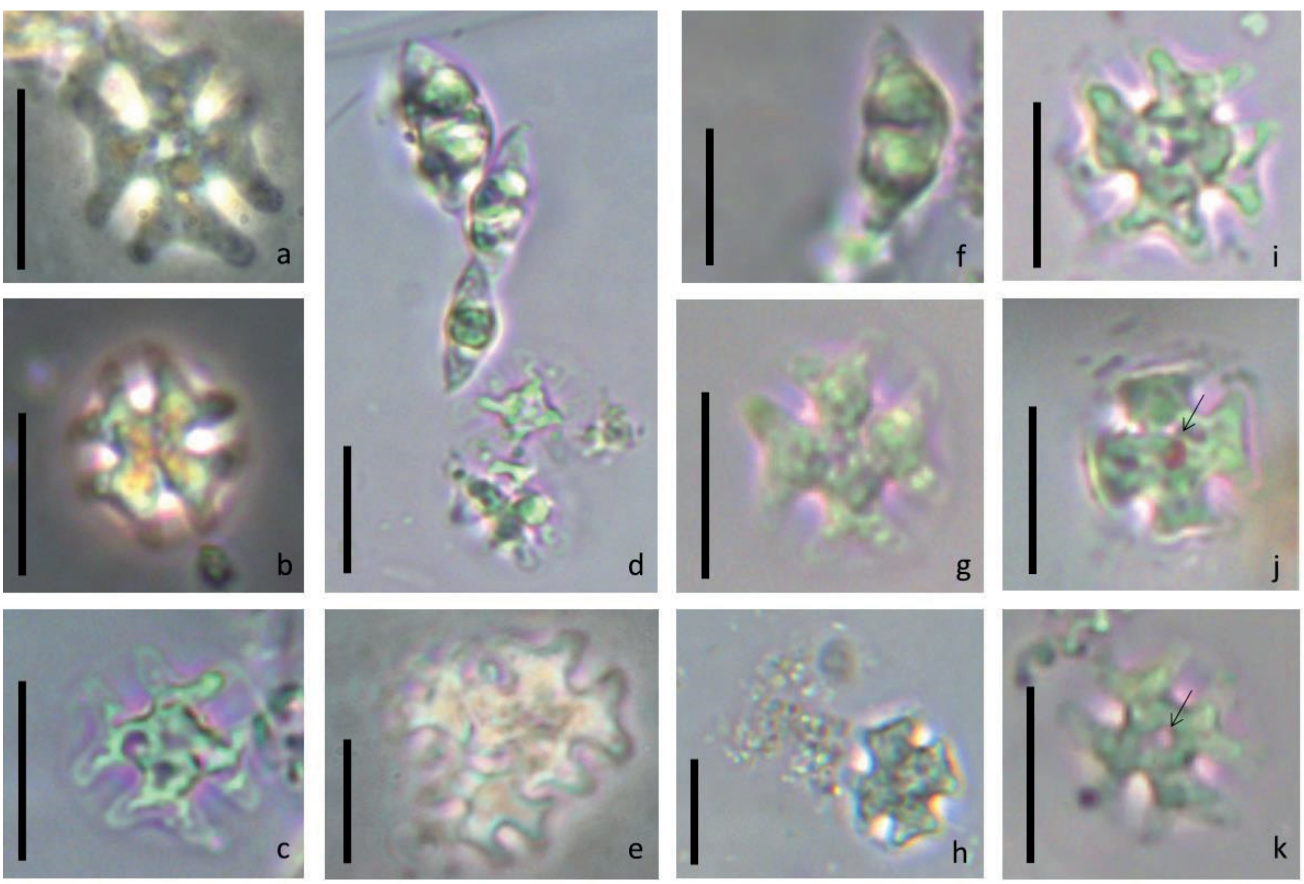

Figure 1. a-k. Different aspects of *Isthmochloron neustonica* Zalocar & Pizarro. a-c: front view (figures a and b in phase contrast). b-c: cells surrounded by mucilage. d-e: general appearance of a group of cells. f: side view. g-k: front view. h: presence of bacteria among organisms. j-k: presence of central vacuole (arrows). scale = 10 µm.

and increase of nitrogen concentrations. These environmental characteristics, though, have favored other phytoplankton groups, appear to have benefited the maintenance *I. neustonica*. On May 26, 2010 (84 days of grow-out period) the species density was 4.7×10^4 ind mL^{-1} and represented 59% of total density

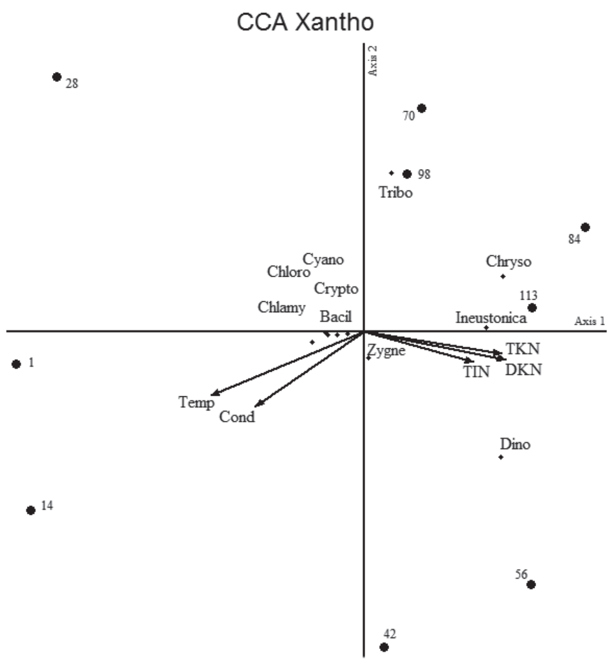

Figure 2. Biplot representation of canonical correspondence analysis (CCA) of density phytoplankton groups, *I. neustonica*, and seven environmental variables, in Nile tilapia production system (*O. niloticus*) during grow-out period. Phytoplankton groups correlation with axes 1 and 2, and the respective codes are given in Table 1. Score abbreviation: Temp: water temperature, Cond: electrical conductivity, TKN: total Kjeldahl nitrogen, DKN: dissolved Kjeldahl nitrogen, TIN: total inorganic nitrogen, TP: total phosphorus and DP: dissolved phosphorus (n = 9).

(figure 3 a, b, respectively), and was classified as dominant, no other species was classified as dominant throughout the study period. The environmental characteristics at the time of sampling were: water temperature 21.4 °C, pH 7.74, electrical conductivity 62.7 µS cm^{-1}, dissolved oxygen 6.75 mg L^{-1}, turbidity 27.7 NTU, NKT 418.7 µg L^{-1}, DKN 169.3 µg L^{-1}, TIN 135.36 µg L^{-1}, TP 153.1 µg L^{-1}, DP 47.7 µg L^{-1}, PO_4-P 12.7 µg L^{-1} and TSS 10.91 mg L^{-1} (figure 4). Shallow artificial system and high concentrations of nutrients appear to be important characteristics for the maintenance of species, as cited by Zalocar & Pizarro (1993), while the density recorded by the authors of of 2×10^7 ind mL^{-1}, was higher than observed in this study. (Insert Figure 4).

Isthmochloron neustonica was found in high density (see figure 3) as a short duration blooming (time interval up to 15 days), providing the water surface a delicate and bright film appearance, similar characteristic to reported by Zalocar & Pizarro (1993). This feature of the system surface was not recorded on other sampling days, leading to the conclusion that this is as a feature of the neuston community. This feature gives it a particular characteristic concerning environment because it forms a film on the water surface associated with certain physical-chemical properties, such as viscosity, osmotic pressure, light absorption and dispersion, electro kinetic properties, biochemical reactions, photochemical reactions, etc. (Burchard & Dutkiewicz 2007). This community presents a high productivity time related to its ability of keeping the pigment concentration even on the water level, with the possibility of using atmospheric carbon. However, these blooming can be easily broken by an excessive agitation of the water surface (Margalef 1991).

Table 1. Results of Pearson correlations obtained for phytoplankton groups and *Isthmochloron neustonica* and axes 1 and 2 of the CCA.

Bacillariophyceae	Bacil	0.207	-0.044
Chlorophyceae	Chloro	-0.176	-0.261
Chlamydophyceae	Chlamy	-0.741	-0.453
Chrysophyceae	Chryso	0.872	0.314
Cryptophyceae	Crypto	0.818	-0.012
Cyanobacteria	Cyano	-0.288	0.067
Dinophyceae	Dino	0.697	-0.559
Zygnemaphyceae	Zygne	0.486	-0.338
Tribophyceae	Tribo	0.312	0.893
Isthmochloron neustonica Zalocar & Pizarro	Ineustonica	0.985	0.040

On the other hand, environmental factors may have benefited the *I. neustonica* bloom formation and breaking in this study registered on May 26, 2010. Some factors related to practices management may have supported this bloom, such as residence time (four days), fertilizer (with regular superphosphate and urea), and the addition of feed (up to 68.75 kg ha^{-1} day^{-1} recorded at the end of culture). Since the constant input of nutrients observed in the fishpond favored the establishment of several different phytoplankton species, mostly Chlorophyceae and Cyanobacteria. Meteorological data showed sharp decrease in air temperature, due to the presence of rain showers and winds gusty (figure 5) observed seven days before the record of blooming, a fact that may have favored the mixing of the water column at the fishpond.

Additionally, the event favored the establishment of colonizing species (*I. neustonica* in this case), according to Reynolds (1997), species classified as nanoplanktonic (maximum linear dimensions between 2-20 µm) are considered the first aquatic environment colonizers after suffering some kind of disturbance, since they have some peculiar characteristics such as rapid growth, high productivity, high nutrient reserve and rapid light absorption. Ten days after the record of the bloom species, new occurrences of intense rainfalls (accumulated rainfall of 7.7 mm^3) accompanied by wind gusts peaks were observed and this phenomenon may have contributed this time to the *I. neustonica* bloom breaking, favoring the establishment of other colonizing species, in this case of small cyanobacteria like *Chroococcus minutus* (Kützing) Nägeli, *Pseudanabaena mucicola* (Naumann & Huber-Pestalozzi) Schwabe and *Synechocystis aquatilis* Sauvageau and consequently of diatoms: *Fragilaria* cf. *crotonensis* and *Fragilaria familiaris* (Kützing) F. Hustedt. However, other factors such as control of the water supply flow and management practices in fishpond should be considered and they may have contributed to decline of blooms.

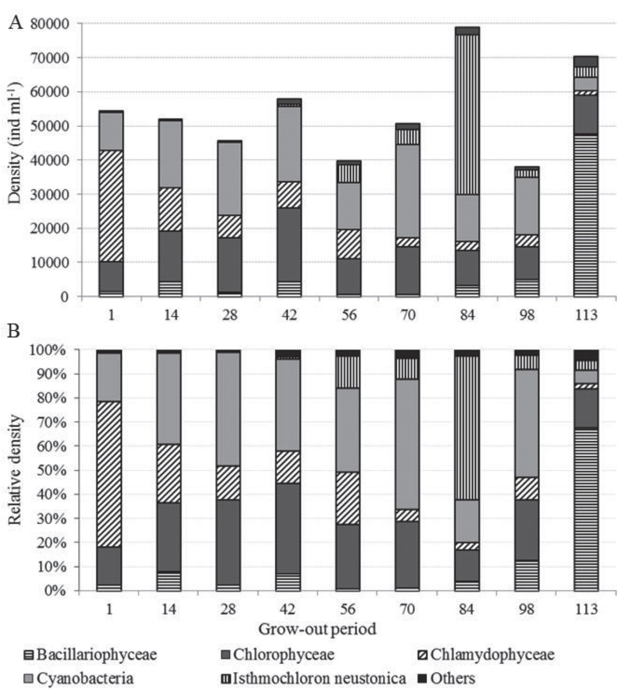

Figure 3. Fortnightly variation in the density (ind ml^{-1}) (a) and the relative density (b) of phytoplankton groups and *Isthmochloron neustonica*, in Nile tilapia production system during grow-out period (n = 9). (Others = Chrysophyceae, Cryptophyceae, Dinophyceae, Zygnemaphyceae and Tribophyceae).

Other factors should be considered, such as herbivory and trophic interactions with zooplankton (Pogozhev & Gerasimova 2011), and fishponds, the presence of Nile tilapia, omnivorous and filter water species, which occupies an intermediate trophic position among primary producers and piscivorous animals (Attayde *et al.* 2007). The fact that the tilapia does not select the prey type during foraging (Panosso *et al.* 2007) may favor the livelihood of organisms with smaller cell size as in the case of *I. neustonica* which has an individual biovolume on 234.3 µm³, that is small when compared with biovolume of the algae of subtropical environments (Fonseca *et al.* 2014) with algae of larger bodies such as the colonial and filamentous Cyanobacteria.

Finally, was attributed to management practices in fishpond, the presence of species with small cell dimensions, adapted to transition stages of aquatic ecosystems, such as *I. neustonica*. The decrease of air and water temperature also favored the first records of *I. neustonica* and the dominance of diatoms species by the end of the culture period. It was not detected dominance of species of Cyanobacteria with specific structures (*i.e.* aerotopes, mucilage, heterocytes etc.) commonly observed in aquaculture farming (Mercante *et al.* 2011, Millan & Sipauba-Tavares 2014). *Isthmochloron neustonica*, was prevalent in environmental conditions that also favor other species, however, as temperature decreases and, probable absence of turbulence on the surface of the water column, could have favored the species. Their biological characteristics, such as small cell size and neuston habit can promote restrictions on their geographical distribution due to the need of environmentally favorable factors for development of the population. Few records in the literature on

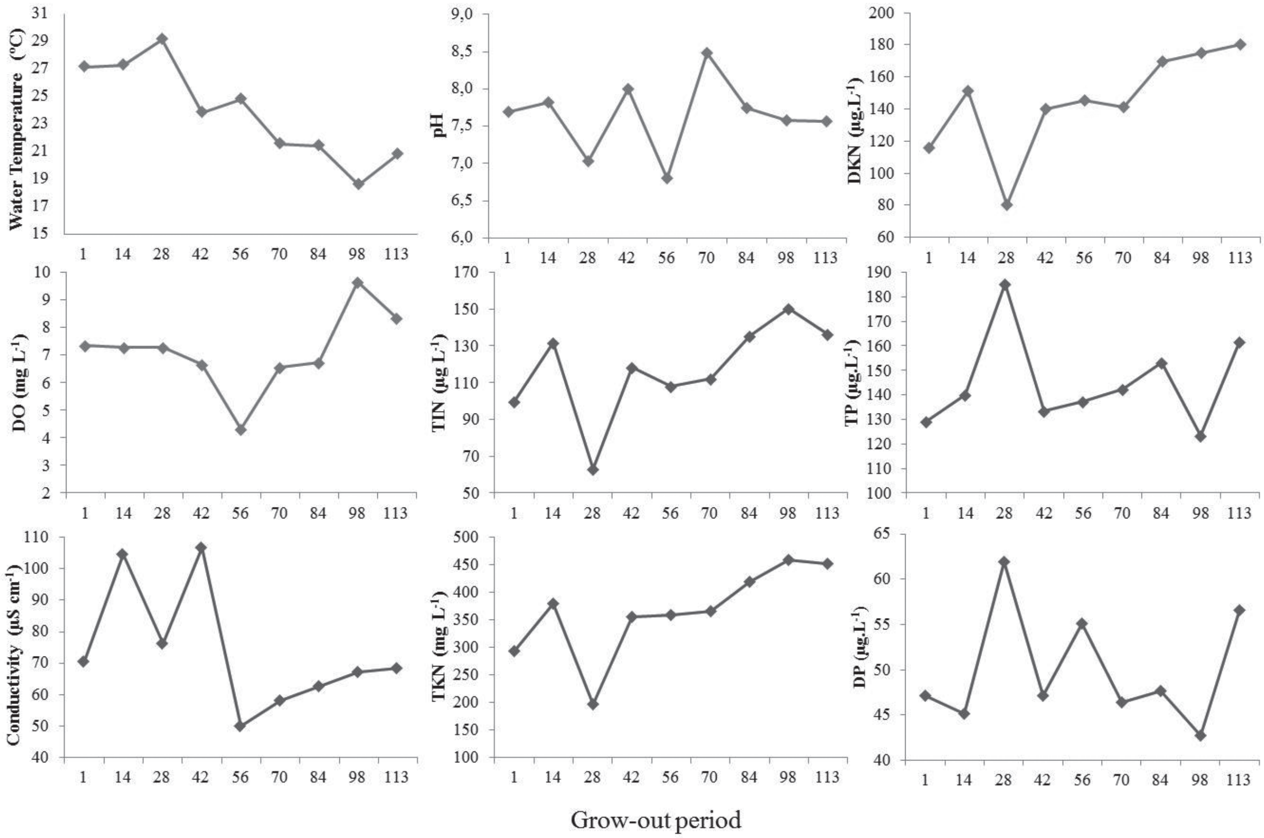

Figure 4. Values of the abiotic limnological variables in Nile tilapia production system during grow-out period (n = 9). (DO: dissolved oxygen, TIN: total inorganic nitrogen, TKN: total Kjeldahl nitrogen, DKN: dissolved Kjeldahl nitrogen, TP: total phosphorus, TP: dissolved phosphorus).

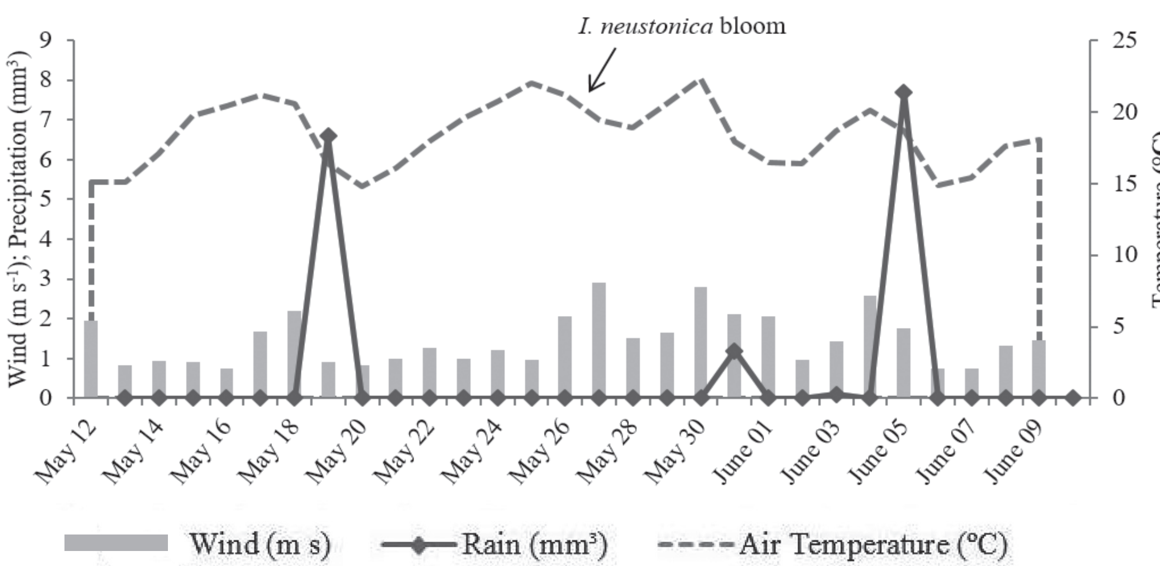

Figure 5. Average values of wind speed (m. s^{-1}) (bars), precipitation (mm^3) (continuous line) and air temperature (°C) (dotted line), during the registration period of *I. neustonica* bloom (arrow). *Source*: CIIAGRO Integrated Center for Agrometeorology information (CIIAGRO 2012).

this species (two) can also be one of the reasons that hamper the identification of the species in subtropical environments.

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