BOLETIM DO INSTITUTO DE PESCA
ISSN 1678-2305 online version
Scientific Article

RESPONSE OF PHYTOPLANKTON TO DIFFERENT CARBON SOURCES AND C:N RATIOS IN TILAPIA FINGERLING CULTURE WITH BIOFLOCS*

Ugo Lima SILVA[1]
Lucas Conceição VIEIRA[1]
Marcus Vinicius Lourenço de MELLO[1]
Elton José de FRANÇA[1]
Dario Rocha FALCON[1]
Eudes de Souza CORREIA[2]

[1]Universidade Federal Rural de Pernambuco – UFRPE, Unidade Acadêmica de Serra Talhada, Gregório Ferraz Nogueira, Av. José Tomé de Souza Ramos, CEP 56909-535, Serra Talhada, PE, Brasil. E-mail: ugolimas@gmail.com (corresponding author)

[2]Universidade Federal Rural de Pernambuco – UFRPE, Departamento de Pesca e Aquicultura, Don Manuel de Medeiros, Av. Dois Irmãos, CEP 52171-900, Recife, PE, Brasil.

*Financial support: Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Received May 31, 2017
Approved November 26, 2017

ABSTRACT

The objectives of this study were to evaluate the dynamics and qualitative and quantitative aspects of the phytoplankton community during the fingerlings production of Nile tilapia (*Oreochromis niloticus*) using biofloc technology. A complete randomized design, with 6 treatments and 4 replications was used. Three carbon sources molasses (M), sugar (S) and cassava starch (CS) at carbon:nitrogen (C:N) ratios of 10:1 and 20:1 were evaluated. Phytoplankton was collected weekly during 63 days using 5 L water samples that were filtered through a 25 µm mesh plankton net. The phytoplankton community was represented by 17 genera of the Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, and Fragilariophyceae classes. *Pseudanabaena* and *Synechococcus* were the main genera responsible for the dominance of the Cyanophyceae class in all treatments. Chlorophyceae was the second most diverse class, whose main genera were *Chlorella* and *Scenedesmus*. Molasses at C:N ratio of 20:1 were the carbon source that most affected the phytoplankton development.

Key words: microalgae; fish culture; *Oreochromis niloticus*; water quality.

RESPOSTA FITOPLANCTÔNICA À DIFERENTES FONTES DE CARBONO E RELAÇÕES C:N NA ALEVINAGEM DE TILÁPIA CULTIVADA COM BIOFLOCOS

RESUMO

O objetivo deste trabalho foi avaliar a dinâmica da comunidade fitoplanctônica durante a alevinagem de tilápia do Nilo, *Oreochromis niloticus*, com tecnologia de bioflocos. Adotou-se um delineamento experimental inteiramente casualizado com 6 tratamentos e 4 repetições, sendo testadas três fontes de carbono: melaço (ME), açúcar (AC) e fécula de mandioca (FE), nas relações carbono/nitrogênio (C:N) 10:1 e 20:1. As coletas de fitoplâncton foram realizadas semanalmente durante 63 dias, em amostras de 5 L de água filtradas numa rede de plâncton com malha de 25 µm. A comunidade fitoplanctônica do ambiente de cultivo esteve representada por 17 gêneros, distribuídos entre as classes Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae e Fragilariophyceae. Em termos de gênero, pode-se concluir que a *Pseudanabaena* e *Synechococcus* foram os principais integrantes responsáveis pela dominância da Cyanophyceae em todos os tratamentos. A Chlorophyceae foi a segunda classe mais diversificada, tendo como principais gêneros *Chlorella e Scenedesmus*. A fonte de carbono que mais influenciou no desenvolvimento do fitoplâncton foi o melaço, na relação carbono:nitrogênio 20:1.

Palavras-chave: microalgas; piscicultura; *Oreochromis niloticus*; qualidade da água.

INTRODUCTION

Nile tilapia was the most widely cultivated fish species in Brazil in the last decade, and present desirable characteristics, high acceptance by consumers, and high commercial value, especially in developing countries (FAO, 2014). Moreover, this species is rustic, precocious and has omnivorous habit; it uses satisfactorily high levels of plant protein and therefore it is recommended for fish farming because it is easily adapted to food management practices and tolerates high storage densities in intensive breeding systems (BEVERIDGE, 1996; AVNIMELECH, 2009).

The concentration of food and residues, such as organic material, nutrients, and suspended solids, in intensive production systems is high, causing eutrophication and reduction of oxygen, and affecting the water turbidity (LIN and YI, 2003). Therefore, new, more sustainable production techniques are being implemented around the world, with very promising, productive, and efficient technologies (AVNIMELECH, 2009; COLLAZOS-LASSO and ARIAS-CASTELLANOS, 2015).

Biofloc technology (BFT) is one of the most appropriate and promising alternatives for a sustainable development of aquaculture (AVNIMELECH, 2009; KUHN *et al*., 2009). BFT systems are characterized by minimal or no water renewal, inducing the development of microbial communities by using organic carbon sources to stimulate the growth of heterotrophic bacteria, which assimilate inorganic nitrogen (PÉREZ-FUENTES *et al*., 2016).

The bacteria in the bioflocs use nitrogen compounds dissolved in the water, incorporating them, generating a microbial biomass that can be used as food source for fishes (THOMPSON *et al*., 2002; WASIELESKY JUNIOR *et al*., 2006; AZIM and LITTLE, 2008). Feed costs is reduced in BFT systems; thus, it is considered an environmentally friendly aquaculture practice; moreover, its effluents present small amounts of nitrogen compounds at the end of the productive cycle, reducing risks of eutrophication of water bodies (AVNIMELECH, 2009).

The natural food in the bioflocs is important for the nutrient cycling because it is composed of several microorganisms bacteria, protozoa, microalgae, zooplankton, benthic invertebrates, plant material, and debris (MARTÍNEZ-CORDOVA *et al*., 2003; SILVA *et al*., 2008). The BFT may affect positively the tilapia larval performance (EKASARI *et al*., 2015). The natural food is the best option for the initial feeding of fish larvae and fingerlings due to its nutritional content; the plankton has enzymes that are essential for their growth and survival, especially in their first days of life (EKASARI *et al*., 2015).

Phytoplankton is the first link between abiotic and biotic environments, and is the main entry of matter and energy into the trophic chain through the primary assimilation; thus, it is an important ecological component in the characterization and definition of the environmental dynamics of aquatic systems (MARGALEF, 1983). Different than algae, microbial populations are more stable and independent from light conditions (AVNIMELECH, 2009).

The main problems of fertilization programs are inconstancy of C:N:P ratios; application of excessive fertilizers causing excessive growth of undesirable algae; deficiency of other essential macro and micronutrients to phytoplankton; the use of poor quality fertilizers; and deposition of phosphorus in the bottom sediment of the nurseries. Nutrients are important for the phytoplankton ecology and composition, and can modify qualitatively and quantitatively the phytoplankton communities, especially phosphorus and nitrogen. The algal biomass does not increase linearly; this is understandable due to their complex chemical, physical, and biological interactions (FRANCESCHINI *et al*., 2010).

Thus, the objectives of this study were to evaluate the dynamics and qualitative and quantitative aspects of the phytoplankton community during the fingerlings production of Nile tilapia (*Oreochromis niloticus*) using biofloc technology, and different carbon sources and carbon-nitrogen (C:N) ratios, and monitor the water quality physical, chemical, and biological parameters of the aquatic environment.

METHODS

The experimental cultivation of the Nile tilapia (*Oreochromis niloticus*) fingerlings was carried out during 63 days in 24 circular 1000 L, glass fiber tanks, using a volume of 800 L.

The tanks were filled with water from an artesian well; the water was filtered in a 200 µm filter and the tanks were aerated individually with four porous rocks per tank fed by a radial compressor (2.0 HP). These tanks were placed in an outdoor area, with reduced natural light to 75% by a shade screen (Sombrite®), and covered with mesh lids to avoid the fishes to escape. The water of the tanks was not exchanged, but they were refilled due to evaporation.

A complete randomized design with 6 treatments and 4 replications was used. Three carbon sources molasses (M), demerara sugar (S) and cassava starch (CS) at carbon:nitrogen (C:N) ratios of 10:1 and 20:1 were evaluated.

Dolomitic limestone (150 mg L^{-1} of $CaCO_3$) was applied weekly to maintain the alkalinity of the water. The amount of limestone was determined by the formula {[(Ideal Value × (mg L^{-1} $CaCO_3$) – Analyzed Value × (mg L^{-1} $CaCO_3$)] × (Limestone reactivity power)$^{-1}$ × Tank volume (L)}, thus, the amount of limestone (mg tank^{-1}) used was {[(150 – Analyzed Value) × 0.64^{-1}] × 800}.

The carbon sources were added daily to induce a heterotrophic medium, with amounts (ΔCarbon) calculated based on the established carbon:nitrogen (C:N) ratios, amount of nitrogen available in the feed as ammonia (ΔN), and content of carbon in the sources used (%C), according to Equations 1 and 2, adapted from AVNIMELECH (2009). $\Delta Carbon = [\Delta N \times (C:N)] \times \%C^{-1}$ (Equation 1); $\Delta N = QFeed \times \%NFeed \times \%NExcretion$ (Equation 2).

Wherein %C is the percentage of carbon present in carbohydrate sources 0.22; 0.31, and 0.46 g g^{-1} of carbon for molasses, sugar, and cassava starch, respectively, ΔN was determined by assuming that QFeed is the quantity of feed offered daily, %NFeed is the amount of nitrogen added into the system (%Crude Protein × 6.25^{-1}), and %NExcretion is the ammonia rate in the water resulted direct from fish excretion or indirectly from microbial degradation of organic nitrogen residues approximately 50% of the nitrogen of the feed.

Sexually reversed fingerlings of Nile tilapia with average weight of 1.68 ± 0.05 g were obtained from a commercial fish farm and stored in experimental tanks at density of 300 fingerlings m^{-3}. The fishes were fed *ad libitum* four times a day 07:30h, 10:30h, 13:30h, and 16:30h with extruded feed at diameter of 1.0 mm. The centesimal composition of the offered diet had 10% moisture, 45% crude protein, 8% ethereal extract, 2.8% crude fiber, 17% mineral matter, 3.3% calcium, and 1.5% phosphorus.

The water temperature (°C), dissolved oxygen (mg L^{-1}), and pH were monitored daily with a multiparameter probe (YSI ProPlus). The water transparency (cm) was evaluated using a Secchi disk, and its salinity was evaluated using an optical refractometer (Atago S10).

The water samples were collected weekly for analyses of total ammonia (mg L^{-1} N-NH_4+NH_3), nitrite (mg L^{-1} N-NO_2^-), nitrate (mg L^{-1} N-NO_3^-), inorganic phosphate (mg L^{-1} P-PO_4^{3-}), alkalinity (mg L^{-1} $CaCO_3$), and sedimentable solids (mL L^{-1}). Total ammoniacal nitrogen (TAN), nitrite, nitrate, inorganic phosphate, and alkalinity were measured with a photometer (YSI 9500). Sedimentable solids (SS) were evaluated using Imhoff cones, with sedimentation time of 15 to 20 minutes.

Phytoplankton collections were carried out weekly. Five liters of water was collected from each tank using a beaker in the bottom-surface direction and filtered through a 20 µm mesh plankton net. These samples were packed in 300 mL labeled containers, and 37% formaldehyde was added to a final concentration of 4% formol.

The phytoplankton community was identified and counted through direct method. Qualitative and quantitative analyses of phytoplankton were carried out using an optical microscope, based on the specialized literature (BICUDO and BICUDO, 2004; SANT'ANNA *et al*., 2012). The abundance of each group of organisms was evaluated by the number of cells (mL^{-1}) given by Cells (mL^{-1}) = (D×C') × (C"×C"')$^{-1}$ (APHA, 1995), wherein D is the number of cells in the analyzed aliquot, C' is the volume of sample concentration (100 mL), C" is the volume of the analyzed aliquot (0.2 mL), and C"' is the volume filtered through the collector (5000 mL).

All fishes were weighed at the beginning and end of the experiment, using a precision scale (± 0.01 g) to determine their final average weight (g), feed conversion factor, survival (%), and productivity (fingerlings m^{-3}).

A Canonical Correspondence Analysis (CCA) establishing the relationships between the seasonal variation of the phytoplankton richness with the environmental variables and experimental treatments and a diversity analysis were carried out using the PAST 2.17 program.

The Shapiro-Wilk normality and Bartlett's homoscedasticity tests were applied at 5% significance to verify the sample normality, and homogeneity of variances, respectively. Analysis of variance (ANOVA Criterion 1) at 5% significance was used to verify differences between treatments, which was complemented, when necessary, with the Tukey's test at 5% significance. The statistical analyses are in accordance with ZAR (1996).

RESULTS

The physical-chemical variables of water quality evaluated during the experiment (Table 1) were adequate for the studied fish species. The water salinities (0.3±0.8 g L^{-1}) of the treatments

Table 1. Water quality variables recorded during 63 days of Nile tilapia (*Oreochromis niloticus*) fingerlings production using biofloc technology with different carbon sources Sugar (S), Cassava starch (CS), and Molasses (M) and carbon:nitrogen (C:N) ratios (10:1 and 20:1).

Variable	C:N ratio	Carbon sources		
		S	CS	M
Temperature (°C)	10:1	23.1±0.7^{a}	23.0±0.7^{a}	23.0±0.7^{a}
	20:1	23.2±0.8^{a}	23.1±0.7^{a}	23.2±0.7^{a}
pH	10:1	8.0±0.1^{a}	8.1±0.1^{a}	8.1±0.1^{a}
	20:1	8.0±0.1^{a}	8.1±0.1^{a}	8.1±0.1^{a}
Dissolved oxygen (mg L^{-1})	10:1	7.1±0.2^{a}	7.3±0.3^{a}	7.1±0.3^{a}
	20:1	6.7±0.4^{a}	7.1±0.2^{a}	6.8±0.3^{a}
Transparency (cm)	10:1	18.9±8.9^{a}	28.4±7.8^{b}	13.1±5.5^{a}
	20:1	14.0±7.0^{a}	18.9±7.0^{b}	9.5±4.0^{a}
Alkalinity (mg L^{-1} $CaCO_3$)	10:1	233.2±32.6^{Aa}	198.0±17.0^{Aa}	225.0±18.1^{Aa}
	20:1	272.7±34.6^{Bab}	223.7±5.7^{Bb}	301.8±11.2^{Ba}
Total ammoniacal nitrogen	10:1	1.9±0.8^{a}	1.6±0.4^{a}	1.7±0.1^{a}
(mg L^{-1})	20:1	1.9±0.3^{a}	1.9±0.4^{a}	2.0±0.3^{a}
Nitrite (mg L^{-1} N-NO_2)	10:1	0.5±0.1^{a}	0.5±0.4^{a}	0.6±0.0^{a}
	20:1	0.5±0.0^{a}	1.1±0.5^{a}	0.7±0.1^{a}
Nitrate (mg L^{-1} N-NO_3)	10:1	13.6±3.4^{aA}	13.6±1.3^{aA}	12.4±1.1^{aA}
	20:1	6.8±0.6^{aB}	11.3±0.2^{aB}	9.3±1.1^{aB}
Inorganic phosphate	10:1	5.7±0.9^{a}	6.6±0.3^{a}	6.7±0.9^{a}
(mg L^{-1} P-PO_4)	20:1	5.9±1.2^{a}	7.6±1.4^{a}	6.5±1.6^{a}
Sedimentable solids (mL L^{-1})	10:1	80.3±17.7^{Aab}	3.5±1.1^{Ab}	162.3±70.8^{Aa}
	20:1	124.2±32.7^{Bab}	5.1±2.1^{Bb}	201.8±80.4^{Ba}

Means followed by with different lowercase letters in the same row differ significantly by the Tukey's test ($P < 0.05$). Means followed by different uppercase letters differ significantly for C:N ratios (10:1 and 20:1) by the Tukey's test ($P < 0.05$).

were similar. The water transparency was significantly higher when using cassava starch as carbon source (Table 1).

The total ammonia (1.9 mg L^{-1}) and nitrite (0.7 mg L^{-1}) of the treatments were similar ($P > 0.05$). The used carbon sources affected the microbial floc formation, which presented significantly higher sedimentable solids in treatments with sugar (102.2 mL L^{-1}) and molasses (182.0 mL L^{-1}) when compared to cassava starch (4.4 mL L^{-1}). The carbon sources and C:N ratios used affected significantly the alkalinity and nitrate in the water of the experimental treatments ($P < 0.05$).

The phytoplankton community during the experiment was represented by 17 genera of the Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, and Fragilariophyceae classes (Table 2).

The Cyanophyceae was the most diverse class, regardless of the treatment, and the dominant class in the temporal scale, presenting higher percentages than the other classes (Figure 1, Table 2). Regarding the genera, *Pseudanabaena* (Figure 2d) and *Synechococcus* were the main responsible for the dominance of the Cyanophyceae class (>67%) in all treatments (Table 2).

Chlorophyceae was the second most diverse class (Figure 1). *Chlorella* and *Scenedesmus* were the most abundant genera of microalgae (Figure 2a); especially in the treatment tanks with cassava starch (Table 2).

The Bacillariophyceae, Euglenophyceae, and Fragilariophyceae classes were represented by only one genus each, *Cymbella*, *Euglena*, and *Asterionella*, respectively (Table 2). The most abundant genera found were *Limnococcus* (Figure 2b), *Synechococcus* and *Pseudanabaena*; but none of them were present in 100% of the samples. The treatments with cassava starch presented the greatest diversity of genera (Table 2).

According to the diversity analysis, treatments with sugar at C:N ratio of 10:1 (S10) and 20:1 (S20) had the highest diversity (1.9 and 2.2, respectively) and equitability (0.7) of species. The treatments with molasses at C:N ratio of 10:1 (M10), and cassava starch at C:N ratio of 10:1 (CS10) presented the highest indexes of dominance of species (0.75 and 0.61, respectively). The Canonical Correspondence Analysis (Figure 3) explained 84.7% of the total variability of the data in axes I and II, and showed that transparency ($r = 0.3353$) and phosphate ($r = -0.8662$) had significant correlations ($P < 0.01$) with the phytoplankton richness in the treatments.

Regarding the zootechnical performance of the tilapia fingerlings (Table 3), the treatments presented similar ($P > 0.05$) results of final weight, feed conversion, survival, and productivity.

Table 2. Relative phytoplankton abundance (%) during 63 days of Nile tilapia (*Oreochromis niloticus*) fingerlings production using biofloc technology with daily addition of different carbon sources, and different carbon-nitrogen (C:N) ratios.

	S10	S20	CS10	CS20	M10	M20
Cyanophyceae	**93**	**95**	**78**	**67**	**91**	**84**
Aphanocapsa spp.	0	0	4	4	0	0
Chroococcus spp.	2	1	5	2	4	5
Limnococcus spp.	3	3	13	12	7	4
Romeria spp.	0	1	0	0	0	0
Synechococcus spp.	4	11	20	23	6	5
Synechocystis spp.	2	1	9	9	3	2
Planktolyngbya spp.	2	3	3	0	0	3
Pseudanabaena spp.	80	74	24	16	70	64
Non-identified genus	0	1	0	1	1	1
Chlorophyceae	**2**	**3**	**16**	**28**	**7**	**12**
Chlorella spp.	1	1	7	7	1	3
Coelastrum spp.	0	1	3	2	4	0
Scenedesmus spp.	0	0	4	17	0	1
Tetrastrum spp.	0	0	2	1	1	7
Non-identified genus	1	1	0	1	1	1
Bacillariophyceae	**5**	**2**	**4**	**4**	**2**	**3**
Cymbella spp.	5	2	4	4	2	3
Fragilariophyceae	**0**	**0**	**1**	**0**	**0**	**1**
Asterionella spp.	0	0	1	0	0	1
Euglenophyceae	**0**	**0**	**1**	**1**	**0**	**0**
Euglena spp.	0	0	1	1	0	0
Total	**100**	**100**	**100**	**100**	**100**	**100**

M10 = Molasses at C:N ratio of 10:1; S10 = Sugar at C:N ratio of 10:1; CS10 = Cassava starch at C:N ratio of 10:1; M20 = Molasses at C:N ratio of 20:1; S20 = Sugar at C:N ratio of 20:1; CS20 = Cassava starch at C:N ratio of 20:1.

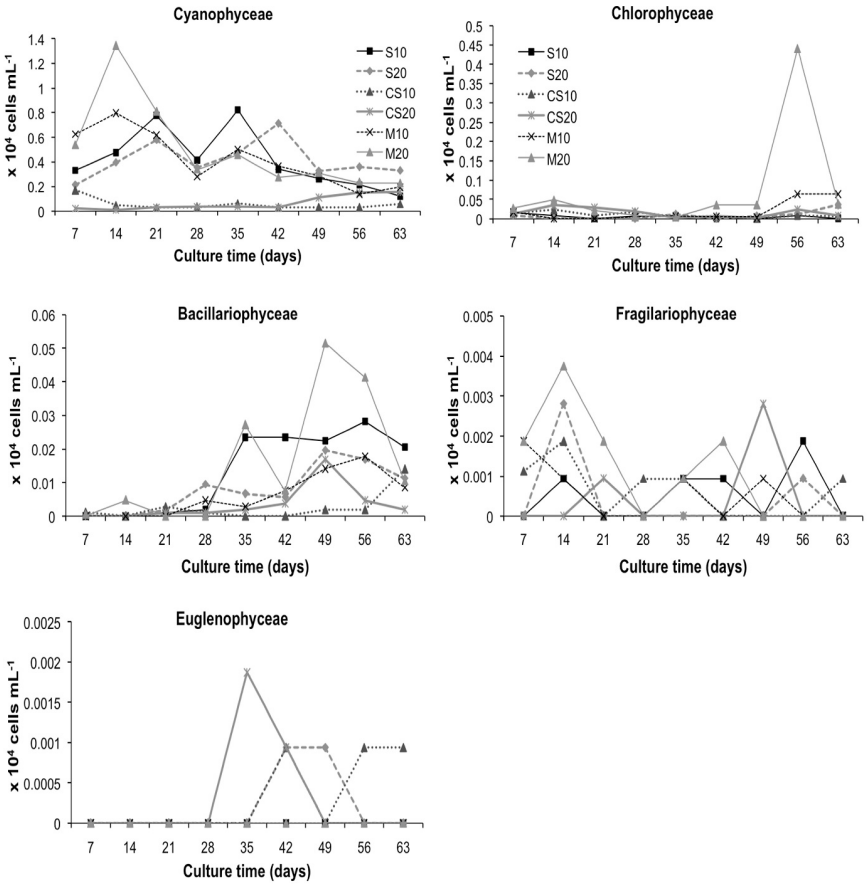

Figure 1. Variation of phytoplankton population densities during 63 days of Nile tilapia (*Oreochromis niloticus*) fingerlings production using biofloc technology represented by the Bacillariophyceae, Chlorophyceae, Cyanophyceae, Fragilariophyceae, and Euglenophyceae classes.

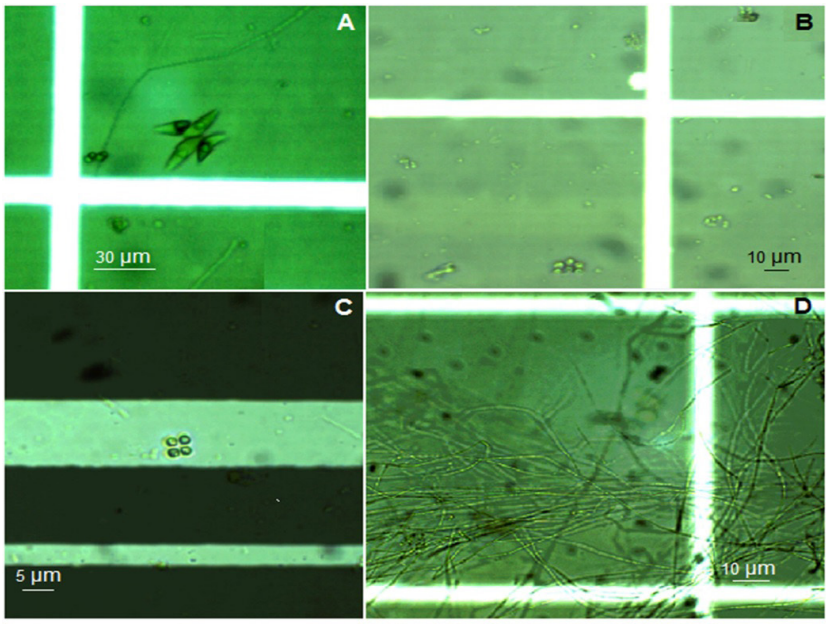

Figure 2. Microalgae found in Nile tilapia (*Oreochromis niloticus*) fingerlings production using biofloc technology. A) *Scenedesmus spp.*; B) *Limnococcus spp.*; C) *Chroococcus spp.* and D) *Pseudanabaena spp.*

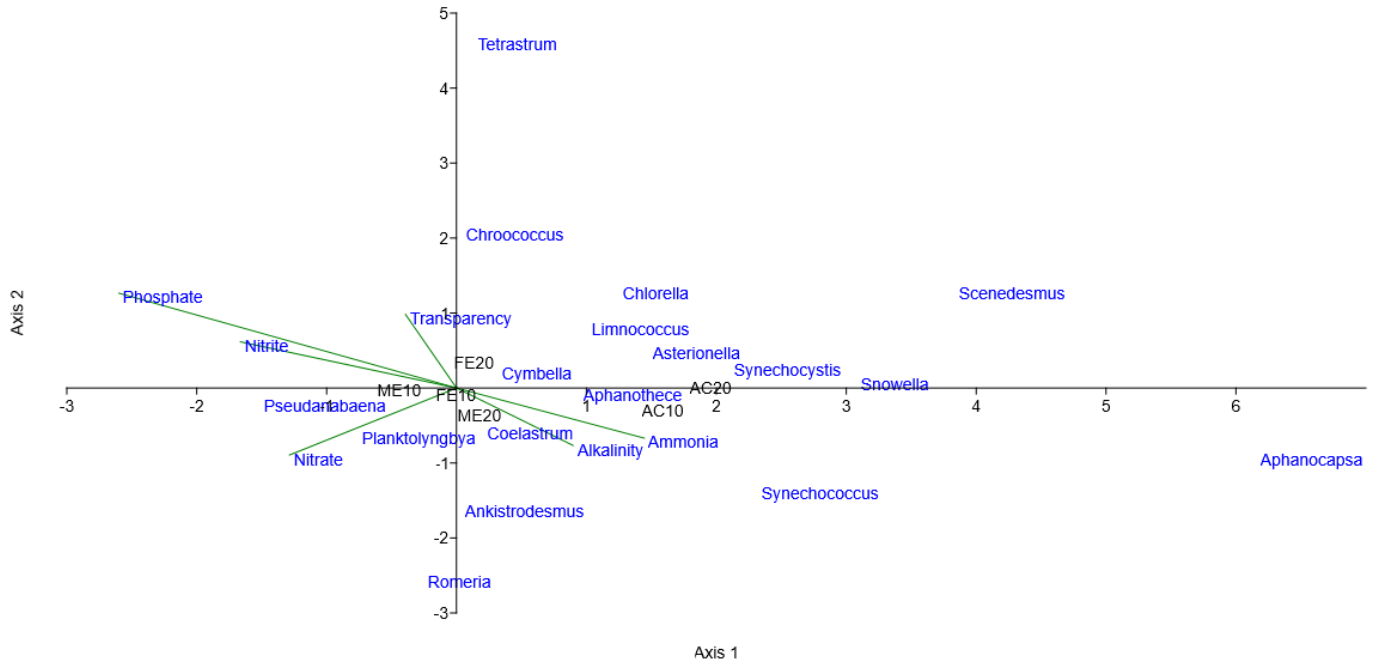

Figure 3. Canonical Correspondence Analysis (CCA) diagram for richness of phytoplankton species as a function of environmental variables alkalinity, ammonia, nitrite, nitrate, phosphate, and transparency in experimental treatments.

Table 3. Means (±SD) of zootechnical performance variables of Nile tilapia (*Oreochromis niloticus*) fingerlings reared for 63 days without water renewal, using sugar, cassava starch, and molasses as carbon sources.

Variable	Carbon sources		
	Sugar	Cassava starch	Molasses
Final weight (g)	13.5±1.4	12.4±0.1	13.0±1.4
Feed conversion factor	1.6±0.0	2.1±0.4	2.1±0.6
Survival (%)	29.3±6.8	28.2±5.1	30.4±1.6
Productivity (fingerlings m^{-3})	87.9±20.6	84.6±15.3	91.2±4.7

Feed conversion factor = (Amount of offered feed) × $(\text{Biomass gain})^{-1}$. Means with absence of letters in the rows did not differ significantly by the Tukey's test ($P > 0.05$).

DISCUSSION

The treatments had similar water temperature (23.1 ± 0.7°C), which remained below the thermal comfort range for the evaluated fish species (Table 1). However, it had no effect on the phytoplankton development and densities in any of the treatments.

According to EBELING *et al*. (2006), increases in microbial and phytoplankton biomasses affect the water pH; however, it remained around 8.2 in the water environments of the present study (Table 1) due to the addition of limestone.

The dissolved oxygen needs especial attention in systems using biofloc because of the bacteria requirements to assimilate inorganic nitrogen and degrade organic matter (EBELING *et al*., 2006). The average dissolved oxygen in the present study was 7.0 mg L^{-1}.

Water transparency is connected to phytoplankton density. The treatments with molasses, and sugar had significantly lower transparency than those with cassava starch. The organic carbon applied in these treatments was more easily assimilated by the heterotrophic bacteria as a source of energy, thus creating a less propitious environment for the phytoplankton development due to the obstruction of sunlight (VIDAL *et al*., 2005).

SANTOS *et al*. (2008) reared marine shrimp without water renewal and with addition of different quantities of molasses and found dominance of the Cyanophyceae class represented mainly by the *Pseudanabaena* genus over the other classes identified. PEREIRA-NETO *et al*. (2008) evaluated nurseries with marine shrimp and found dominance of the Cyanophyceae class. Similarly, the dominance of this class and the *Pseudanabaena* genus was found in the present study, showing the greater effect of molasses on phytoplankton density, compared to the other carbon sources used sugar, and cassava starch.

The treatment with sugar at C:N ratio of 10:1 had the highest total ammoniacal nitrogen content (5.3 mg L^{-1}). This result explains

the higher density of phytoplankton of the Cyanophyceae class in this treatment on thirty-fifth day of cultivation. In the twenty-first day, the density of phytoplankton of the Cyanophyceae class was also high in this treatment; this was probably due to the decreased density of phytoplankton of the other classes.

The treatment with cassava starch at C:N ratio of 20:1 had the highest inorganic phosphate content (13.1 mg L^{-1}). This result explains the second highest phytoplankton density of the Cyanophyceae class found in this treatment on the fifty-sixth day of cultivation.

According to MARGALEF (1983), succession of species in aquatic environments starts with small flagellates and diatoms, followed by dinoflagellates. Species of the phylum Cyanobacteria are opportunistic microorganisms that grow under extreme environmental conditions. Therefore, the phytoplankton dynamics in nurseries even in artificial environments may be similar.

The increased diversity of genera in ponds may be connected to their fertilization, because a short-term change in the conditions of a natural environment causes ecological succession of some species that dominate the nursery, thus reducing the growth of microalgae in the environment (PEREIRA-NETO *et al*., 2008).

Algae from the Cyanophyceae class, mainly of the *Pseudanabaena* genus, were dominant over the other classes identified. According to CALIJURI *et al*. (2006), species of the phylum Cyanobacteria have some advantages over other microalgae regarding the dominance in aquatic environments, such as capacity of store phosphorus and other nutrients, assimilate light under low-intensity light, and absorb atmospheric nitrogen, greater competitiveness for nutrients, higher reproductive speed, and capacity of accumulating gas in vesicles, which allows them to move and adjust themselves to the water column.

According to FRANCESCHINI *et al*. (2010), the abundance of cyanobacteria is direct linked to the concentration of organic nitrogen. This explains the dominance of the Cyanophyceae class in the present study, since cultivations without water renewal have several sources of organic nitrogen, such as degradation of feed leftovers, decomposition of dead organisms, and excretion of animals. However, there are few ways of reducing nitrogen, since the water of the nurseries remains in circulation throughout the cultivation period (AVNIMELECH, 2009).

Some filamentous cyanobacteria have specialized cells heterocysts and akinetes for fixation of molecular nitrogen (N_2). Cyanobacteria species with these cells have a relevant competitive advantage that may be a key factor for the dominance of this class in the present study. The fixation of N_2 by cyanobacteria improves significantly the nitrogen in the environment due to their death, consumption by protozoa and other animals, and the entry more nitrogen into the trophic aquatic environment (LOURENÇO, 2006; TUNDISI and TUNDISI, 2008).

Most algae assimilates nitrogen in oxidized form (nitrates or ammonium ions) or even in amine form (amino acids), except some cyanophytes that directly fix gas nitrogen. Ammonia has advantage over nitrates; it does not require reduction for assimilation and therefore saves energy. When both forms are present, ammonia ions are preferred to nitrates (MARGALEF, 1983).

Fish mortality may be associated with cyanobacterial bloom with presence of biotoxins in coastal zones (ISMAEL, 2012), and cultivation of aquatic organisms (RODGERS JUNIOR, 2008). Cyanobacteria bloom in fish farming can cause production losses; it compromises the water quality, causing the death of fishes, mainly due to the presence algae species of the genera *Microcystis, Anabaena, Aphanocapsa*, and *Cylindrospermopsis* (ELER *et al*., 2009). The high ammonia, nitrite, and sedimentable solid contents, combined with the high abundances of algae of the Cyanophyceae class may have contributed to the low survival of the Nile tilapia fingerlings reared under the no water renewal system used.

CONCLUSIONS

The use of molasses, sugar, or cassava starch does not affect the zootechnical performance of Nile tilapia fingerlings. The use of molasses at C:N ratio of 20:1 resulted in a higher phytoplankton density than the use of sugar, or cassava starch as carbon sources to induce the heterotrophic medium. The use of cassava starch resulted in a lower phytoplankton density; however, it promoted a greater diversity of phytoplankton genera. No ecological succession was identified, but 17 microalgae genera were identified, with the *Pseudanabaena* genus showing permanent dominance in all treatments.

ACKNOWLEDGEMENTS

The authors thank Professor Yllana Marinho for her guidance during the phytoplankton analysis and the Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE) for granting a doctoral scholarship for the first author.

REFERENCES

APHA – American Public Health Association. 1995 *Standard methods for the examination of water and wastewater*. 19th ed. Washington: APHA/AAWWA/WEF.

AVNIMELECH, Y. 2009 *Biofloc technology: a pratical guide book*. Baton Rouge: The World Aquaculture Society. 182p.

AZIM, M.E.; LITTLE, D.C. 2008 The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture (Amsterdam, Netherlands)*, *283*(1-4): 29-35. **http://dx.doi.org/10.1016/j.aquaculture.2008.06.036**.

BEVERIDGE, M.C.M. 1996 *Cage aquaculture*. 2nd ed. Oxford: Fishing News Books. 346p.

BICUDO, C.E.M.; BICUDO, D.C. 2004 *Amostragem em limnologia*. 2. ed. São Carlos: RiMa. 371p.

CALIJURI, M.C.; ALVES, M.S.A.; SANTOS, A.C.A. 2006 *Cianobactérias e cianotoxinas em águas continentais*. São Carlos: RiMa. 118p.

COLLAZOS-LASSO, L.F.; ARIAS-CASTELLANOS, J.A. 2015 Fundamentos de la tecnología biofloc (BFT). Una alternativa para la piscicultura en Colombia: una revisión. *Orinoquia (Universidad Tecnologica de los Llanos Orientales)*, *19*(1): 77-86. **http://dx.doi.org/10.22579/20112629.341**.

EBELING, J.M.; TIMMONS, M.B.; BISOGNI, J.J. 2006 Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture (Amsterdam, Netherlands)*, *257*(1-4): 346-358. **http://dx.doi.org/10.1016/j.aquaculture.2006.03.019**.

EKASARI, J.; RIVANDI, D.R.; FIRDAUSI, A.P.; SURAWIDJAJA, E.H.; ZAIRIN JUNIOR, M.; BOSSIER, P.; DESCHRYVER, P. 2015 Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. *Aquaculture (Amsterdam, Netherlands)*, *441*(1): 72-77. **http://dx.doi.org/10.1016/j.aquaculture.2015.02.019**.

ELER, M.N.; CAMPAGNA, A.F.; MINILLO, A.; RIBEIRO, M.A.R.; ESPÍNDOLA, E.L.G. 2009 Water quality, toxicity and Gill lesions caused by intraperitoneally administered water-bloom crude extract in *Brycon cephalus* (Gu, 1896; Characidae) from free-fishing ponds in São Paulo state, Brazil. *Acta Limnologica Brasiliensia*, *21*(1): 89-100.

FAO – Food and Agriculture Organization. 2014 *Fishstat plus: universal software for fisherystatistical time series*. Version 2.3. Rome: FAO. Available from: <http://www.fao.org/fishery/ statistics/software/fishstat/en>. Access on: 10 out. 2015.

FRANCESCHINI, I.M.; BURLIGA, A.L.; REVIERS, B.; PRADO, J.F.; RÉZIG, S.H. 2010 *Algas: uma abordagem filogenética, taxonômica e ecológica*. Porto Alegre: Artmed. 332p.

ISMAEL, A.A. 2012 Benthic bloom of cyanobacteria associated with fish mortality in Alexandria waters. *Egyptian Journal of Aquatic Research*, *38*(4): 241-247. **http://dx.doi.org/10.1016/j.ejar.2013.01.001**.

KUHN, D.D.; BOARDMAN, G.D.; LAWRENCE, A.L.; MARSH, L.; FLICK JUNIOR, G.L. 2009 Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. *Aquaculture (Amsterdam, Netherlands)*, *296*(1-2): 51-57. **http://dx.doi.org/10.1016/j.aquaculture.2009.07.025**.

LIN, C.K.; YI, Y. 2003 Minimizing environmental impacts of fresh water aquaculture and reuse of pond effluents and mud. *Aquaculture (Amsterdam, Netherlands)*, *226*(1-4): 57-68. **http://dx.doi.org/10.1016/S0044-8486(03)00467-8**.

LOURENÇO, S.O. 2006 *Cultivo de microalgas marinhas: princípios e aplicações*. São Carlos: RiMa. 606p.

MARGALEF, R. 1983 *Limnología*. Barcelona: Ediciones Omega. 1010p.

MARTINEZ-CORDOVA, L.R.; CAMPANA TORRES, A.; PORCHAS-CORNEJO, M.A. 2003 Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microcosms. *Aquaculture Nutrition*, *9*(3): 155-160. **http://dx.doi.org/10.1046/j.1365-2095.2003.00235.x**.

PEREIRA-NETO, J.B.; DANTAS, D.M.M.; GÁLVEZ, A.O.; BRITO, L.O. 2008 Avaliação das comunidades planctônicas e bentônicas de microalgas em viveiros de camarão (*Litopenaeus vannamei*). *Boletim do Instituto de Pesca*, *34*(4): 543-551.

PÉREZ-FUENTES, J.A.; HERNÁNDEZ-VERGARA, M.P.; PÉREZ-ROSTRO, C.I.; FOGEL, I. 2016 C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture (Amsterdam, Netherlands)*, *452*(1): 247-251. **http://dx.doi.org/10.1016/j.aquaculture.2015.11.010**.

RODGERS JUNIOR, J.H. 2008 *Algal toxins in pond aquaculture*. Washington: United States Department of Agriculture, Cooperative State Research, Education, and Extension Service. (Southern Regional Aquaculture Center, 4605).

SANT'ANNA, C.L.; TUCCI, A.; AZEVEDO, M.T.P.; MELCHER, S.S.; WERNER, V.R.; MALONE, C.F.S.; ROSSINI, E.F.; JACINAVICIUS, F.R.; HENTSCHKE, G.S.; OSTI, J.A.S.; SANTOS, K.R.S.; GAMA-JUNIOR, W.A.; ROSAL, C.; ADAME, G. 2012 *Atlas de cianobactérias e microalgas de águas continentais brasileiras*. São Paulo: Instituto de Botânica, Núcleo de Pesquisa em Ficologia. 175p. Available from: <www.ibot.sp.gov.br>. Access on: 10 out. 2015.

SANTOS, D.R.; MELO, F.P.; COSTA, W.M.; SILVA, U.L.; LIMA, J.P.V.; CORREIA, E.S. 2008 Melaço na produção de fitoplâncton em tanques berçário estáticos de *Litopenaeus vannamei*. In: CYRINO, J.E.P.; FURUYA, W.M.; RIBEIRO, R.P.; SCORVO FILHO, J.D. *Tópicos especiais em biologia aquática e aquicultura III*. Jaboticabal: Sociedade Brasileira de Aquicultura e Biologia Aquática. cap. 28. p. 295-305.

SILVA, U.L.; CAMPOS, S.S.; CORREIA, E.S. 2008 Efeitos de fertilizantes orgânicos e inorgânicos na abundância macro e meiobentos e na qualidade da água do cultivo do camarão *Litopenaeus vannamei* (Boone, 1931). *Atlântica*, *31*(1): 23-33.

THOMPSON, F.L.; ABREU, P.C.; WASIELESKY, W. 2002 Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture (Amsterdam, Netherlands)*, *203*(3-4): 263-278. **http://dx.doi.org/10.1016/S0044-8486(01)00642-1**.

TUNDISI, J.G.; TUNDISI, T.M. 2008 *Limnologia*. São Paulo: Oficina de Textos. 631p.

VIDAL, L.; MENDONÇA, R.F.; MARINHO, M.M.; CESAR, D.; ROLAND, F. 2005 Caminhos do carbono em ecossistemas aquáticos continentais. In: ROLAND, F.; CESAR, D.; MARINHO, M. *Lições de limnologia*. São Carlos: RiMa. p. 193-243.

WASIELESKY JUNIOR, W.; ATWOOD, H.; STOKES, A.; BROWDY, C.L. 2006 Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture (Amsterdam, Netherlands)*, *258*(1-4): 396-403. **http://dx.doi.org/10.1016/j.aquaculture.2006.04.030**.

ZAR, J.H. 1996 *Biostatistical analysis*. New Jersey: Prentice Hall. 662p.