Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(4): 1113 – 1134 (2022)
www.ejabf.journals.ekb.eg

Assessment of production, physiological responses, and economic efficiency of the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) reared at different stocking densities in a polyculture system

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ARTICLE INFO
Article History:
Received: July 27, 2022
Accepted: Aug. 16, 2022
Online: Aug. 23, 2022

Keywords:
Aquaculture,
Stocking density,
Tilapia,
Fish production,
Growth

ABSTRACT
A field study was carried out to evaluate common practices in the study area with regard to determining the appropriate stocking density, SD for the culture of Nile tilapia, *Oreochromis niloticus* in a polyculture system with thin-lipped mullet, *Chelon ramada*, and African catfish *Clarias gariepinus* for 129 days. Thus, fish reared in earthen ponds (400 m^3 in volume of each) at low SD (T_1, 5.62 fish m^{-3}), and high SD (T_2, 11.12 fish m^{-3}), and assessment of their effects on productive performance, body chemical composition, physiological responses, and economic efficiency. Fish reared at low SD (T_1) attained a high ($P \leq 0.05$) growth rate, the best-feed conversion ratio, improved hematological, and serum biochemical parameters and body composition of fish compared to those reared at high SD (T_2). Fish in T_1 also achieved high fish weight categories, and the best economic efficiency parameters than those reared in T_2. Thus, based on the obtained results under the cultivation conditions in the fish farm area, it could be concluded that the suitable SD is 5.62 fish m^{-3} (T_1) for rearing Nile tilapia in a polyculture system with thin-lipped mullet, and African catfish in earthen ponds.

INTRODUCTION

Globally, aquaculture contributes 46% of global fish production and is one of the fastest growing food sectors in the world **(FAO, 2020)**. Tilapias are second only to carps in importance in the aquaculture industry. The Nile tilapia, *Oreochromis niloticus* is the most widely distributed fish in aquaculture with 8.0% annual growth **(FAO, 2020)**. In Egypt, aquaculture is exhibiting the strongest growth sector of any fisheries related activity in the country and as a result, aquaculture is considered as the only viable tool for reducing the gap between production and consumption of fish (**GAFRD, 2014**). Thus, aquaculture now accounts for nearly 80.5 percent of total fish production in Egypt, with private farms producing an estimated 99% **(GAFRD, 2019)**. In order to ensure food safety and sustainability, aquaculture systems and technologies aim to improve productivity and minimize environmental effects **(Bakeer *et al*., 2008)**. Because Egypt has limited freshwater sources, it is possible to develop aquaculture production through higher fish stocking densities, artificial feeding, and especially through the popular polyculture system of different fish species **(Abdel-Hakim *et al*., 2012)**. Earthen ponds are the major fish-farming system in Egypt. Generally, ponds are supplied by water and irrigated with drainage canals and are stocked with Nile tilapia monocultures or polycultures that include tilapia, mullet, and carp **(GAFRD, 2014)**. In this respect, **Ponce-Marbán *et al*. (2006)** suggest that polyculture is one method to increase fish production yields by maximizing the use of available resources to the maximum extent possible. In Egypt, fish farmers prefer the tilapia-mullet polyculture system. The positive effect of a tilapia–mullet polyculture system is attributed to trophic divergence between species **(Abdel-Tawwab *et al*., 2005)**.

In aquaculture, stocking density (SD) is considered to be one of the important factors that effect on natural food availability, the efficient utilization of food resource, fish growth, feed utilization and total fish yield in ponds (**Khattab *et al.*, 2004**). Fish intensification was also found suitable to overcome the problem of land shortage (**Chakraborty and Banerjee, 2010**). The full utilization of space for maximum fish production through intensive culture can improve the profitability of the fish farm (**Ellis *et al*., 2002**). Higher growth rate and yield of aquaculture species can be attained when farmers culture fish at an optimum SD, which will eventually result into high growth for economic benefits (**Shoko *et al*., 2016**). Although high SD in aquaculture systems may be led to increase of fish production, but it may have some negative effects on physiological, hormonal and immune responses of aquatic species (**Kpundeh *et al*., 2013; Jumah, 2020**). In tilapia, the effect of SD have been documented on different sizes including fry (**El-Sayed, 2002; Ferdous *et al*., 2014**), fingerlings (**Shourbela *et al*., 2021; Ani *et al*., 2022**), sub-adults (**Bakeer *et al*., 2007; Dawood *et al*., 2020**), and adult (**Diana *et al*., 2004; Zaki *et al*., 2020**). Generally, **Suresh (2003)** indicated that *O. niloticus* is suitable for culturing at high stocking densities (5–10 fish m^{-2}) for achieving high productivity.

The difference in fish species cultured under the polyculture system leads to the best utilization of the available natural foods produced in fish ponds **(Greglutz, 2003)**. Many studies indicate that polyculture system gives the highest growth performance and survival rate of reared fish species compared to those in monoculture system **(El-Sagheer *et al*., 2008; Mehrim *et al*., 2016, 2018)**. In polyculture system, Nile tilapia, *O. niloticus* is usually reared alongside different species like common carp, *Cyprinus carpio* **(Abdel-Hakim *et al*., 2012)**, silver carp, *Hypophthalmichthys molitrix* **(Mehrim *et al*., 2016)**, striped mullet, *Mugil cephalus* **(Tahoun *et al*., 2013; Mehrim *et al*., 2018)**, African catfish, *Clarias gariepinus* **(Shoko *et al*., 2016),** and European eel, *Anguilla anguilla* **(Abdel-Hakim *et al*., 2001)**. Several studies showed the importance of determining the appropriate stocking density, especially in polyculture system, which is reflected in their productivity and profitability **(Uddin *et al*., 2007; Abdel-Hakim *et al*., 2014; Shoko *et al*., 2016)**. Regarding SD of tilapia, experimental culture of tilapias at stocking densities of 5–10 fish m^{-2} has demonstrated that intensive tilapia farming is feasible in earthen ponds (**Suresh, 2003**). Regardless of the suitability of *O. niloticus* culture at high densities, limited studies have demonstrated stocking densities up to 9 fish m^{-2} at production level in earthen ponds (**Diana *et al*., 1996**). On the other hand, in recent years the fish farms especially scattered in the study area show many problems, including some malpractices, the spread of diseases, low productivity, and consequently large financial losses for the farmers. Therefore, the current study aims to assess the status in one of the fish farms to identify the causes of these problems, regarding an assessment study to evaluate the common malpractices in the study area with regard to determining the appropriate SD for the culture of Nile tilapia, *O. niloticus* in a polyculture system with thin-lipped mullet, *Chelon ramada* , and African catfish, *C. gariepinus* for 129 days. In addition, to evaluate the effects of such fish SD on growth performance, feed conversion ratio, physiological responses, chemical examination of the body, fish production, and economic efficiency of Nile tilapia reared under two stocking densities (low SD, T_1, and high SD, T_2) in a polyculture system in earthen ponds.

MATERIALS AND METHODS

1. The experimental fish

This study was carried out at a private fish farm, El-hajj Eid Shaban (San El-Baharia, El-Hosania, Al-Sharqia Governorate, Egypt). Nile tilapia fingerlings (monosex) and thin-lipped mullet were acquired from the same farm, with an average initial body weight of 82 ± 1.62 g $fish^{-1}$ and 80 ± 2.49 g $fish^{-1}$, respectively. The catfish was acquired from a fish seller in El-Mataria, Dakahlia Governorate, Egypt with an average initial body weight of 56 ± 3.50 g $fish^{-1}$. All fish were adapted on the experimental conditions for two weeks. After that, fish were distributed into two treatments that differed in stocking density. Each treatment includes two earthen ponds (20 m long × 20 m wide × and 1.0 m deep, for a total volume of 400 m^3). Fish in both experimental treatments were reared under a polyculture system of Nile tilapia, thin-lipped mullet, and African catfish at two stocking densities as shown in **Fig. 1**. Where, the first treatment (T_1; low stocking density, LSD) consisted of (2000, 200, and 50; equivalent per fadden to 14000, 1400, and 350 fish; 5.62 fish m^{-3}; or equal 15750 fish $faddan^{-1}$), while the second treatment (T_2; high stocking density, HSD) consisted of (4000, 400, and 50; equivalent per fadden to 28000, 2800, and 350 fish; 11.12 fish m^{-3}; or equal 31150 fish $faddan^{-1}$), respectively.

In the present study, water quality parameters as water temperature and pH were measured once/week. Where, water dissolved oxygen (DO), total ammonia nitrogen (TAN), and NO_3 levels were measured every two weeks. The water quality parameters of both experimental treatments are displayed in **Table 1**.

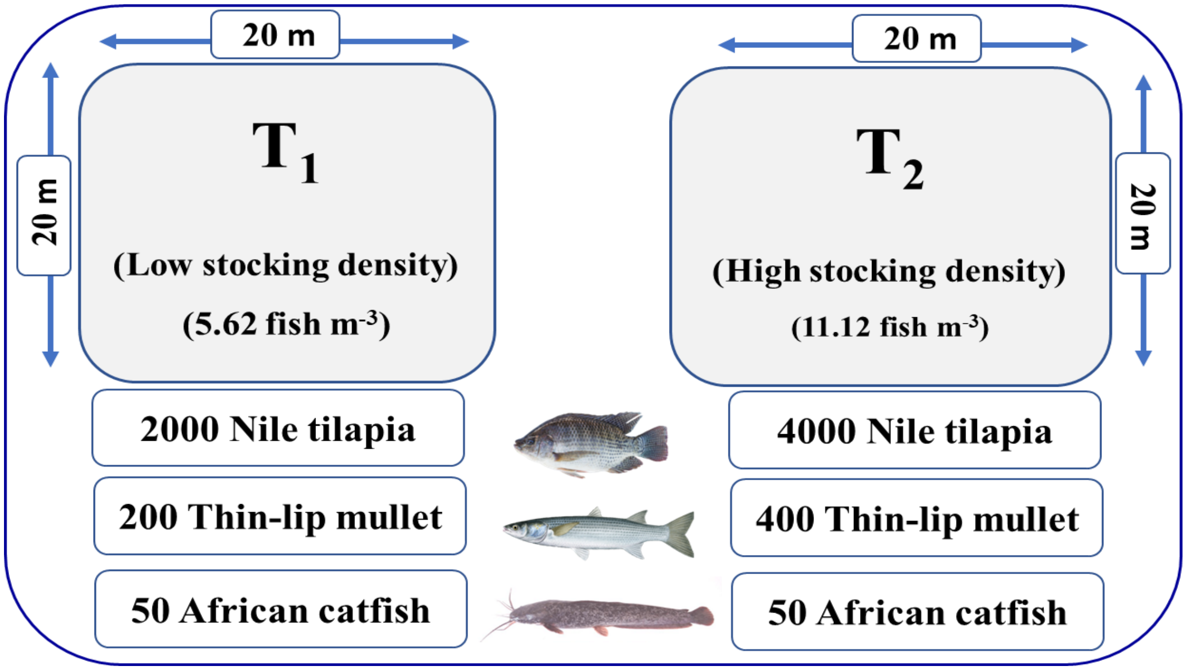

Figure 1. Details of the experimental design

Table 1. Water quality parameters of Nile tilapia, thin-lipped mullet, and African catfish reared at different stocking densities in a polyculture system (mean ± SD)

Parameter	Experimental treatment		*P*- value
	T_1	T_2	
Temperature (°C)	29.40±2.17	29.40±2.17	1.000
Dissolved oxygen (mg L^{-1})	7.85±0.55^a	7.01±0.40^b	0.0011
pH	8.00±0.26	8.00±0.26	1.000
TAN (mg L^{-1})	2.57±0.45^b	3.21±0.54^a	0.0528
NO_3 (mg L^{-1})	0.13±0.09^b	0.58±0.17^a	0.0399

Mean in the same row having different small letters are significantly different ($P \leq 0.05$). TAN: total ammonia nitrogen.

2. Feeding system

All fish groups were fed the same commercial pelleted diet comprising 30% crude protein and bought from Aller Aqua Industrialization and Trade Factory belonging to the industrial zone 6 of October, Cairo, Egypt. This commercial diet contained soybean meal (46%); yellow corn; wheatgrass grinders; herring fish meal (60%); limestone; corn gluten (60%), dicalcium phosphate, a mixture of mineral salts, and a mixture of vitamins, fish oil, and soybean oil, according to the manufacturer's formula. The chemical composition of the commercial diet is shown in **Table 2**. The diet was daily presented to fish by hand two times per day (8.30 am and 15.00 pm). The percentage of the feeding rate was 3% of the total biomass of body weight of Nile tilapia in each pond.

Table 2. Chemical composition (% on dry matter basis) of the experimental diet

Nutrient composition	%
Dry matter (DM, %)	87.04
Crude protein (CP, %)	30.09
Crude fat (CF, %)	8.34
Ash (%)	7.83
Total carbohydrate (%)	53.74
Gross energy (TE, KJ Kg^{-1} DM)	1985.3
Protein / energy ratio** (P/E, mg CP KJ^{-1} GE^{*})	15.16

* GE (Gross energy, KJ 100 g^{-1} DM) = (CP × 23.64) + (CF × 39.54) + (total carbohydrate × 17.57) was calculated according to **NRC (2011)**.
** P/E ratio (mg crude protein KJ^{-1} GE) = (CP/GE) × 1000.

3. Experimental measurements

3.1. Growth performance parameters

At the end of the experiment fish in each earthen pond were weighted to calculate the growth performance parameters according to Lovell , (2001) as showen in the following equations;

Total weight gain (TWG, g) = $\mathbf{FW\ (g) - IW\ (g)}$.

Average daily gain (ADG, g $fish^{-1}$ day^{-1}) = $\frac{\mathbf{TWG}}{\mathbf{T}}$.

Relative growth rate (RGR, %) = $\frac{\mathbf{TWG}}{\mathbf{IW}} \times \mathbf{100}$.

Specific growth rate (SGR, % day^{-1}) = $\left[\frac{(\ln FW - \ln IW)}{T}\right] \times \mathbf{100}$.

Where: FW: Final weight (g); IW: Initial weight (g); T: The experimental period (day).

3.2. Hematological parameters

At the end of the experiment, nine fish in each treatment were randomly taken and anesthetized with clove oil extract (50 mg L^{-1}) to get the blood samples. Blood was collected from the fish caudal peduncle, which were received in small plastic vials containing heparin (5 mL at each collection) to determine the hematological parameters. Hemoglobin (Hb) was measured using commercial colorimetric kits (Diamond Diagnostic, Egypt). Total red blood cells (RBCs × 10^6 mm^{-3}), blood platelets (PLT, × 10^3 mm^{-3}), total white blood cells (WBCs × 10^3 mm^{-3}), and WBCs differential were counted according to **Dacie and Lewis (1995**) on an Ao Bright – Line Hämocytometer model (Neubauer improved, Precicolor HBG, Germany). Meanwhile, mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, %) were calculated according to **Beutler *et al*. (2000)**. In addition, packed cell volume (PCV, %) was measured according to **Stoskopf (1993)**.

3.3. Serum biochemical parameters

To get serum, other blood samples (5 mL) were collected in dry small plastic vials at the room temperature, and then centrifuged at 3500 × g for 20 min. Several serum biochemical components, including creatinine, uric acid, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, total cholesterol, triglycerides, glucose, and cortisol levels were determined as methods described by **Doumas *et al*. (1971)** and **Tietz (1990)**. In accordance with **Doumas and Biggs (1972)**, serum globulin was estimated by subtracting albumin from total protein. These serum biochemical parameters were determined by calorimetrically testing with the kits manufactured by Diagnostic System Laboratories, Inc., USA.

3.4. The chemical compostion of the fish body

At the beginning ten fish (n = 10) as a pooled sample were randomly collected, where at the end of the experiment five fish (n = 5) in each treatment were collected and kept frozen (–20 °C) till the proximate analysis of the whole fish body was done according to **AOAC (2016)**.

3.5. Graded size parameters of Nile tilapia

At the end of the study, the total yield of Nile tilapia in each pond was categorized according to body weight (size) into five categories: extra-large weight: ranged 445–500 g fish^{-1}; super weight: ranged 305–315 g fish^{-1}; number 1: weight ranged 210–215 g fish^{-1}; number 2: weight ranged 100–110 g fish^{-1}, and number 3: weight ranged 50–90 g fish^{-1}. In addition, the total fish production and total return for each treatment were also estimated.

4. Statistical analysis

The obtained data such as growth performance, and chemical composition of the whole fish body in each of Nile tilapia, thin-lipped mullet, and African catfish, hematological and serum biochemical, fish production, and economic efficiency parameters were exposed to one-way examination of variance (ANOVA) using SAS™ software for windows version 9.1.3 (**SAS, 2006**), and were statistically compared for the significance at $P \leq 0.05$.

RESULTS

1. Growth performance of Nile tilapia and thin-lipped mullet

The effect of stocking density on the growth performance parameters of Nile tilapia and thin-lipped mullet is shown in **Table 3**. Nile tilapia and thin-lipped mullet reared at

LSD in T_1 showed significant increase of all growth performance parameters compared to those reared at HSD in T_2 ($P \leq 0.05$).

Table 3. Growth performance parameters of Nile tilapia and thin-lipped mullet reared at different stocking densities in a polyculture system

Parameter	Experimental treatment		*P*- value
	T_1	**T_2**	
Nile tilapia			
FW (g)	361.5±1.15^{a}	348.8±0.87^{b}	0.0009
TWG (g)	279.5±1.15^{a}	266.8±0.87^{b}	0.0009
ADG (g fish^{-1} day^{-1})	2.17±0.01^{a}	2.07±0.01^{b}	0.0009
RGR (%)	340.9±1.41^{a}	325.3±1.04^{b}	0.0009
SGR (% day^{-1})	1.15±0.00^{a}	1.12±0.00^{b}	0.0008
Thin-lipped mullet			
FW (g)	153.5±1.44^{a}	143.5±0.87^{b}	0.0040
TWG (g)	73.50±1.44^{a}	63.50±0.87^{b}	0.0040
ADG (g fish^{-1} day^{-1})	0.57±0.01^{a}	0.49±0.01^{b}	0.0040
RGR (%)	91.90±1.79^{a}	79.40±1.10^{b}	0.0039
SGR (% day^{-1})	0.51±0.01^{a}	0.45±0.01^{b}	0.0040

Mean in the same row having different small letters are significantly different ($P \leq 0.05$). T_1: Low stocking density, and T_2: high stocking density; FW: Final body weight; TWG: Total weight gain; ADG: Average weight gain; RGR: Relative growth rate; SGR: Specific growth rate.

2. Hematological parameters of Nile tilapia

The effect of stocking density on hematological parameters of Nile tilapia reared in a polyculture system is shown in **Table 4**. There were no significant differences between the experimental treatments in Hb, PCV, RBCs, PLT, MCH, and MCHC ($P \geq 0.05$). The same trend was observed in WBCs, neutrophils, and eosinophils counts. Nile tilapia reared in LSD (T_1) showed significant ($P \leq 0.05$) increase of MCV, lymphocytes, and monocytes counts compared to those reared in HSD treatment (T_2).

3. Serum biochemical parameters

Data in **Table 5** showed the effect of stocking density on serum biochemical parameters of Nile tilapia reared at different stocking densities in a polyculture system. No significant ($P \geq 0.05$) differences were observed in serum creatinine, uric acid, and urea levels, as well as total protein, albumin, globulin, total cholesterol, and glucose concentrations ($P \leq 0.05$). Nile tilapia in T_2 showed significant increase of liver function enzymes (AST, ALT, and ALP), triglycerides, and concentration of cortisol compared to T_1 ($P \leq 0.05$).

4. Chemical composition of fish body

The effect of stocking density on the chemical composition of the bodies of Nile tilapia and thin-lipped mullet reared in a polyculture system is displayed in **Table 6**. For

Nile tilapia, the contents of dry matter, ash, and crude protein significantly ($P \leq 0.05$) decreased, while crude fat significantly increased in fish reared at LSD (T_1) compared to those reared at HSD (T_2). The same pattern was observed in thin-lipped mullet regarding dry matter, ash, crude protein, and crude fat, where the ash content of thin-lipped mullet does not differ significantly between treatments ($P \geq 0.05$).

Table 4. Hematological parameters of Nile tilapia reared at different stocking densities in a polyculture system

Parameter	Experimental treatments		*P*- value
	T_1	T_2	
Hb (g dL^{-1})	9.38±0.32	9.20±0.27	0.6770
PCV (%)	37.67±2.42	33.46±0.85	0.1665
RBCs ($\times10^6$ mm^{-3})	2.42±0.12	2.34±0.07	0.5871
PLT (%)	32.00±1.89	32.00±3.82	0.0472
RBCs indices			
MCV (fl)	152.7±4.39^a	141.6±1.46^b	0.0391
MCH (pg)	38.33±0.82	39.48±0.81	0.8562
MCHC (%)	25.95±1.35	27.23±1.14	0.1400
WBCs differential			
WBCs ($\times10^3$ mm^{-3})	70.95±4.74	62.15±4.41	0.2415
Neutrophils	1.50±0.39	2.00±0.08	0.2328
Lymphocytes	91.70±1.77^a	86.82±1.88^b	0.0022
Monocytes	4.00±0.52^a	2.90±0.48^b	0.0113
Eosinophils	2.32±0.36	1.92±0.32	0.2867

Mean in the same row having different small letters are significantly different ($P \leq 0.05$). T_1: Low stocking density, and T_2: high stocking density; Hb: Hemoglobin; PCV: Packed cell volume; RBCs: Red blood cells; PLT: blood platelets; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBCs: White blood cells.

5. Graded size of Nile tilapia

Data in **Fig. 2** showed the different categories of produced Nile tilapia reared under two stocking densities in a polyculture system. Fish reared at LSD (T_1) has significantly ($P \leq 0.05$) increased the percentage of fish categories as extra-large, super, and number 1 and has decreased ($P \leq 0.05$) the percentage of categories number 2 and number 3 compared to those reared in HSD (T_2).

6. Total production and total return

Data in **Fig. 3** demonstrated the economic efficiency parameters of the experimental treatments reared in a polyculture system. Fish reared at LSD (T1) revealed a significant ($P \leq 0.05$) decreased of total cost, and significantly increased total output, net return, and economic efficiency compared to those reared at HSD (T2).

Table 5. Serum biochemical parameters of Nile tilapia reared at different stocking densities in a polyculture system

Parameter	Experimental treatment		*P*- value
	T_1	**T_2**	
Kidney functions parameters			
Creatinine (mg dL^{-1})	0.28±0.02	0.24±0.03	0.9180
Uric acid (mg dL^{-1})	1.01±0.05	0.96±0.05	0.3046
Urea (mg dL^{-1})	12.28±0.33	12.94±0.20	0.3225
Liver functions parameters			
AST (U L^{-1})	230.3±14.32^{b}	356.5±17.85^{a}	0.0001
ALT (U L^{-1})	218.0±20.14^{b}	237.3±19.43^{a}	0.0155
ALP (U L^{-1})	74.17±5.77^{b}	92.31±4.57^{a}	0.0007
Total protein (g dL^{-1})	2.79±0.25	2.80±0.34	0.9767
Albumin (g dL^{-1})	2.14±0.08	1.96±0.10	0.0660
Globulin (g dL^{-1})	0.65±0.09	0.84±0.08	0.7970
Lipid profile			
Total cholesterol (mg dL^{-1})	147.5±7.17	143.8±5.94	0.6162
Triglycerides (mg dL^{-1})	428.5±5.00^{b}	454.0±5.44^{a}	0.0079
Stress biomarkers			
Glucose (mg dL^{-1})	188.6±6.91	184.3±9.98	0.7062
Cortisol (µg dL^{-1})	24.53±1.49^{b}	30.24±1.70^{a}	0.0077

Mean in the same row having different small letters are significantly different ($P < 0.05$). T_1: Low stocking density, and T_2: high stocking density; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: Alkaline phosphatase.

Table 6. Chemical composition (% on dry matter basis) of Nile tilapia and thin-lipped mullet bodies reared at different stocking densities in a polyculture system

Parameter	Experimental treatment		*P*- value
	T_1	**T_2**	
Nile tilapia			
Dry matter (%)	25.22±0.43^{b}	27.62±0.79^{a}	0.0241
Crude protein (%)	53.75±0.53^{b}	58.53±0.49^{a}	0.0001
Crude fat (%)	32.69±0.46^{a}	27.05±0.46^{b}	0.0001
Ash (%)	13.57±0.31^{b}	14.43±0.07^{a}	0.0212
Thin-lipped mullet			
Dry matter (%)	23.89±0.10^{b}	25.92±0.71^{a}	0.0182
Crude protein (%)	49.40±0.44^{b}	51.81±0.30^{a}	0.0012
Crude fat (%)	39.56±0.49^{a}	36.99±0.44^{b}	0.0029
Ash (%)	11.05±0.13	11.20±0.19	0.5163

Mean in the same row having different small letters are significantly different ($P < 0.05$). T_1: Low stocking density, and T_2: high stocking density.

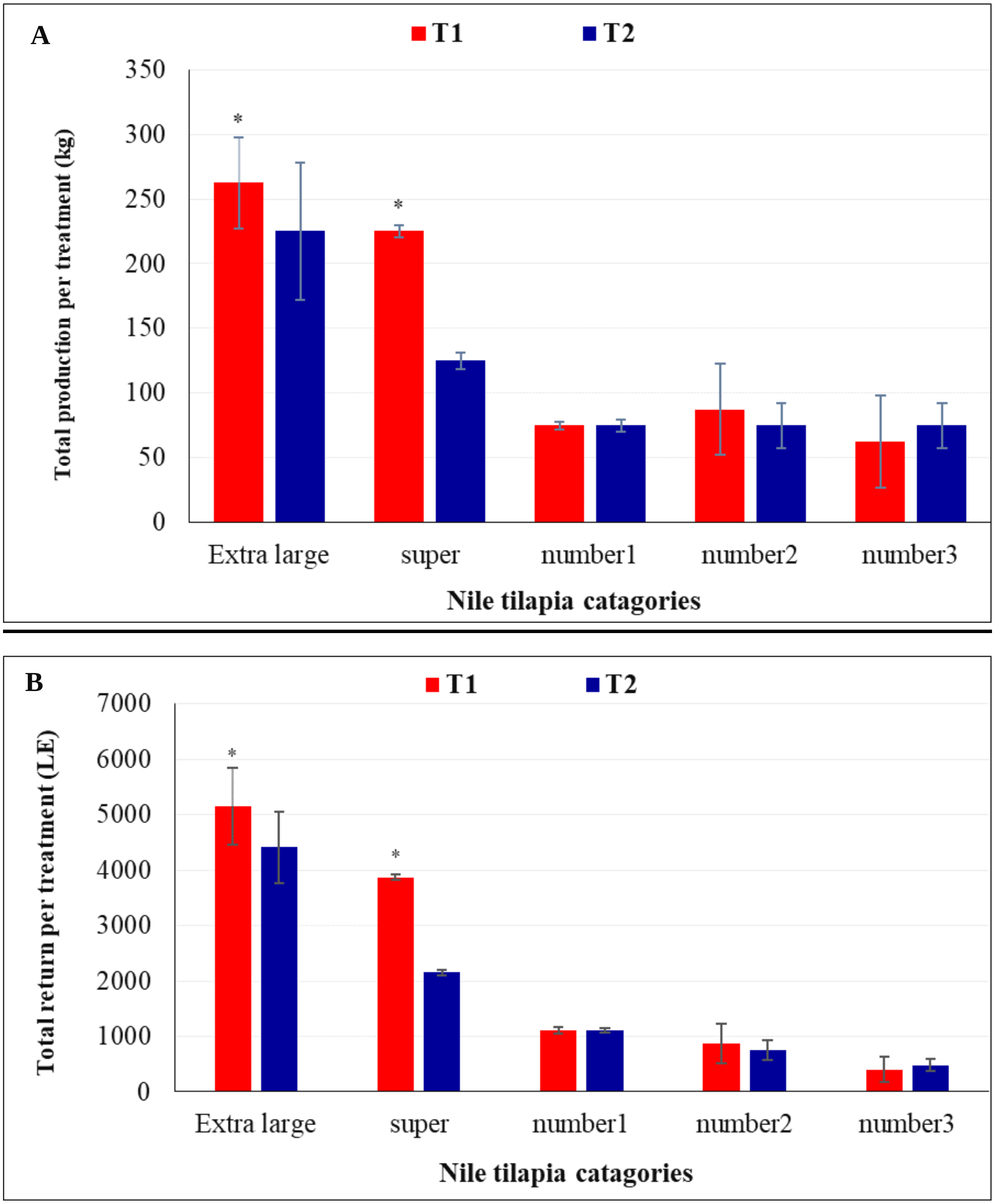

Figure 2. Total production and total return of different categories of produced Nile tilapia reared at different stocking densities in a polyculture system (A) T_1 (low stocking density) and (B) T_2 (high stocking density). Extra-large: weight ranged 445–500 g; Super weight: ranged 305–315 g; Number 1: weight ranged 210–215 g; Number 2: weight ranged 100–110 g, and Number 3: weight ranged 50–90 g.

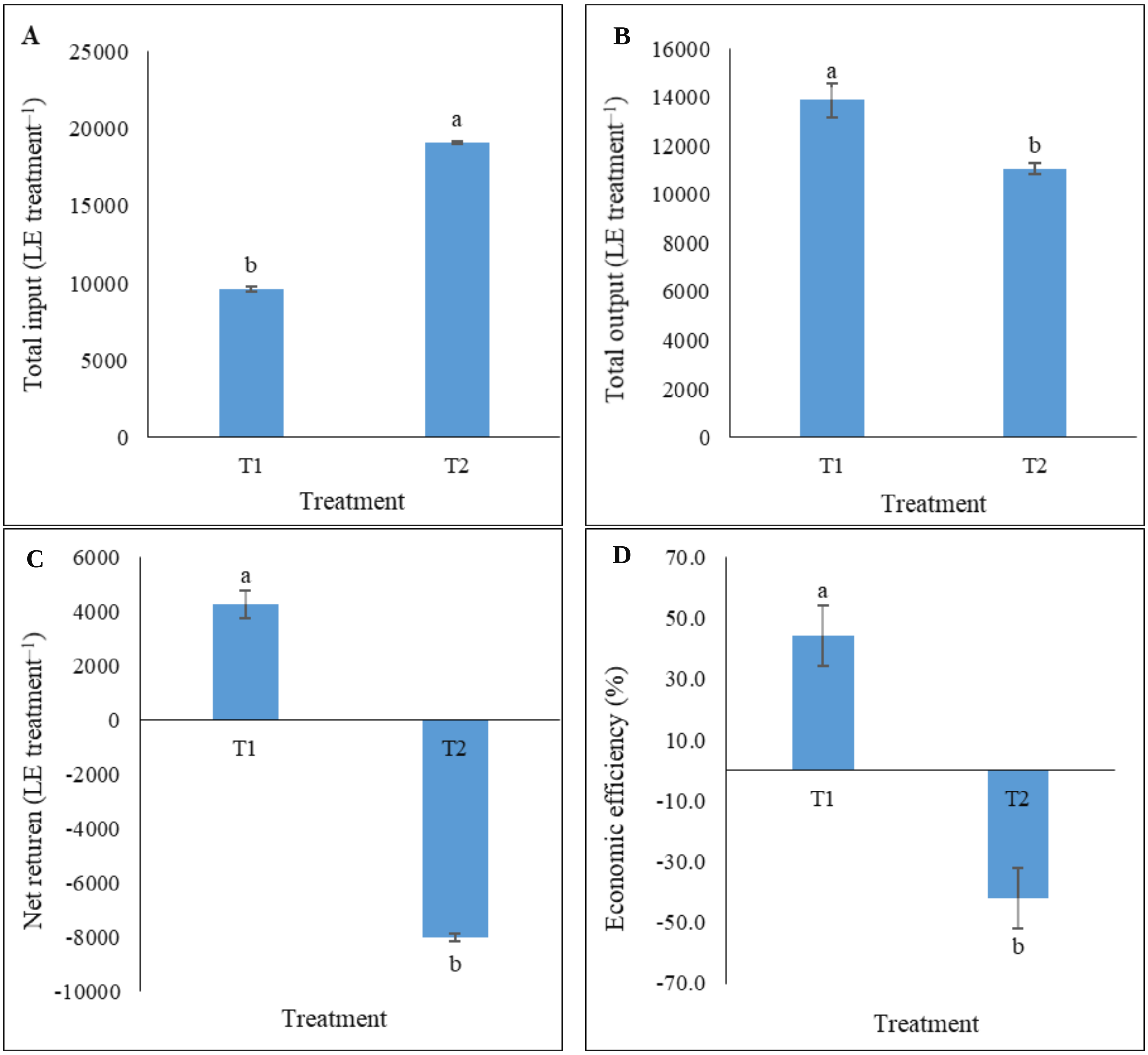

Figure 3. Economic efficiency parameters of the experimental treatments reared in a polyculture system. (A) total input (LE treatment^{-1}) = total feed costs + total fingerlings price (the prices of feed was 9.250 LE kg^{-1}; The price of fingerlings: 0.442 LE for Nile tilapia; 1.5 LE for Thin-lipped mullet; 0.375 LE for catfish); (B) total outputs (LE treatment^{-1}) = fish price × total fish production; (C) net return per treatment (LE) = total outputs – total costs; (D) economic efficiency per treatment (%) = (net return / total costs) × 100.

DISCUSSION

Enhancing the growth performance of the fish is one of the most crucial goals of aquaculture. Therefore, most aquaculture systems aim to increase growth performance and profitability (**Uddin *et al.*, 2007)**. In the current study, Nile tilapia and thin-lipped mullet reared in LSD (T_1) significantly increased the growth performance parameters compared to those reared in HSD (T_2). The same trend was observed in fish reared under different culture systems, such as polyculture in earthen ponds **(Abdel-Hakim *et al.*,**

2014; Shoko *et al*., 2016; Mehrim *et al*., 2018), in recirculating aquaculture systems (RAS) **(Refaey *et al*., 2018)**, in cages **(Mehrim *et al*., 2016)**, in biofloc system (**Zaki *et al*., 2020**), and in aquaponics system (**Ani *et al*., 2022)**. Fish reared in HSD may lose growth due to crowding stress, which triggers an increase in energy demand as a result of physiological responses to cope with stress, and reducing available energy for growth **(Qi *et al*., 2016)**. These results harmonize with serum cortisol levels **(Table 5)** in the present study that increased with increasing the SD, as well as decreased crude fat **(Table 6)** of the fish body to cope with crowding stress. It should be noted that tilapia ponds suffer from the phenomenon of random breeding. Therefore, to control the tilapia community and maintain the population density inside the ponds by cultivating some predators such as catfish, which fed new hatching tilapia fry **(Abdel-Tawwab, 2005)**.

Similarly with the obtained findings herein, **Shoko *et al*. (2016)** stated that the cultivation of *O. niloticus* at LSD in both mono- and polyculture systems will achieve higher growth rate, yield and economic benefits than HSD as reported elsewhere (**Shoko *et al*., 2003; Uddin *et al*., 2007**). In addition, **Mehrim *et al*. (2017)** also established these drastically effects of HSD (80 fish m^{-3}) on all growth and feed efficiency parameters of *O. niloticus*. More recently, **Ani *et al*. (2022)** confirmed the drastic effects of HSD on growth performance, and feed utilization of *O. niloticus* reared at an aquaponics system. Generally, the rate of growth of fish is highly variable, because it is greatly dependent on a variety of interacting environmental factors such as water temperature, levels of DO and ammonia, salinity, and photoperiod (**Brogowski *et al*., 2005**). Moreover, the severe effects of HSD on growth performance parameters of fish may be related to increasing SD, which can increase the competition among fish for space and limited food availability, which consequently reduce growth performance of stocked fish (**Quiros, 1999**). Furthermore, increased SD can compromise water quality in fish ponds, which can affect both the feed intake, and conversion efficiency of the fish (**Ellis *et al*., 2002**), that led to weakened growth. In this respect, **Schwedler and Johson (2000)** confirmed that at optimum SD, aggressiveness is reduced and instead energy can be channel to growth of fish.

Hematological profiles are essential tools for assessing fish health and physiological responses (**Fazio, 2019**). The results of the present study showed there are no significant differences in most tested hematological parameters between experimental treatments. This could be explained by the fish's ability to adapt to SD conditions, especially with the long experimental period, which lasted 129 days (18 weeks). In the same line, other studies stated that no significant differences of hematological parameters were found of *O. niloticus* (**Hastuti and Subandiyono, 2020**), or silver carp, *Hypophthalmichthys molitrix* (**Kamal and Omar, 2011**) reared at HSD. In fish, HSD may cause severe stress, which in turn may suppress immune responses of fish and make them more susceptible to disease (**Silveira, 2010**). Thus, in the present study MCV, monocytes, and lymphocytes are significantly decreased of *O. niloticus* reared at HSD (T_2) compared to those reared at

LSD (T_1). This mean that increasing SD led to adversely effects on immune responses of reared fish. In accordance with current findings, **Mehrim (2009) and Kpundeh *et al*. (2013)** who reported significantly decreased hematological indices (RBCs, WBCs, Hb, PCV, and blood platelets) by increasing SD of *O. niloticus*. In a recent study, **Zaki *et al*. (2020)** also stated that over SD (60 fish m^{-3}) adversely affects RBCs, Hb, and PCV values of *O. niloticus*. Moreover, the adversely effects of increasing SD on hematological parameters were also detected in other fish species like, gilthead sea bream, *Sparus aurata* (**Montero *et al*., 1999**); rainbow trout, *Oncorhynchus mykiss* (**Yarahmadi *et al*., 2015**), and great sturgeon, *Huso huso* (**Naderi *et al*., 2017**).

Regarding the composition of bodies of Nile tilapia and thin-lipped mullet, the results showed increased dry matter and crude protein contents and decreased crude fat content with increasing SD. Low-fat content in fish reared at HSD (T_2) may result from increased lipid metabolism to meet the increased energy demand from dense stocking, where lipids are a valuable source of energy for fish **(Menezes *et al*., 2015)**. As SD increased, some fish species' lipid content decreased **(Abdel-Hakim *et al*., 2014; Refaey *et al*., 2018)**. These findings are principally related to the harmful effects of increasing SD on growth performance **(Table 3)**, and grading size of Nile tilapia **(Fig. 2)** in the present study. Furthermore, improvements in the body composition of fish reared in polyculture compared to fish reared in monoculture conditions are previously reported **(Khalil *et al*., 2008; Mehrim *et al*., 2016)**. In the same line with the current findings, **Mehrim *et al*. (2018)** reported that the body composition of *O. niloticus* reared in polyculture systems significantly improved compared to those reared in monoculture system. This may be linked to the polyculture system based on cultured fish species feeding at different levels of the food chain in a pond **(Milstein *et al*., 2002)**, which leads to increased efficiency in utilizing ecological resources and obtaining the maximum standing crop of a pond by allowing a wide range of available food in pond volume **(Hassan, 2011)**. Where, tilapias are considered as filter feeders fish, because they can efficiently harvest plankton from the water. They can also digest more efficiently the plant protein (**Popma and Masser, 1999**).

In the current study, no statistically significant differences were observed between the experimental treatments in kidney functions parameters (creatinine, uric acid, urea), serum proteins (total protein, albumin, and globulin), and total cholesterol and glucose levels. This is due to the fish's ability to adapt to different culture conditions (stocking density), especially with the long period of the culture with approximately five months. On the other hand, *O. niloticus* reared at HSD in T_2 treatment led to an increase in the activity of liver function enzymes (AST, ALT, and ALP), triglycerides, and cortisol levels in serum compared to those reared at LSD (T_1). The activity of AST, ALT, and ALP enzymes is a good indicator for liver function **(Lemaire *et al*., 1991)**, especially in crowding stress conditions **(Refaey *et al*., 2018)**. In this study, increased activities of liver enzymes indicate the occurrence of hepatic dysfunction, which is mainly related to crowding stress, as well as increasing water TAN and NO_3 **(Table 1)** as observed in HSD (T_2). This result is in agreement with those obtained by **Sadhu *et al*. (2014)** in Asian seabass. AST and ALT are the major bio-indicators of exposed fish to stress conditions, like HSD (**Luo *et al*., 2013**). Thus, **Aly *et al*. (2009**) reported that HSD caused higher AST or ALT as the stress biomarkers of *O. niloticus*, as well as they concluded that SD of tilapia up to 6 fish m^{-3} exhibited an adversely effect on the liver functions. Similarly, with the current findings, **Refay *et al*. (2018)** found that AST and ALT activities were elevated in channel catfish reared at HSD, where the same authors suggesting that the weakening of liver function because reared fish at HSD. In a recent study, **Jumah (2020)** also suggested the same findings of plasma AST, and ALT of *O. niloticus* reared at HSD compared to those reared at LSD. Although, in some cases the drastic effects of HSD are either temporary (**Garr *et al*., 2011**), or absent (**Southworth *et al*., 2009**), where **Portz *et al*. (2006**) suggested that some fish species like tilapias can tolerate extreme crowding.

On the other side, the increase of serum cortisol hormone may be confirmed that fish reared at HSD were exposed to stress conditions, which is reflected in an increase in the metabolic rates of fats, and an increase in the levels of triglycerides as well. These results are consistent with the lower body fat content of the fish reared at HSD compared to those reared at LSD **(Table 6)**. The elevation of serum glucose, triglyceride and total cholesterol levels of fish reared in HSD could result from energy metabolism by gluconeogenesis pathways (**Barton and Iwama, 1991**), causing the augmentation of available energy to cope the stressful status by HSD. Similarly, with the current findings, **Lupatsch *et al*. (2010)** stated that serum cortisol significantly increased of European sea bass, *Dicentrarchus labrax* reared at HSD. The same trend of plasma cortisol of *O. niloticus* reared at HSD was recently described by **Jumah (2020)**. Inversely with the obtained findings herein, **Zaki *et al*. (2020)** reported that serum cortisol was significantly decreased by increasing SD of *O. niloticus*. Additionally, triglyceride is an important energy parameter of blunt snout bream, *M. amblycephala* reared under HSD conditions (**Qi *et al*., 2016**). Similarly, with the current findings, **Refay *et al*. (2018)** stated that serum triglyceride significantly increased of channel catfish reared at HSD compared to those reared at LSD.

From the production and economic point of view, fish reared at LSD (T_1) achieved significantly high total production, and the biggest categories of reared *O. niloticus*, which consequently led to high economic efficiency compared to those reared at HSD (T_2). These results are not surprising, where fish reared at LSD (T_1) achieved high growth performance parameters, and physiological responses than those reared at HSD. Similarly with the obtain findings herein, **Mehrim *et al*. (2016)** stated that *O. niloticus* reared in polyculture system with 12% silver carp achieved the highest total production, the differentiation of the fish size, total output (LE), total profit (LE), and economic efficiency (%) compared to those reared in monoculture system. In this respect, **Milstein *et al*.** , **(2002)** recommended that stocking two or more fish species could increase the maximum standing yield of a pond by allowing a wide range of available food, and the pond volume to be applied. In contrast with the current findings, **Abdel-Hakim *et al*. (2014)** reported that the total pond production increased (P<0.05) with increasing SD of fish. **Hassan and Mahmoud_(2011)** previously confirmed these findings also. These conflicts between obtained data of different studies may be related with the experimental management, fish species, their SD, period, or system of fish cultivation.

Economic benefit analysis in aquaculture is an essential tool to evaluate the profitability of the culture system, to determine efficiency of resource allocation and management practices (**Shoko *et al*., 2016**). The current findings are in agreement with those previously reported by **Uddin *et al*. (2007)** and **Shoko *et al*. (2016)**. The cultivation of the Nile tilapia and planktivorous fish like silver carp or mullet fish species in a polyculture system can improve the water quality, as well as helps to gain an extra yield of silver carp or mullet without incurring additional costs, which makes aquaculture more profitable to farmers (**Sarkar *et al*., 2006, 2008**). Thus, the current findings in the present study confirmed the superiority of polyculture system regarding economic efficiency among other fish culture systems. In this regards, **Essa *et al*. (2008)** suggested that polyculture system of Nile tilapia and silver carp was more productive (12.26 kg m^{-3}), and economic than monoculture system of Nile tilapia (5.77 kg m^{-3}). Generally, the polyculture system is considered an economic fish production system regarding its positive effects on water quality, growth performance, and feed utilization parameters, besides improved immune responses and the health status of fish as well. In addition, income above variable cost and net returns were significantly higher from polyculture than monoculture system (**Shoko *et al*., 2016**). Inversely with the current findings, **Abdel-Hakim *et al*. (2014)** reported that the total returns, and economic efficiency significantly increased with each increase of SD of fish. In a previous study, **Mehrim (2009)**, and **Hassan and Mahmoud (2011)** confirmed these findings also. In this regard, **North *et al*. (2006) and Lupatsch *et al*. (2010)** reported that SD is one of the vital factors determining fish production and profitability in aquaculture, as well as the same authors confirmed that HSD might cause chronic stress and reduce fish welfare.

CONCLUSION

Based on the obtained results under the cultivation conditions in the fish farm area (San El-Baharia, El-Hosania, Al-Sharqia Governorate, Egypt), it could be concluded that the appropriate stocking density is 5.62 fish m^{-3} (T_1) for rearing Nile tilapia, *O. niloticus* in a polyculture system with thin-lipped mullet, *C. ramada* and African catfish, *C. gariepinus* for 129 days in earthen ponds. It can also be recommended that more field studies are needed to increase SD of fish reared in the polyculture system, not only with these fish species, but also with other fish species.

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