



Are artificial semi-dry wetlands efficient in wastewater treatment from different fish densities and for lettuce production?

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Received: 13 April 2021 / Revised: 8 August 2021 / Accepted: 24 September 2021
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Abstract

This study aimed to determine artificial semi-dry wetlands' performance to wastewater treatment from fish farming and *Lactuca sativa* production in an aquaponics system. This trial model observed the effects of different stocking biomass on the growth rate of *Colossoma macropomum* and yield of two *L. sativa* varieties. A factorial design consisting of three fish density treatments in quadruplicate and two Lettuces variety was used. Nine hundred and sixty fingerlings with 8.35 ± 0.91 g were stocked at three stocking biomass treatments in quadruplicate: 40 fingerlings or 334 g m^{-3} ; 80 fingerlings 668 g m^{-3} ; 120 fingerlings or 1002 g m^{-3} . Delice American Lettuce and Purple Crested Lettuce were cultured in the semi-dry wetlands. The final mass (g), consumed food, feed conversion ratio, and yield of fingerling were statistically different between the tested densities, but survival did not. The artificial semi-dry wetlands were efficient on water treatment and the recovery of nutrients in all fish densities trials, and it has potential as support for lettuce production. The recovery of nutrients was shown on the increment of the number of leaves, total fresh mass (g), and lettuces yield by area (kg m^{-2}) in both varieties of plants tested. Precocious flowering in 30% of lettuce varieties, incidence of mealybug and whitefly, temperature and deficiency of calcium and magnesium were factors that may have compromised the full lettuce development. The aquaponics system efficiently treated the fish effluent at the densities tested, being appropriate for fish farming and lettuces production.

Keywords Biodiversity preservation · *Colossoma macropomum* · Cleaner Tambaqui production · Intensive production · Sustainability of water · Water treatment

Introduction

According to Diana (2009), no food production system now in use is truly sustainable from an energy and biodiversity perspective—all food production systems generate wastes, require energy, use water, and change land cover. Aquaculture has some positive impacts on biodiversity as reduction pressure on overexploited wild stocks, boosts natural production and species diversity, replacing more destructive resource uses. On the negative side, species that escape from aquaculture can become invasive in areas where they are nonnative and effluents from aquaculture can cause eutrophication and may transmit diseases to wild species as for example observed in USA (Diana 2009) and Thailand (Sampantamit et al. 2020). Pollution of local waters that supply aquaculture systems threatens aquaculture itself as well as biodiversity. In this context, the development of less polluting aquaculture systems becomes a scientific and technological challenge that needs to be achieved.

Editorial responsibility: Rangabhashiyam S.

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Aquaponics is an emerging technology for food cultivation that integrates an aquaculture recirculation system and hydroponics. This technology conceptually is an excellent ecology recycling system formed by solid effluent decanters, biological filters, and a hydroponics section where waste organics of water are transformed by microorganisms into nutrients for plants, supporting their growth and water pollution reduction (Sace and Fitzsimmons 2013; Zou et al. 2016; Pinho et al. 2017). The pollution reduction on aquaculture farms is crucial events for biodiversity preservations from rivers and lags of word, especially in fragile region as Amazon.

In numerous world regions, aquaponics has been projected for small private installations. Aquaponics studies in Brazil and other tropical countries have grown in the last decade, but it is still sparse and incipient compared to other countries (Jordan et al. 2018; Lima et al. 2019a). Currently, numerous Brazilian researchers have suggested this technology as an ecologic alternative for food production on a small scale to meet the family farming in many urban centers, including in the Amazon region (Geisenhoff et al. 2016; Jordan et al. 2018; Da Rocha et al. 2017; Lima et al. 2019a).

The nutrient film, floating raft, and media-based bed are techniques of aquaponics. Media-based bed aquaponics has more surface area for microbes than the other two methods having efficiency in recovery of nutrients and elimination of toxic substances for fish, being the most used in the world (Love et al. 2014). Also, this aquaponics model can reduce the need of aeration, being an excellent choice, showing higher yields and less energy cost (Fang et al. 2017).

An aquaponics requires numerous equipment (pumps, aerators, loggers) and knowledge for a mercantile operation. However, it is possible to design and operate low-tech aquaponic systems with a media-based bed or aerated bio-filters using low-cost materials as ceramsite or associations with lignocellulosic material media. A modified aquaponic system with biological aerated filter—BAFs can balance the nutrient deficiency and afford additional environmental and economic benefits (Zhang et al. 2020). In organic effluent treatment, Ullah et al. (2021) suggests that semiconductor-based photocatalysis present in natural inorganic clays with a higher surface area can be used as an effective adsorbent material. This material probably can be combined with other traditional media used in aquaponic systems improving water quality treatment.

The aquaponic systems with a media-based bed require less skill to work and still yield good family operation results (Lima et al. 2019a). Media-based bed aquaponics is structured similarly to an artificial wetland, but it has a soaked and dry cycle different from observed in the artificial wetland that is a full-time drenched (Love et al. 2014; Trang and Brix 2014). Recently, a new designation was given to the media-based aquaponics by Lima et al. (2019a), which

he called an aquaponic system with the artificial semi-dry wetland.

Although Lima et al. (2019a) using artificial semi-dry wetland have demonstrated efficacy in the recovery of nutrients of shrimp culture effluents in an aquaponic system, is questioned if a similar aquaponic system has the same efficiency on water treatment of *Colossoma macropomum* Cuvier, 1818 in different nutrient loads (densities) and the production of two lettuces varieties.

Colossoma macropomum is one crucial Amazonian fish species that has been an issue of interest for researchers and farmers due to its adaptation to intensive production, fast growth, very rusticity and resistance, acceptance into the industrial feed, and high value of its meat (Da Costa et al. 2019). It is exploited in great artisanal and commercial fishing and is heavily farmed in aquaculture, both in South America and in tropical and subtropical areas of other continents (Wood et al. 2017). Thus, the *C. macropomum*, due to be a very rustic and resistant fish, has a good feed conversion, tolerates high stocking densities, has a technological package of cultivation, advanced and widespread in many countries, besides having an excellent commercial price, and can be a good option in the aquaponics.

This work demonstrates the efficiency of a low-cost production system using artificial semi-dry wetlands to recover nutrients from fish farming wastewater and integrated production of lettuces at the family level. This trial model also observed the effects of different stocking biomass on the growth rate of Tambaqui and the yield of two lettuces varieties. The results contribute to the achievement of Sustainable Development Objective (SDG) No. 2—Zero Hunger, agreed at the United Nations Conference on Sustainable Development, held in Rio de Janeiro in 2012, which seeks to end hunger, achieve food security and improved nutrition and promote sustainable agriculture.

Materials and methods

Experimental setup and procedure

The experiment design was factorially consisting of three density treatments in quadruplicate and two Lettuces variety of treatments. The experimental setup summarized in Fig. 1 is an adaptation of Lima et al. (2019a) at the Laboratory of Aquaculture and Fishing from the Agroforestry Research Center of Amapá—Embrapa Amapá (Amapá State, Brazil). The aquaponic experimental system consisted of tree identical aquaponic units, allowing replication of experimental treatments. Each aquaponic unit consisted of four fish culture tanks ($1 \text{ m}^3 \text{ tank}^{-1}$), a conical sedimentation tank (0.1 m^3), a circular holding tank (0.2 m^3), and a constructed semi-dry wetland ($0.2 \text{ m} \times 1.0 \text{ m} \times 4.0 \text{ m}$) used as a hydroponic

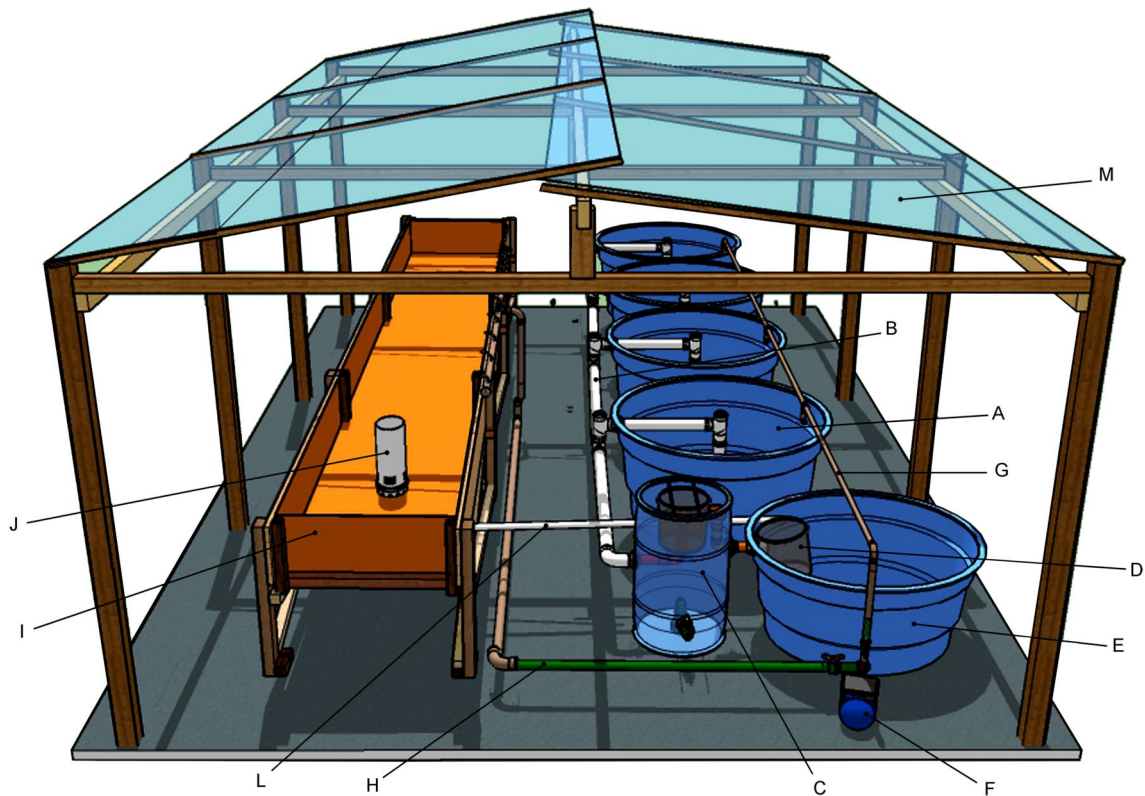


Fig. 1 A layout of the aquaponic system's design construction using artificial semi-dry wetlands (Adapted from Lima and Bastos 2020): fish tanks (A); drain piping (B); decanter filter (C); suspended solids filter (D); aerated biological filter (E); water pump (F); supply pipe of

fish tanks (G); semi-dry wetland supply pipe (H); semi-dry wetland (I); bell siphon (J); semi-dry wetland drainage pipe (L); solar cover (M)

sub-system and biofilter. The substrate of constructed semi-dry wetland was gravel with diameter of 2–3 cm. In this system, two submersible pumps with a flow rate of 5000 L and 1200 L per hour, started by a timer every 15 min, controlled the water intermittent flux in each aquaponics unit to the fish tank artificial semi-dry wetland. The amount water in the semi-dry wetland was 7.2 m³ each day and it was transferred to the sump via gravity by a bell siphon. The fish tank and decanter tank bottom were daily siphoned. The water pH buffering was daily, adding 50 g hydrated lime, as Lima et al. (2019a; b) suggested. The oxygenation network was supported by a radial compressor (3 hp) connected to the fish tank and at the artificial semi-dry wetland by four air stones by m⁻². The fish were acclimatized for 1 week and then subjected to the treatments; feed twice per day was administered (9 a.m. and 4 p.m.), ad libitum.

Nutrients and water quality parameters

Water from each sump and fish tank was sampled at about 10 a.m., about 1 h after feeding twice a week. Total ammonia nitrogen (TAN mg L⁻¹), nitrite nitrogen (NO₂-N mg L⁻¹), nitrate nitrogen (NO₃-N mg L⁻¹), total phosphorous (TP

mg L⁻¹), potassium (K₂O mg L⁻¹), magnesium (Mg²⁺ mg L⁻¹) and calcium (Ca²⁺ mg L⁻¹) were recorded using Multiparameter Photometer for Water Analysis (HANNA™, Model HI83200). The temperature (°C), conductivity (μS cm⁻¹), turbidity (NTU), hydrogen ionic potential (pH), total dissolved solids (TDS, ppm), and dissolved oxygen (DO, mg L⁻¹) were assessed using Multiparameter Water Quality Checker (HORIBA™, Model U-50).

Fingerling farming

Nine hundred and sixty *C. macropomum* fingerlings with an average mass of 8.35 ± 0.91 g were farmed between March 25–July 13, 2018 at three density trials with four replicate: 40 fingerlings or 334 g m⁻³; 80 fingerlings or 668 g m⁻³ and 120 fingerlings or 1002 g m⁻³. The fingerlings were fed using commercial extruded food (GUABI™) with the following composition 36.3% protein, 10.6% ash, 6.8% lipid, 4.6% fiber, and 18.6 kJ g⁻¹ gross energy. The fingerlings at 9 a.m., and 4 p.m. daily were fed at a 3% body mass rate. Two sizes of extruded food were administered to the *C. macropomum* fingerlings, being 2.4 mm were for the first 60 days and 4 mm until ending the culture. The fish



performance was evaluated based on mean final mass (g), food consumption (kg), feed conversion ratio-FCR (feed consumed \times biomass -1), survival rate (%), and fish yield (kg m $^{-3}$).

Plant response to recovered nutrients

Lactuca sativa—the cultivars Delice American lettuce—DAL (Isla) and Purple Crested Lettuce -PCL (Isla) were used. Thirty-two selected lettuces seedlings with 15 days of germination were transplanted to the artificial semi-dry wetland, spaced 0.25 m between profiles and 0.25 m between plants (Lima et al. 2019a). The number of leaves per plant and fresh mass of leaves (g plant $^{-1}$) was used for estimates lettuces growth. The lettuce yield (g m $^{-2}$) was estimated based on shoot mass, considering the adopted plant population (16 plants m $^{-2}$). The two-lettuce culture was gathered 36 days after transplanting, one in April 07-May 12, 2018, and the other in May 13-July 17, 2018.

Statistical analysis

All data were calculated into mean and standard deviation. The *C. macropomum* performance [mean mass (g), total biomass (kg), food consumption, feed conversion ratio, survival (%), and productivity (kg ha $^{-1}$)], growth lettuce, and water parameters data were assessed for normality and homoscedasticity by the Kolmogorov–Smirnov test and Bartlett tests, respectively. The results were expressed as mean value \pm standard deviation. ANOVA was applied, followed by Tukey's test ($P < 0.05$) when normality and homoscedasticity requirements were met. Recovery of nutrients and

water quality data were not assuming a normal distribution and homoscedasticity, that is why mathematical transformations (ln) were performed, and ANOVA was applied, followed by Tukey's test ($P < 0.05$). Growth lettuce data were submitted to two-way ANOVA, followed by the Holm-Sidak method ($P < 0.05$).

Results and discussion

Nutrients and water quality parameters

The temperature during the experiment remained stable and did not differ among the densities. PH values fluctuated in this work; it was slightly alkaline in 40 fingerlings and almost neutral in 80 and 120 fingerling densities. Electrical conductivity, nitrite, nitrate, potassium, and magnesium differed statistically between 40 and 120 fingerling treatments. Total solids and oxygen dissolved decreased with the density increase and lower density concentrations with 80 and 120 fingerlings. Turbidity, TAN, phosphate, alkalinity, and calcium value increased with a density among all treatments (Table 1).

Figure 2A–F details the dynamics of temperature, pH, conductivity, dissolved oxygen, TDS, and turbidity parameters during the study period. The temperature varied within a narrow range between 25 and 30 °C, close to 27 °C in all treatments (Fig. 2A). The water pH remained relatively stable until the 20th day of cultivation, after which this parameter presented acidifying tendencies, which were controlled with the addition of hydrated lime. In Fig. 2B, periods of low and gradual pH levels were standard in all treatments.

Table 1 Physicochemical parameters and primary nutrients recovered from the water of *Colossoma macropomum* fingerling tank, reared at different densities in aquaponics using artificial semi-dry wetlands

Parameters	Unit	Treatments			F	P
		40 fingerlings	80 fingerlings	120 fingerlings		
Temperature	°C	27.16 \pm 0.93 ^a	27.12 \pm 0.94 ^a	27.10 \pm 0.94 ^a	0.0887	0.915
pH		7.48 \pm 0.23 ^a	7.09 \pm 0.26 ^b	7.04 \pm 0.35 ^b	20.787	< 0.001
Conductivity	μ S cm $^{-1}$	694.01 \pm 148.3 ^a	809.40 \pm 130.5 ^{ab}	848 \pm 125.3 ^b	4.866	0.008
Turbidity	NTU	4.35 \pm 1.51 ^a	5.94 \pm 1.46 ^b	8.24 \pm 1.82 ^c	37.553	< 0.001
DO	Mg L $^{-1}$	6.82 \pm 0.58 ^a	6.08 \pm 0.55 ^b	5.91 \pm 0.90 ^b	5.256	0.005
TDS	Ppm	0.51 \pm 0.13 ^a	0.68 \pm 0.14 ^b	0.73 \pm 0.17 ^b	6.228	0.001
TA	mg L $^{-1}$	0.65 \pm 0.41 ^a	1.11 \pm 0.55 ^b	1.89 \pm 0.74 ^c	22.901	< 0.001
NO $_2$ -N	mg L $^{-1}$	0.40 \pm 0.26 ^a	0.58 \pm 0.25 ^{ab}	0.74 \pm 0.23 ^b	10.171	< 0.001
NO $_3$ -N	mg L $^{-1}$	28.04 \pm 14.11 ^a	42.30 \pm 20.96 ^{ab}	50.80 \pm 22.18 ^b	7.370	0.001
PO $_4$ $^{-3}$	mg L $^{-1}$	6.57 \pm 3.53 ^a	12.52 \pm 3.63 ^b	15.87 \pm 6.93 ^c	12.537	< 0.001
K $_2$ O	mg L $^{-1}$	2.75 \pm 2.09 ^a	4.10 \pm 3.03 ^{ab}	5.97 \pm 3.45 ^b	6.441	0.003
Alkalinity	CaCO $_3$ mg L $^{-1}$	14.58 \pm 5.34 ^a	22.19 \pm 6.40 ^b	27.57 \pm 7.38 ^c	21.629	< 0.001
Calcium	mg L $^{-1}$	0.42 \pm 0.22 ^a	0.69 \pm 0.21 ^b	1.01 \pm 0.37 ^c	23.476	< 0.001
Magnesium	mg L $^{-1}$	0.74 \pm 0.22 ^a	0.93 \pm 0.22 ^{ab}	1.16 \pm 0.33 ^b	13.056	< 0.001

Within each column, mean values (\pm standard errors) followed by dissimilar superscript lowercase letters are significantly different



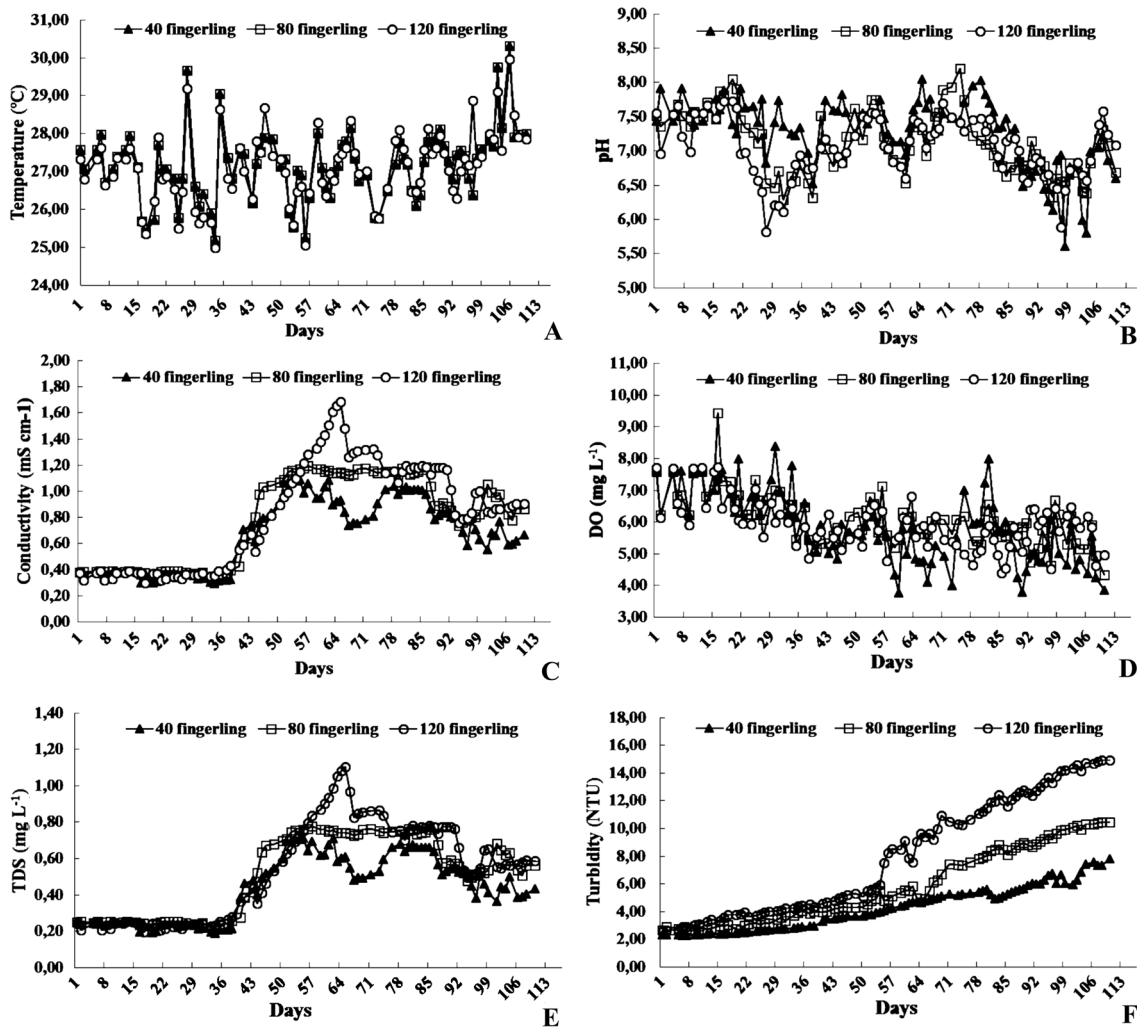


Fig. 2 Detail of dynamics of primary nutrients recovered and water quality parameters from *Colossoma macropomum* farmed in different stocking densities in aquaponics with artificial semi-dry wetlands. **A** Temperature, **B** pH, **C** conductivity, **D** dissolved oxygen, **E** TDS, and **F** turbidity

Conductivity and TDS values were relatively low and stable until the 35th day. They then increased substantially until the 55th day of cultivation, where they started a modest downward trend in all treatments (Fig. 2C, E). Dissolved oxygen values and turbidity showed an antagonistic behavior in all densities. While DO concentrations had an apparent reduction throughout the trial, turbidity values continued to rise, probably reflecting fish growth (Fig. 2D, F).

The dynamics of TAN, nitrite, nitrate, and phosphate parameters are detailed in Fig. 3A–D. TAN values in 40 and 80 fingerlings treatments had a substantial increase until the seventh day of cultivation, followed by a considerable fall. On the other hand, in the 120 fingerlings density, this parameter only showed a reduction around the sixth day of cultivation. Throughout the crop, there was a clear tendency to decrease the ammonia concentrations in all the treatments. Besides, it is possible to find a distinct variation in ammonia concentrations with increased density (Fig. 3A).

Nitrite concentration showed different values between all densities, a similar oscillation pattern (Fig. 3B). Only from the sixth trial day, the instruments detected nitrate, showing increased concentration up to the 41st day, followed by a slight decrease until the 56th day. Nitrate concentrations increased from the 56th to the 72nd day peaked close to 100 mg L^{-1} in the treatments with 80 and 120 fingerlings. Still, in the 40 fingerlings density, they follow a stable oscillation between 16 and 30 mg L^{-1} . Despite differences in concentration, nitrate values in all treatments follow similar oscillation patterns (Fig. 3C). Phosphate concentrations exhibited growing and different values among the treatments throughout this trial, reaching peaks of 13 mg L^{-1} , 22 mg L^{-1} , and 27 mg L^{-1} with 40, 80, and 120 fingerlings, respectively (Fig. 3D).

The potassium values increased to the 91st day of cultivation, and subsequently, it decreased in all treatments, showing a similar oscillation pattern (Fig. 4A). On the other hand,

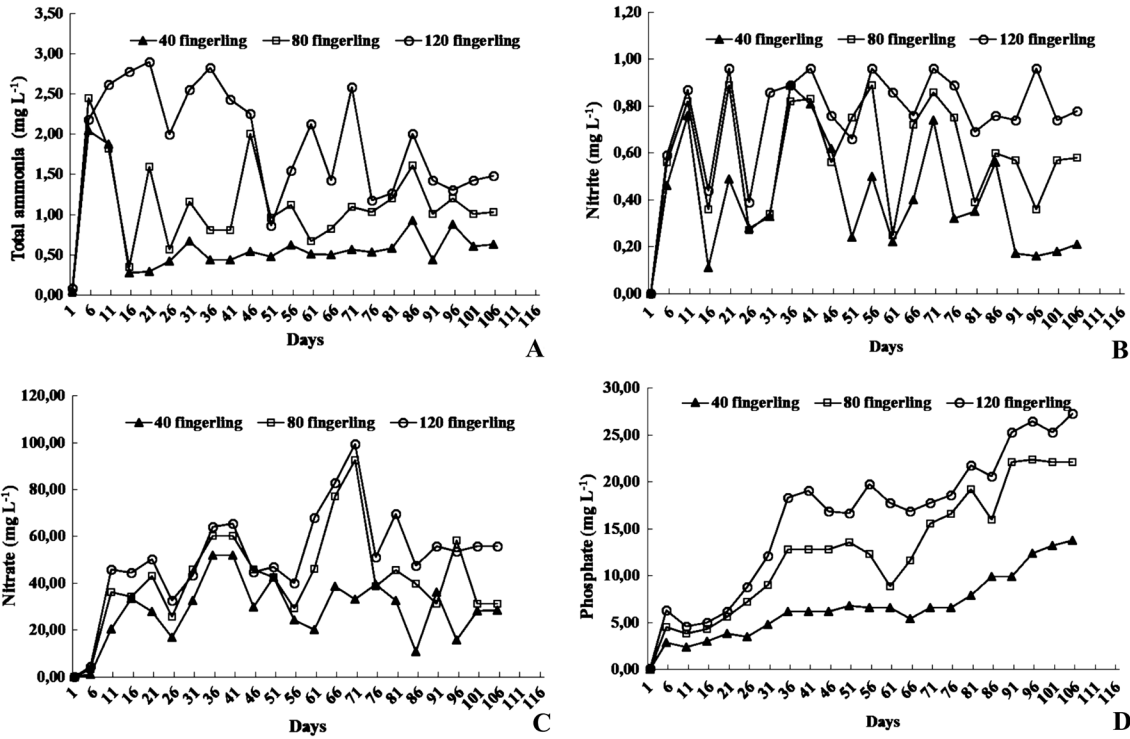


Fig. 3 Detail of dynamics of primary nutrients recovered and water quality parameters from *Colossoma macropomum* farmed in different stocking densities in aquaponics with artificial semi-dry wetlands. A total ammonia, B nitrite, C nitrate, and D phosphate

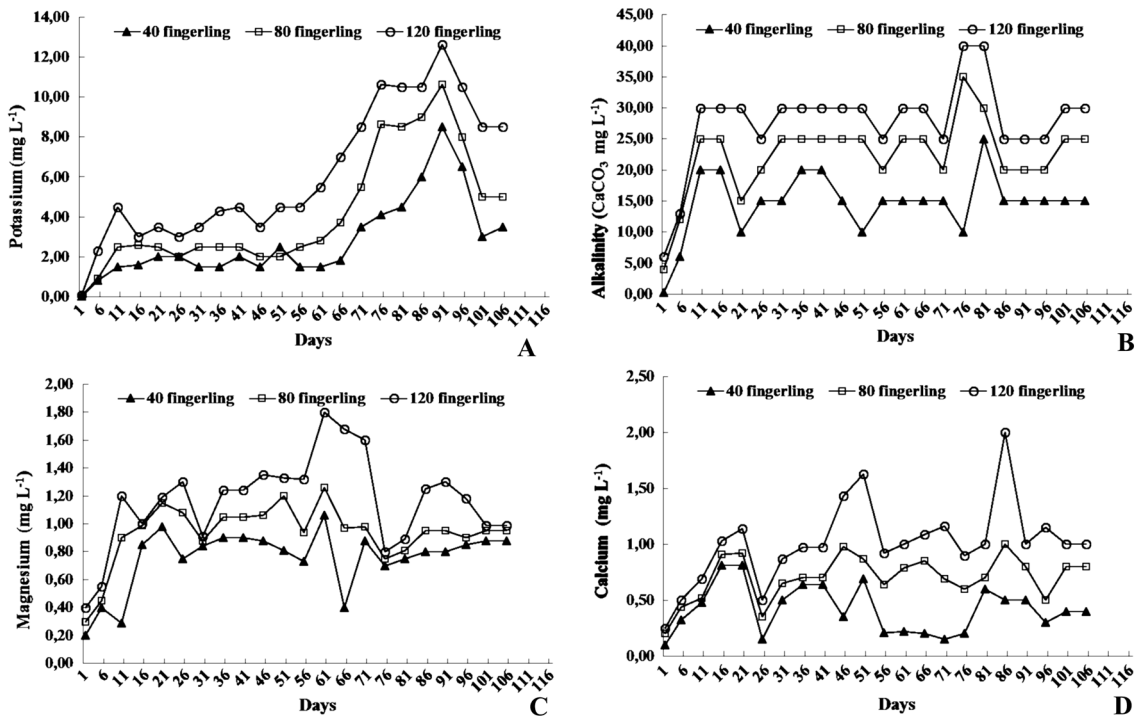


Fig. 4 Detail of dynamics of primary nutrients recovered and water quality parameters from *Colossoma macropomum* farmed in different stocking densities in aquaponics with artificial semi-dry wetlands. A potassium, B alkalinity, C magnesium, and E calcium

the Alkalinity values increased in the densities tried only to the 11th day; subsequently, it showed a similar oscillation pattern (Fig. 4B). Magnesium and calcium concentrations fluctuated strongly throughout the trial but remained close to the intensity recorded in 16 to 21st days of the experiment (Fig. 4C, D).

Fish growth response

The initial fish mass not differed between the densities tried. Density harms the final weight (g) of *C. macropomum* fingerling, reduced with increasing biomass (Table 2). The consumed food was affected by increasing density differing between the treatments. The mean total fish diets were 4.01 kg, 7.53 kg, and 11.10 kg, respectively, in 40, 80, and 120-fingerling treatments. Feed conversion ratio differed significantly between the treatments, being 1.14 ± 0.08 , 1.32 ± 0.01 , and 1.39 ± 0.08 , respectively. The total fish yield also differed between the treatments, showing 3.52, 6.16, and 7.99 kg m^{-3} , respectively, in 40, 80, and 120-fingerling

treatments. However, survival did not significantly differ among treatments (Table 2).

Growth of lettuces

The results suggest that the recovered nutrients from *C. macropomum* farming have the potential for hydroponic lettuce crops. Two-way ANOVA analysis showed that fish stock density has a synergic effect on the recovered nutrients and, consequently, the number of leaves, total fresh mass (g), and lettuce yield (kg m^{-2}). Both lettuce varieties cultivated in water from fish tanks with the density of 120 fingerlings had a higher number of leaves than the lettuce cultivated in the 40 and 80 fingerlings densities (Tables 2, 3) and did not differ in this parameter among themselves. The total fresh mass and yield were higher in the 120 fingerlings density in both lettuce varieties (Tables 3, 4).

Nevertheless, the total fresh mass and yield did not differ among lettuce varieties. In all treatments, the lettuces showed symptoms of precocious flowering. Mealybug and

Table 2 Performance (mean \pm standard deviation) of *Colossoma macropomum* fingerling farmed at different storage densities in an aquaponics system for 110 days using artificial semi-dry wetlands with lettuce

	Treatments			F	P
	40 fingerlings	80 fingerlings	120 fingerlings		
Initial mass (g)	8.85 ± 1.07^a	8.13 ± 0.65^a	8.08 ± 0.98^a	0.187	0.918
Final mass (g)	90.89 ± 5.05^a	80.29 ± 2.16^b	68.59 ± 1.73^c	44.723	<0.001
Food consumers (kg)	4.01 ± 0.01^a	7.53 ± 0.01^b	11.10 ± 0.04^c	34.323	<0.001
FCR	1.14 ± 0.08^a	1.32 ± 0.01^b	1.39 ± 0.08^c	13.065	0.002
Fish yield (kgm^{-3})	3.52 ± 0.23^a	6.16 ± 0.59^b	7.99 ± 0.53^c	24.343	<0.001
Survival (%)	96.88 ± 2.39^a	95.94 ± 2.77^a	97.08 ± 4.33^a	0.139	0.872

Within each column, mean values (\pm standard errors) followed by dissimilar superscript lowercase letters are significantly different

Table 3 Growth response of lettuce varieties cultured between April 07–May 12, 2018 using the recovered nutrients of *Colossoma macropomum* fingerling tank at different densities in an aquaponics system using artificial semi-dry wetlands

Treatments	Variety	Number of leaves plant ⁻¹	Parameters	
			Total fresh mass (g)	Yield (kg m^{-2})
40 fingerlings	DAL	13.14 ± 4.52^{aA}	81.56 ± 32.00^{aA}	1.28 ± 0.46^{aA}
	PCL	15.48 ± 5.10^{aA}	91.28 ± 33.92^{aA}	1.42 ± 0.44^{aA}
80 fingerlings	DAL	17.61 ± 4.17^{bA}	100.58 ± 32.73^{aA}	1.43 ± 0.43^{aA}
	PCL	18.61 ± 3.31^{bA}	88.79 ± 42.92^{aA}	1.5 ± 0.69^{aA}
120 fingerlings	DAL	19.30 ± 3.48^{cA}	112.36 ± 38.32^{bA}	1.80 ± 0.55^{bA}
	PCL	20.20 ± 3.31^{cA}	125.24 ± 49.67^{bA}	2.03 ± 0.49^{bA}
Value of F and P density		F = 10.042, P < 0.001	F = 7.133, P = 0.001	F = 7.133, P = 0.001
Values of F and P lettuce variety		F = 3.295, P = 0.073	F = 1.914, P = 0.170	F = 1.916, P = 0.171
Value de F and P density \times variety		F = 0.214, P = 0.808	F = 0.0461, P = 0.955	F = 0.0463, P = 0.958

SD1 with 40 fingerlings (334 g m^{-3}); SD2 with 80 fingerlings (668 g m^{-3}) and SD3 with 120 fingerlings (1002 g m^{-3})

Values reported are mean \pm SD for 32 lettuce replicates. Capital letters indicate differences between the varieties of lettuce and lowercase letters between fingerling densities

Table 4 Growth response of lettuce varieties reared between May 13–July 17, 2018 using nutrient-rich water of *Colossoma macropomum* fingerling tank at different stocking densities in an aquaponics system using artificial semi-dry wetlands

Treatments	Variety	Number of leaves plant ⁻¹	Parameters	
			Total fresh mass (g)	Yield (g m ⁻²)
40 fingerlings	DAL	14.13 ± 4.62 ^{aA}	80.86 ± 35.00 ^{aA}	1.29 ± 0.56 ^{aA}
	PCL	16.28 ± 5.10 ^{aA}	90.38 ± 33.92 ^{aA}	1.44 ± 0.54 ^{aA}
80 fingerlings	DAL	17.21 ± 4.17 ^{bA}	100.28 ± 30.79 ^{aA}	1.44 ± 0.53 ^{aA}
	PCL	18.21 ± 3.51 ^{bA}	89.99 ± 32.92 ^{aA}	1.6 ± 0.49 ^{aA}
120 fingerlings	DAL	19.50 ± 3.08 ^{cA}	113.26 ± 28.32 ^{bA}	1.85 ± 0.45 ^{bA}
	PCL	20.00 ± 3.61 ^{cA}	126.04 ± 43.67 ^{bA}	2.22 ± 0.69 ^{bA}
Value of F and P density		F = 11.062, P < 0.001	F = 8.143, P = 0.001	F = 7.933, P = 0.001
Values of F and P lettuce variety		F = 3.995, P = 0.083	F = 1.984, P = 0.173	F = 1.996, P = 0.174
Value de F and P density × variety		F = 0.318, P = 0.838	F = 0.0475, P = 0.985	F = 0.472, P = 0.978

SD1 with 40 fingerlings (334 g m⁻³); SD2 with 80 fingerlings (668 g m⁻³) and SD3 with 120 fingerlings (1002 g m⁻³)

Values reported are mean ± SD for 32 lettuce replicates. Capital letters indicate differences between the varieties of lettuce and lowercase letters between fingerling densities

whitefly were observed in about 30% of the plants in the two growing seasons in both lettuce varieties.

Discussion

Nutrients and water quality parameters

Numerous biological and abiotic processes are related to the recovery of nutrients and maintenance of physicochemical water parameters in aquaponics (Shi et al. 2011). These processes occur systematically and continuously throughout the entire system, either by chemical reactions or through the intermediation of bacteria and other microorganisms free in the water or adhered to the surface edges of the growing tanks, pipelines, and biological media. In general, processes occur in increasing flux over time and tend to stabilize according to the support capacity of the aquaponic system. The results demonstrated that artificial semi-dry wetlands were useful in recovering nutrients from *C. macropomum* culture for the lettuces. The control of hazardous organic compounds for *C. macropomum* culture was adequate in all treatments, showing similar elements to reported by Lima et al. (2019a) using similar constructed semi-dry wetland with lettuce (*Lactuca sativa* L.) on treating wastewater of culture of Amazon River shrimp (*M. amazonicum*). Consequently, the treatment system using artificial semi-dry wetlands reduced fish farming effluent emission, that without proper treatment, it will pollution of waters natural that supply aquaculture systems threaten aquaculture itself as well as biodiversity in marginal rivers.

Water dissolved oxygen, temperature, and pH are one of the most critical parameters responsible for optimum aquatic animals' growth, plant growth, and reduction of nitrogen

compounds, indicating the excellent performance of nitrifying bacteria in the system as observed in other studies (Zou et al. 2016; Jordan et al. 2018; Lima et al. 2019a). According to Nuwansi et al. (2020), water quality parameters can be improved in aquaponics systems if the hydraulic loading rate is optimized. In this work, the dissolved oxygen, temperature, and pH were adequate to *C. macropomum* growth rates resembling another intensive system (Sousa et al. 2016; Saint-Paul 2017; Da Costa et al. 2019), which is within the range for vegetal production reported in other aquaponic studies (Sace and Fitzsimmons 2013; Zou et al. 2016; Jordan et al. 2018; Lima et al. 2019a; Nuwansi et al. 2020). Comparing the data with studies on lettuces production in the hydroponics system, was possible observe that the temperature did not remain recommended for this crop. Lettuces cultured in temperatures above 24 °C sometimes result in premature inflorescence initiation, decreasing the uptake and concentration of leaf mineral nutrients (Sublett et al. 2018). It means that water temperature was a limiting factor for lettuce varieties tried the same that it had resistance to precocious flowering symptoms.

According to Genuncio et al. (2012), the increase in the flow rate is a factor that might favor lower temperatures and higher DO concentrations in nutritive solution due to less exposure to the solution during the time of increased incidence of solar radiation. Lima et al. (2019a) suggested the simultaneous use of a timer control and a bell siphon as essential to keep a reasonable interval of the flow of water between the fish tank and semi-dry wetland and as a strategy of reducing the temperature. In this work, this strategy was successful and allowing the temperature to be kept close to 27 °C, thus below the average values obtained by Lima et al. (2019a). Moreover, temperature, DO, pH, nitrogen compounds, and alkalinity mean values recorded in this study



corroborated with typical values recorded in aquaponics systems (Zou et al. 2016; Fang et al. 2017; Pinho et al. 2017; Jordan et al. 2018).

The *C. macropomum* is not exceptionally tolerant to high environmental ammonia, in contrast to their extreme tolerance to hypoxia, ion-poor water, and low pH (Wood et al. 2017), indicating that these species have good potential for aquaponics and provided that ammonia and other nitrogenous compounds are kept at an acceptable level as observed in present study.

Unlike traditional hydroponics, composition control of nutrients in aquaponics is complicated because of many of the metabolic processes performed by different aquatic organisms' (Lima et al. 2019a). The results suggest that the pH value influenced the interaction between the dissolved ions represented by electrical conductivity and total dissolved solids. However, the pH effects were not evident concerning the nitrogen compounds (Ammonia, Nitrite, and Nitrate) and alkalinity, similar to the effect observed by Lima et al. (2019a; b). On the other hand, pH reduction caused phosphate increase, especially in the treatment with 80 and 120 fingerlings. Cerozi and Fitzsimmons (2016) recommended in aquaponics systems pH at a 5.5–7.2 range for optimal phosphate availability and uptake by plants. Phosphate and nitrogen compounds concentration in this work is more related to the addition of feed in the *C. macropomum* culture tanks than to pH.

Increases in density contribute to increasing nitrogen compounds in the aquaponic system using semi-dry wetland, especially nitrate (NO_3^-) (Lima et al. 2019a). This fact is corroborated in this work. However, the total ammonia and nitrite concentration data were below the suggested values for the *C. macropomum* cultivation in an intensive system (Santos et al. 2014; Silva and Fujimoto 2015; Da Costa et al. 2019; Lima et al. 2019b), indicating that water treatment with semi-dry wetland was efficient.

Nitrate concentration was satisfactory to lettuce production at all density treatments. The maintenance of ammonia and nitrite concentrations at relatively low levels and the increase in nitrate concentrations could indicate a well-developed microbial community. That semi-dry wetland with gravel media is bedded adequate for an aquaponics system (Lima et al. 2019a). However, numerous works recommend alternative beds with agricultural by-products used as media to develop the microbial community in an aquaponics system, besides the possibility of converting waste to wealth (Boxman et al. 2016; Oladimeji et al. 2020).

Phosphorus is a crucial nutrient in the metabolism of plants and animals. In plants, phosphorus plays a vital role in cell energy transfer, respiration, and photosynthesis. In contrast, in animals, this element is a structural component of nucleic acids of genes and chromosomes, as well as of many coenzymes, phosphoproteins, and phospholipids

(Cerozi and Fitzsimmons, 2017). The phosphorus concentrations in water that limit Tambaqui's development are still unknown. However, there are indicators in the literature for other species that can be a reference point. Tilapia, for example, can easily withstand concentrations of up to 400 mg L^{-1} , with 40 mg L^{-1} being the safe concentration for the species (Cagol et al. 2016). Like this, the concentrations of phosphate recorded in this work are adequate for the development of *C. macropomum* and compatible with values indicated for aquaponics systems (Cerozi and Fitzsimmons 2017).

TDS values recorded in this work were within the appropriate range for the *C. macropomum* biology and standard requirements of the lettuce with excellent TDS performance among 560 and 840 ppm in other aquaponics systems (Sace and Fitzsimmons 2013). The TDS values obtained in this study are higher than those observed by Lima et al. (2019a) for shrimps, probably due to the higher amount of waste generated by *C. macropomum*.

Potassium in plants also plays an essential role in various metabolic reactions, including the accumulation of nutrients such as iron and chlorophyll in the shoot and the synthesis of hormones that aid in vegetative development (Roosta 2014). In aquatic organisms, potassium has similar importance in cultured water because it is related to osmoregulation mechanisms and muscle activities (Al-Saadi 2017). To date, there are no recommendations for the potassium concentration in the aquaponics system. Regardless of the density, the potassium and phosphate concentrations recorded in the present study are below the previous studies' values (Roosta 2014; Nozzi et al. 2018). Regarding *C. macropomum* cultivation, there are no limiting recommendations for potassium concentrations in the literature. However, it is worth noting that very high or deficient potassium concentrations may interfere with osmoregulation processes (Al-Saadi 2017).

The alkalinity observed in this work was not adequate for *C. macropomum* culture in any of the densities, being below the reported values for this same species in other intensive systems (Silva et al. 2013; Santos et al. 2014; Silva and Fujimoto 2015; Sousa et al. 2016; Lima et al. 2019b). In aquaponics, the alkalinity of water is critical due to its direct effect on solution pH and its impact on plant growth and quality, including interveinal chlorosis in the youngest leaves and stunted growth (Roosta 2011). These effects were not observed in this study. High alkalinity reduces Fe's solubility due to the high pH associated with the consumption of H^+ by HCO_3^- (Roosta 2011). The values of alkalinity found in this work were higher than observed by Lima et al. (2019a), studying an aquaponics system with semi-dry wetlands and similar to reported by Da Rocha et al. (2017) studying lettuce production in aquaponic and biofloc systems, indicating that the alkalinity was satisfactory for lettuce culture in all treatments.



Calcium and magnesium are key nutrients for plant physiology and aquatic animals. These elements can negatively affect the metabolism and, therefore, both productivity performance in very low or very high concentrations. The results indicate that the calcium and magnesium concentrations were deficient, even after the addition of hydrated lime, not being compatible with the real needs of lettuce and fish, being lower than values reported in the literature (Jordan et al. 2018; Nozzi et al. 2018; Oladimeji et al. 2020).

Growth of lettuces

Constraints to the uses of aquaculture farming water for lettuce production have been related to the temperatures above 25 °C, low macro- and micronutrient concentration, low dissolved oxygen, and high-suspended solids values. (Sikawa and Yakupitiyage 2010; Sace and Fitzsimmons 2013). The nutrient solution flow is a relevant variable at the nutrient availability and water retention in a hydroponic system (Genuncio et al. 2012; Guimarães et al. 2016). The nutrient solution flow done by submersible pumps used in this work probably provided a rate of the nutrient solution flow and dissolved oxygen satisfactory for lettuces production. However, this vegetal has not reached its productive potential if compared to hydroponics systems reported in the literature (Guimarães et al. 2016), indicating that temperature, among other factors, may have affected its performance.

Although we didn't use any substrate, the leaves number, total fresh mass, and yield of the lettuce varieties cultured in water with 120 fingerlings were relatively similar to the values reported by Jordan et al. (2018) in aquaponic and hydroponic systems using phenolic foam. However, compared to lettuces cultured in a substrate composed of coconut shell fiber, the parameters leaves number, total fresh mass, and yield were adequate. Considering that the concentrations of recovered nutrients were also like that reported by Jordan et al. (2018), it can be inferred that the presence and type of substrate has a high impact on plant yield and water quality. This theory is supported by a recent study by Oladimeji et al. (2020), suggesting that different growth media could affect the nutrient uptake and diminish or increase the plants' growth efficiencies in an aquaponics system. Aerated biofilters with different media could affect the concentrations of recovered nutrients in aquaponics (Zhang et al. 2020).

Interactions between fish stock density and leaf number, total fresh mass, and yield were observed in this trial. Both lettuce varieties responded positively to the increase in biomass and the increased availability of nutrients. Although no additional nutrient was included in this trial, except hydrated lime for pH regulation, the lettuce biomass obtained was near the values reported by Guimarães et al. (2016), who grew lettuce plants hydroponically using saline wastewater from fish farming, supplemented with a fertilizer solution.

The results support the potential use of *C. macropomum* tank water for supplying a family lettuce production. However, for commercial lettuce production, the use of substrate and additional nutrients is necessary. Another point to be evaluated is the use of lettuce varieties that are more resistant to high temperatures and adapted to the Amazon climate. Both lettuce varieties tested in this trial shown precocious flowering symptoms, probably due to high temperatures. Besides, pests' occurrence may have favored lettuces' poor performance compared to results obtained in aquaponics by Jordan et al. (2018).

Fish growth response

The stocking density management is vital to the success of *C. macropomum* production, affecting numerous zootechnical parameters as feeding, survival, growth, behavior, health, and could negatively influence final yield (Santos et al. 2014; Silva and Fujimoto 2015; Da Costa et al. 2019). The present results corroborate this theory. The effects recorded in this trial include mass growth retardation, FCR, and consumed food increased. It is suggesting that *C. macropomum* farmed in small tanks can be sensible to the intraspecific competition. Nevertheless, surprisingly, survival was not negatively influenced, and productivity has increased with increasing stocking density.

Although the zootechnical performance in this trial has been negatively affected by increasing stocking density, these parameters were compatible, and even superior, to those recorded in the literature for other intensive systems of *C. macropomum* production (Silva et al. 2013; Santos et al. 2014; Silva and Fujimoto 2015; Sousa et al. 2016). The results suggest that Tambaqui juveniles with 8.08 ± 0.98 g can be cultured in aquaponic systems with high survival and productivity using densities in 120-fingerlings m^{-3} .

The *C. macropomum* performance in this work was similar to observed for its hybrid tambacu (*Piaractus mesopotamicus* X *Colossoma macropomum*) (Santos et al. 2017; Verly et al. 2017) and pacu *P. mesopotamicus* (Pinho et al. 2017), especially for growth, fish yield, and survival parameters. This trial's fish yield suggests that *C. macropomum* is a suitable species for the aquaponics system. 80 and 120 fingerlings densities are most productive. It is recommended for the rearing of this species at a cycle of up to 110 days.

Conclusion

The aquaponic system using artificial semi-dry wetlands effectively recovered nutrients for lettuces, and water treatment at the densities tested, removing critical pollutants from *C. macropomum* farming. It can be applied to other fish species of economic interest.



Colossoma macropomum showed an inverse relationship between stocking density and growth, consumed food, feed conversion ratio, suggesting that high stocking densities could negatively influence the final yield of this fish. But survival was not affected, contrary to the hypothesis of higher survival in low density.

Water nutrient concentration, the density of fish, and lettuce production showed a strong association. Except for calcium and magnesium, water nutrient from 80 and 120 fingerlings tanks was satisfactory to lettuces nutrition; even so, these varieties showed low yield compared to traditional hydroponics.

No relationship was observed between stocking density and lettuce variety.

The temperature probably was the cause of precocious flowering observed in both varieties of lettuce. The substantial incidence of Prague agricultural as mealybug and whitefly may have compromised the full lettuce culture and not just the availability of nutrients.

The fingerling farming effluent may be managed in-home family lettuce production without soil—the nutrients like calcium and magnesium for commercial-scale in this system. Lettuce varieties were more resistant to precocious flowering symptoms by high temperatures, and pests' control can better this plant's performance in this aquaponics system.

Acknowledgements The authors are grateful for all the support. National Council for Scientific and Technological Development—CNPq (Project No. 444367/2014-4) and Brazilian Agricultural Research Corporation, code SEG no 03.13.09.011.00.00 supported the present study.

Authors' contributions All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by J de FL, AMB, SSD, and URA dos S. J de FLI wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials The authors are willing to share the raw data of the referred manuscript with other interested scientists.

Declarations

Ethics approval and consent to participate All authors have consent and authorization to participate in the construction and publication of scientific information presented in this manuscript. This work was developed following the ethical principles recommended by the Brazilian College of Animal Experimentation (COBEA) and with the approbation from the Ethics Committee in the Use of Animals of Embrapa Amapá (# 008—CEUA/CPAF-AP). The biological accessions presented in this work were duly registered in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) under the code A0D6DC0.

Consent for publication The authors' consent to the publication of the complete scientific information presented in this manuscript. We are assuming full responsibility for the accuracy of the data and results.

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