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Short Communication

Effect of stocking density on growth performance and oxygen consumption of Nile tilapia (*Oreochromis niloticus*) under greenhouse conditions

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ABSTRACT. The effects of stocking density on oxygen consumption and growth in Nile tilapia (*Oreochromis niloticus*) under greenhouse conditions were investigated in this experiment. Fingerlings with 1.57 ± 0.14 g mean body weight were stocked in 972 L, rectangular plastic tanks placed inside a polyethylene greenhouse 504 m^2, at three densities 90 (T1), 180 (T2), and 270 (T3) ind m^{-3} (biomass of 0.14, 0.28, and 0.42 kg m^{-3}). Fish were feeding with a commercial diet for Nile tilapia (Api-Tilapia 1, MaltaCleyton® with 50% protein and 12% lipid) to apparent satiation, three times a day, for 60 days. Fish in each treatment were selected randomly in order to measure aerobic metabolism using closed respirometric chamber technique with constant temperature and volume (1 L). It was found that growth rate of T1 (0.38 g day^{-1}; 4.65% day^{-1}) and T2 (0.21 g day^{-1}; 3.68% day^{-1}) was significantly higher than T3 (0.11 g day^{-1}; 2.67% day^{-1}). Oxygen consumption measurements over 24 h showed that a significant difference exist between treatments T1 = 350 ± 170, T2 = 260 ± 170, and T3 = 200 ± 130 (mgO_2 kg^{-1} h^{-1}). Thus, according to the results the culture of tilapia at a density of 90 ind m^{-3} can be used for increase production of *O. niloticus* under greenhouse conditions.

Keywords: *Oreochromis niloticus*, density, growth, oxygen consumption, aquaculture.

Efecto de la densidad de siembra sobre el crecimiento y el consumo de oxígeno de la tilapia del Nilo (*Oreochromis niloticus*) bajo condiciones de invernadero

RESUMEN. El efecto de la densidad y el consumo de oxígeno sobre el crecimiento de la tilapia (*Oreochromis niloticus*) fueron analizados. Alevines con un peso húmedo promedio de 1,57 ± 0,14 g se colocaron en estanques rectangulares de plástico de 972 L, dentro de un invernadero de polietileno con un área de 504 m^2, las densidades utilizadas fueron 90 (T1), 180 (T2), and 270 (T3 ind m^{-3} (con una biomasa promedio total por tanque de 0,14, 0,28 y 0,42 kg m^{-3}). Para la alimentación se utilizó alimento comercial (Api-Tilapia 1, MaltaCleyton® con 50% proteína y 12% de lípidos), que se suministró hasta la aparente saciedad tres veces al día durante un periodo de 60 días. Al finalizar este periodo se seleccionaron aleatoriamente individuos de cada tratamiento para determinar el metabolismo aerobio, que se determinó por el método de cámaras semicerradas mantenidas a temperatura constante y volumen conocido (1 L). Se determinó que la tasa de crecimiento de T1 (0,38 g $día^{-1}$; 4,65% $día^{-1}$) y T2 (0,21 g $día^{-1}$; 3,68% $día^{-1}$) fue significativamente más alta que T3 (0,11 g d^{-1}; 2,67% $día^{-1}$). El consumo de oxígeno en el ciclo de 24 h mostró diferencias significativas entre los tratamientos T1 = 350 ± 170, T2 = 260 ± 170, and T3 = 200 ± 130 (mgO_2 kg^{-1} h^{-1}). De acuerdo con los resultados obtenidos, el cultivo de tilapia bajo condiciones de invernadero a una densidad de 90 ind m^{-3} puede ser utilizado para incrementar la productividad de *O. niloticus*.

Palabras clave: *Oreochromis niloticus*, densidad, crecimiento, consumo de oxígeno, acuicultura.

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Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) occupies an important place in aquaculture and is the main cultured fish in Mexico. Traditionally, its cultivation has been made in semi-intensive and extensive systems (Fitzsimmons, 2000). Currently, some producers have adopted intensive culture in tanks under green-house conditions, using recirculation systems (Soto-Zarazúa *et al.*, 2010, 2011). According to Edward & Domaine (1997) failures in small-scale farmer endeavors are due to inadequate knowledge, such as stocking fry at a very small size and at too high density. Optimal stocking densities have been established using growth performance parameters such as weight gain or survival rate (El-Sherif & El-Feky, 2009a, 2009b; Gullian-Klanian & Arámburu-Adame, 2013). However, this procedure leaves apart important variables such as oxygen consumption which gives an indirect measure of metabolism and physiological condition (Beamish, 1970; Cech, 1990). Oxygen consumption is an indirect measure of metabolism, rather than direct measures of heat released through metabolism calorimetry (Steffensen, 1989; Mamun *et al.*, 2012) which has been widely studied on Nile tilapia (De Silva *et al.*, 1986; Iwama *et al.*, 1997; Kumar *et al.*, 2011). Thus, the purpose of this study was to determine the optimal stocking density on fingerlings of tilapia under greenhouse conditions.

The experiment was performed in an aquaculture system producing Nile tilapia inside a polyethylene greenhouse 504 m^2 in area (18×28 m) in Amazcala, Queretaro State, Mexico. The fish were placed in nine rectangular plastic tanks distributed using a Latin square scheme in order to avoid spatial effects. The tank´s dimensions were of 0.9 m depth, 0.9 m wide and 1.2 m long, with a water storage capacity of 972 L. Three triplicated treatments for each experiment were applied using a fish stocking density of T1 = 90, T2 = 180, and T3 = 270 ind m^{-3}, respectively. The handling of tanks involved the feces removal and weekly partial water changes (30%).

Measures of temperature (ºC), dissolved oxygen (mg L^{-1}), pH, Secchi disk (cm), nitrite (NO_2) and nitrate (NO_3) were recorded weakly throughout the experimental period. The temperature and dissolved oxygen were measured with HQ40D multi dual-input meter, brand HACH, USA, with LDO101-03 probe sensor; and the pH was monitored using the water proof pH tester 10, Brand EUTECH, USA Instruments. Visibility was measured using a common Secchi disk; the nitrite and nitrate were measured with a DR/6000 spectrophotometer (HACH) utilizing the 8153, 8192 Hatch Methods. The water temperature was measurement in tanks while environmental temperature and relativity humidity was recorded with a data logger (WatchDog series 1000, USA) each hour during a whole experiment time. The fish were feed with a commercial diet for Nile tilapia (Api-Tilapia 1, Malta Cleyton® with 50% protein, 12% lipid, 13% ash, 3% fiber, 12% moisture) throughout the experiment. Feeding frequency was adjusted to three portions offered three times daily starting at 08:00, 13:00 and 18:00 h. Meristic data, humid weight (HW) and standard length (SL) were measured with a digital caliper (Truper Stainless steel), this measures were acquired only at the beginning (1 day), and at the end (60 days). After 60 days, all fish of each tank were taken, counted, and weighed with an analytical balance (Sartorius AY303 Milligram Scale). The following parameters were used to evaluate tilapia growth performance according to Kumar *et al*. (1995): body weight gain (*WG*) = 100 (*W*1-*W*0); mean daily body weight gain (ADG) = $(W_1–W_0)/t$; specific growth rate (%/day) (SGR) = $((\ln W_1 - \ln W_0) \times 100)/t)$; survival rate (%) (SR) = $Ni \times 100/N_0$. Where: W_1 = final wet weight, W_0 = Initial wet weight, t = time interval in days, Ni = number of fishes at the end, N_0 = number of fishes initially stocked.

Four individuals from each treatment were randomly selected in order to measure aerobic metabolism (oxygen consumption = QO_2) using closed respirometric chamber technique with constant temperature and a known water volume (Timmons *et al.*, 2002, Soto-Zarazúa *et al.*, 2010). Measurements of dissolved oxygen and temperature were taken every four hours (14:00, 18:00, 22:00, 02:00, 06:00, and 10:00 h) during a 24 h cycle. All the fish used to determine QO_2 were euthanized on ice immediately after finishing the experiment in order to determine its dry weight and to get the relation of the oxygen consumed by biomass (Steffensen, 1989).

Weight, standard length, and performance growth data were analyzed with one-way ANOVA in order to determine the differences between densities for each treatment and least square differences (LSD) this test was applied when significant differences were found. For average standard length and average standard weight data, the coefficient of variability (CV) was calculated as CV = (SD x 100)/mean, in order to compare initial and final data. To determine the effect of density on oxygen consumption a multivariate analysis were used (Statgraphics routine centurion XV, ver. 15.2.06), environmental variables were also considered in order to determine which factor explains variations best.

Water temperature inside the tanks did not show a wide variation as the environmental temperature did, showing an average of 18.50 ± 11.92°C, while the temperature in treatments tanks had an average of 24.68

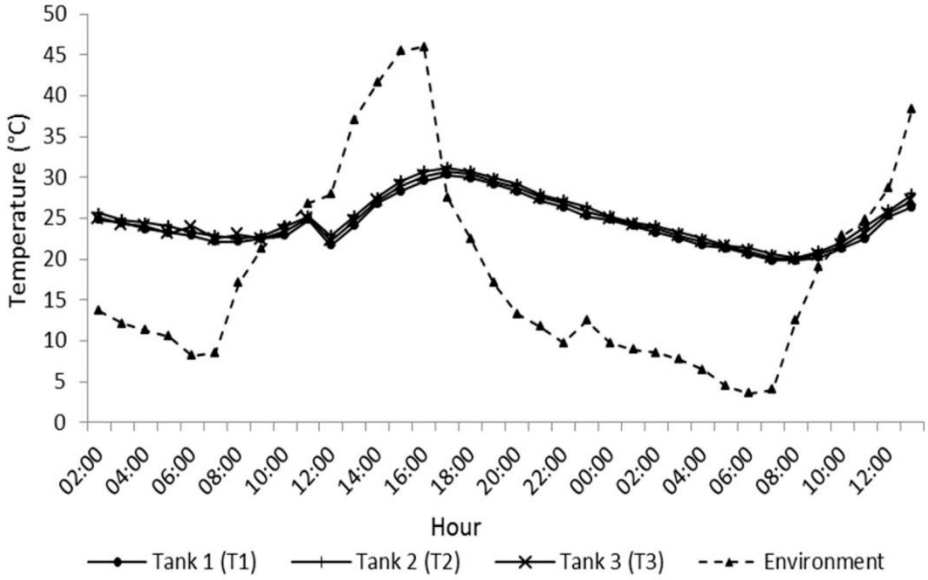

Figure 1. Inside greenhouse temperature cycle (24 h). Treatments (continuous line) and environment (dotted line).

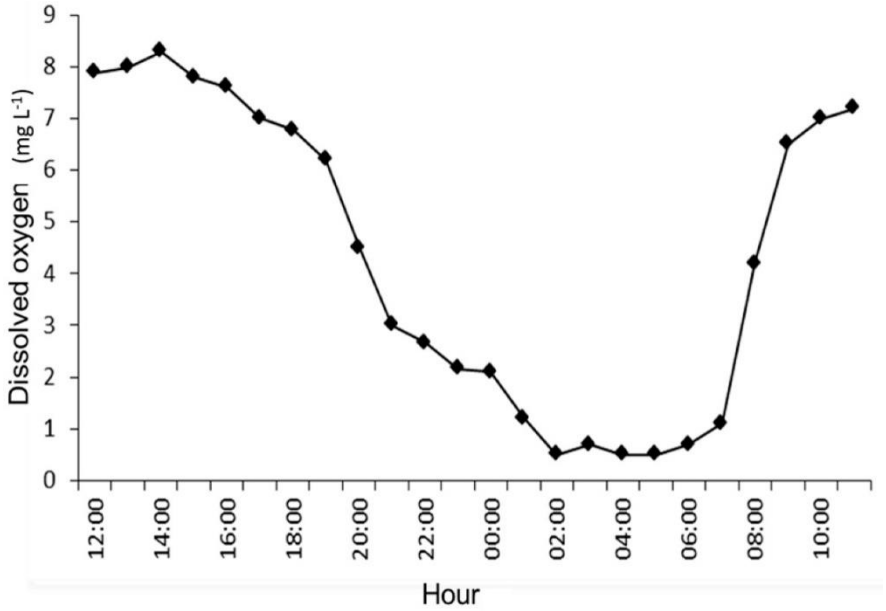

Figure 2. Dissolved oxygen inside the tanks during a 24 h cycle.

± 3.0° C (Fig. 1). Dissolved oxygen in water showed a wide variation during a 24 h cycle (Fig. 2). The minimum concentration was observed at 04:00 h (0.5 mg L^{-1}), and the maximum concentration was registered at 14:00 h (8.3 mg L^{-1}). Physical and chemical parameters measured inside the treatment water during the whole experiment, every week at 12:00 h, are shown in Table 1. All parameters of the water quality showed acceptable values for aquaculture activity (Barker, 2002; Timmons *et al.*, 2002). The visibility in water tanks showed a decrease along time, specifically in high density (T3).

The results of biological measurements showed significant differences ($P < 0.05$) between density treatments 90, 180 and 270 ind m^{-3}, especially in some growth performance parameters. Survival rate (%) showed high value in T2 and minimal value in T3 contrary to weight gain which shows maximum value for T1 and minimal value for T3 (Table 2).

There is no significant difference between initials mean standard lengths values in all treatments, but there is a significant difference ($P < 0.05$) between the finals mean standard lengths (Table 2). T1 is the maximum length and the minimum T3, this behavior is the same

Table 1. Weekly values of physical and chemical parameters in treatment water throughout whole experiment. Minimum (Min), maximum (Max), and average values are shown at the last column for each one of the parameters. T1 = 90 ind m^{-3}, T2 = 180 ind m^{-3}, T3 = 270 ind m^{-3}.

	1	2	3	4	5	6	7	8	Average	Min	Max
Dissolved oxygen (O_2 mg L^{-1})											
T1	7.50	7.20	7.00	6.90	6.80	5.20	5.00	5.50	6.39	5.00	7.50
T2	7.60	7.10	6.80	6.60	6.40	5.30	5.20	5.10	6.26	5.10	7.60
T3	7.50	7.00	6.80	6.50	6.60	5.40	4.30	4.60	6.09	4.30	7.50
Temperature (°C)											
T1	29.6	28.4	30.0	26.0	28.0	29.0	31.0	32.3	29.2	26.0	32.3
T2	30.5	29.4	32.0	28.0	29.0	29.0	32.0	31.4	30.1	28.0	32.0
T3	30.0	30.2	31.0	28.0	28.0	28.5	31.5	31.3	29.8	28.0	31.5
Potential hydrogen (pH)											
T1	8.5	8.8	9.3	9	8.6	8.8	8.7	8.8	8.8	8.5	9.3
T2	8.4	8.8	9.5	9.5	8.8	9	8.8	9.1	8.9	8.4	9.5
T3	8.5	8.9	9.8	9.4	8.9	9.1	9.1	9.1	9.1	8.5	9.8
Nitrate (NO_3^- mg L^{-1})											
T1	1.3	0.11	2.3	2.2	2.4	2.9	3.6	3.7	2.31	0.11	3.70
T2	1.4	0.15	2.5	2.5	3.6	3.1	4.7	4.6	2.82	0.15	4.70
T3	1.3	0.15	2.5	2.3	3.8	3.8	3.65	5.8	2.91	0.15	5.80
Nitrite (NO_2^- mg L^{-1})											
T1	0.03	0.11	0.11	0.12	0.15	0.16	0.14	0.13	0.12	0.03	0.16
T2	0.03	0.2	0.13	0.15	0.15	0.85	0.8	0.9	0.40	0.03	0.90
T3	0.03	0.22	0.15	0.18	0.18	1.16	1.18	1.16	0.53	0.03	1.18
Ammonia (NH_4^+ mg L^{-1})											
T1	0.05	0.11	0.06	0.50	0.70	0.80	0.70	0.75	0.46	0.05	0.80
T2	0.05	0.20	0.20	0.70	0.80	0.70	0.75	0.80	0.53	0.05	0.80
T3	0.05	0.22	0.30	0.90	0.00	0.85	0.80	0.90	0.50	0.00	0.90
Secchi disk (cm)											
T1	90.00	65.00	75.00	50.00	45.00	35.00	30.00	28.00	52.25	28.00	90.00
T2	90.00	50.00	35.00	25.00	20.00	18.00	18.00	18.00	34.25	18.00	90.00
T3	90.00	44.00	15.00	8.00	5.00	5.00	5.00	5.00	22.13	5.00	90.00

Table 2. Growth performance parameters for treatments of stocking density during 60 days (mean ± SD). Mean values for each treatment followed by different superscript letters differ significantly ($P < 0.05$). For final average number (n) data were rounded.

Performance parameters	T1	T2	T3
Initial number (n)	90	180	270
Final mean number (n)	82.6 ± 1.15	166 ± 8	236 ± 8
Initial total weight (g)	135.0 ± 18.0	282.4 ± 17.6	447.9 ± 34.6
Final total weight (g)	2017.09 ± 71.3	2363.8 ± 76.1	1954.6 ± 35.1
Initial individual mean weight (g)	1.50 ± 0.20^a	1.57 ± 0.10^a	1.66 ± 0.13^a
Final individual mean weight (g)	24.41 ± 1.08^a	14.25 ± 1.10^b	8.24 ± 0.72^c
Initial individual mean length (mm)	34.52 ± 1.94^a	33.93 ± 1.40^a	35.62 ± 1.38^a
Final individual mean length (mm)	82.22 ± 3.0^a	66.68 ± 5.04^b	51.88 ± 3.65^c
Length gain (mm)	47.69 ± 4.88^a	32.75 ± 6.34^b	16.26 ± 2.37^c
Length gain (%)	138.94 ± 22.05^a	97.12 ± 22.87^b	45.55 ± 5.24^c
Weight gain (g)	1882.09 ± 55.94^a	2081.48 ± 87.47^a	1507.1 ± 225.48^b
Weight gain (%)	1408.15 ± 161.89^a	740 ± 74.04^b	337.78 ± 50.87^c
Specific growth rate (SGR; % day^{-1})	4.64 ± 0.15^a	3.67 ± 0.2^b	2.67 ± 0.14^c
Survival rate (%)	91.11 ± 1.28^a	92.40 ± 4.32^a	87.65 ± 2.83^a

for the mean humid weights (Table 2). Total mortality for each treatment at the end of the experiment is shown in Table 2. T1 has a 52.5 ± 1.28%; T2 had minimal mean value between treatments 7.52 ± 4.31% and T3 with 12.53 ± 5.12, the analysis showed a significant difference ($P < 0.05$) between T1 and the other treatments.

The determination of oxygen consumption during a 24 h cycle showed a similar behavior for the three treatments (Fig. 3). There is a consumption peak at 02:00 h on the three treatments (T1 = 460 ± 110, T2 = 650 ± 179 and T3 = 600 ± 226 QO_2 kg^{-1} h^{-1}). Rate of daily oxygen consumption was obtained for each treatment (T1 = 8470 ± 170; T2 = 6430 ± 170, and T3 = 4990 ± 130 mg kg^{-1} day^{-1}) and significant differences were founded ($P = 0.008$).

According to the multivariate analysis, the results variations were mainly due to dissolved oxygen (44.4%) and temperature (17.3%), while pH, nitrate, nitrite, visibility and ammonia explain the 38.3%. The main purpose of a greenhouse is to exclude environmental factors, as shown in Figure 1 this is achieved in the present study. While environmental temperature variation was between 5 and 40°C, temperature inside the tanks showed a variation between 20 and 30°C, which is a recommended for Nile tilapia culture (Timmons *et al.*, 2002). Cultivation under greenhouse diminishes the effect of this factor and is a helpful tool for water temperature control. Soto-Zarazúa *et al.* (2011) mentions, the tank position and temperature from the environment and soil inside a greenhouse exert an important influence on the water. However, in this experimental design no significant differences were found for temperature, perhaps for the small space needed for tanks.

As can be seen in Figure 2, dissolved oxygen inside the tanks shows an irregular behavior during a 24 h cycle, minimal values were found during night, between 20:00 and 08:00 h and recommended values were found during the day 08:00 to 20:00 h. Maximum values for oxygen consumed (QO_2) were found during night (02:00 h) with the same behavior for the three treatments; T1 shows minimal consumption (460 mg kg^{-1} h^{-1}) at this time, while treatments T2 and T3 show higher values (650 and 600 mg kg^{-1} h^{-1} respectively). Studies on tilapia metabolism shows a contrary results to the results obtained in this experiment. According to Ross & McKinney (1988) during the light period oxygen consumption is higher than dark period. However, under hypoxic conditions tilapia acquires the oxygen gasping at the water surface, which leads to spend extra energy. Also this behavior could be supported with the results of Mishrigi & Kubo (1978) in an experiment where the main objective was to test the effect of territoriality. According to this author, intraspecific competition modify fish activity and oxygen consumption, so it can be said that increasing density affects metabolism and the recommendation would be to keep low densities. However, mortality was higher in lower density T1 (81.1%) which also could be associated to behavioral conduct found in territorial fish, according to this idea fish have the chance to grow faster in low densities, so biggest fish can chose better conditions inside the tanks.

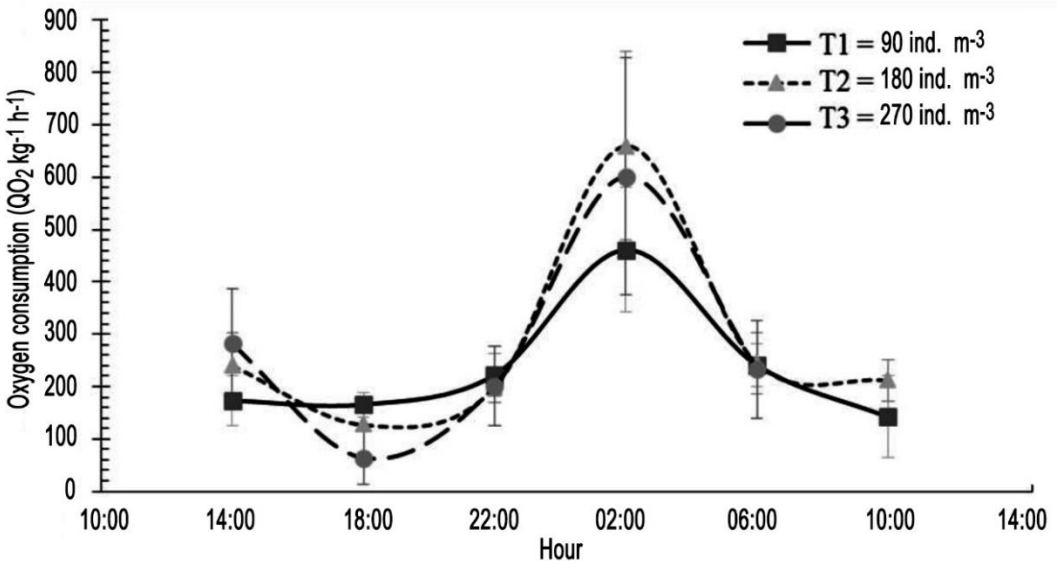

Figure 3. Oxygen consumption (mg kg^{-1} h^{-1}) for each treatment during a 24 h cycle, whisker shows standard error (SE).

Results for final individual mean weight (g), and final individual mean length (mm), clearly show that low density treatments (T1) have maximum value at the end of experiment while high densities (T3) have minimal values (Table 2). Similar results are founded in El-Sayed (2002), experiment in which he worked with larvae of Nile tilapia, *O. niloticus*, at stocking densities of 3, 5, 10, 15, and 20 ind L^{-1} for 30 days and stocked in 20 L fiberglass tanks, in a closed recirculating indoor system. The conclusion was that the optimum density for *O. niloticus* under these conditions was around five ind L^{-1} with a 100% of survival rate. This result could be extrapolated in order to have a recommended density of 5000 ind m^{-3}; however it is only for 30 days. Several investigations mention different results for stocking density according to the system used. Danaher *et al.* (2007) proof the effect of two densities stocking of caged monosex Nile tilapia when is culture in polyculture and mentions that low densities, (100 ind m^{-3}), shows higher weights and better control of quality parameters in water. Osofero *et al*. (2009) suggest a 150 juvenile $cage^{-1}$ with a mean final weight of 82.74 g $fish^{-1}$ in a culture system of bamboo cage. Yakubu (2012) proposed fingerlings stocking density between 300 and 450 ind m^{-3} in water flow through systems. Our results suggest that low densities in a culture under greenhouse are recommendable, specifically for this case a density between 90 and 180 ind m^{-3} should be consider.

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