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Effect of Different Densities on the Rabbitfish (*Siganus rivulatus*) Performance and Health Status Under Biofloc System During the Nursery Phase

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ABSTRACT

A 60-day experiment was organized to determine the optimum stocking density of *Siganus rivulatus* under biofloc system conditions during the nursery stage. The densities of fish samples with 400, 600 and 800fry/ m^3 were subject to evaluation. Nine tanks made of plastic with dimensions of 48 X 35 X 20cm were used for the experiment. The stocked fish fries had an average starting body weight of 0.76 ± 0.03g. All fries were fed a commercial diet containing 40% crude protein (CP) at a rate of 4% of the body weight for six days per week. The highest weight gain (WG), average daily gain (ADG), specific growth rate (SGR), and protein efficiency ratio (PER) were recorded for the fish group stocked at the density of 600fry/ m^3 (2.3, 0.04, 2.32, and 3.27, respectively). In addition, this group showed the best feed conversion ratio (FCR), with a value of 1.22. For histological sections, the histopathological alterations in the gills, liver, and intestines were recorded for the fish group stocked at a density of 800fry/ m^3. Consequently, it could be concluded that, under the biofloc system, the stocking of *S. rivulatus* at medium density (600fry/ m^3) gives the best growth performance, without affecting the welfare of fish throughout the nursery stage.

INTRODUCTION

Fish nursery phase development and habitat are important and critical for population number and activity **(Chambers & Trippel, 2012)**. Knowledge about the requirements of juvenile habitat optimizes growth and survival at the juvenile stage **(Grol *et al.*, 2014)**. Unfortunately, there is limited data in literature on marine fish growth, survival and food of juveniles **(Le Pape & Bonhommeau, 2015)**. It is most likely achievable to increase the amount of fish juveniles by raising the production intensity of fish juveniles in nursery production. Notably, a deficiency of information was recorded on the optimal stocking density for fry in hapas or nursery ponds, which would lead to the highest yields **(Ronald *et al.*, 2014)**. Wastes originated from faeces, uneaten feed and expelled by-products that undergo metabolic processes accumulate rapidly as fish populations grow, which may have a negative impact on water quality **(Suresh & Lin, 1992; Alhassan *et al.*, 2012)**. Recycling aquaculture system (RAS) and bifloc system are examples of the sustainable systems; nevertheless, the older approach is difficult and expensive to implement, while the latter approach owns a great promise as a fish nursery **(Henish *et al*., 2022)** since adding an additional carbon source to the aquaculture system promotes the growth of heterotrophic beneficial bacteria, which in turn, improves water quality **(Suloma *et al*., 2021)**. Being rich in protein, heterotrophic bacteria act as a good food for fish, and at the sametime, they uptake nitrogen through which water quality is improved, water exchange is minimized and the feed cost is reduced **(Avnimelech, 1999; Hargreaves, 2006; Crab *et al*., 2007, 2009, 2010; El-Shafiey *et al.*, 2018; Salama *et al*., 2022)**. The extra natural food provided by heterotrophic bacteria biomass may reduce the competition for food under intensive conditions. The major factor to minimize the stress level posed on fish is the determination of the best stocking density, which helps increasing fish production and profitability **(Samad *et al*., 2005; Mazlum, 2007; Garr *et al.*, 2011; Zhu *et al.*, 2011; Khatune-Jannat *et al.*, 2012)**. The nursery phase of tropical fish farming using a biofloc technology has been limitlessly understood. According to the results of our earlier research, the biofloc system has the potential to serve as a sustainable method for growing *S. rivulatus* at the nursery stage **(Henish *et al*., 2022)**. A steady supply of healthy, disease/ stress resistant juvenile *Siganus rivulatus* is important to be maintained in juvenile production. This investigation was designed to better understand how the *S. rivulatus*'s performance and health could be affected by varying densities of stocking throughout the nursery period under the biofloc system.

MATERIALS AND METHODS

To accomplish the target of this investigation, fish specimens were raised in a lab at Egypt's National Institute of Oceanography and Fisheries (NIOF) in Hurghada.

Experimental fish

The Red Sea was sampled for fish specimens (NIOF). For one week, fish fry specimens were exposed to the controlled environment of the lab to help them adjust. During the adaptation phase, the fish fry samples were given a commercial diet, with 40 percent crude protein.

Experimental design and conditions

Three stocking densities of *Siganus rivulatus* were evaluated, with 15, 20 and 25 fish fries adjusted in 9 plastic tanks, with a capacity of 35L for each, which is equal to 400, 600 and 800 fry/m^3, respectively. On the 1st 3 days of the 60- day experimental study, the dead fish were swapped with live ones. The average starting weight of the fish fries was 0.76 ± 0.03g. For six days a week, the fry samples were given a meal consisting of crude protein equals to 4 percent of their body weight (CP). Each treatment consisted of three plastic tanks (48x35x35cm) that were oxygenated at 5- 6mg/ l, using air stones and a 0.5HP ring blower. The tanks were kept in dark places (no aritificial light source) provided with natural light. To provoke heterotrophic bacteria growth, starch was employed as a carbon source and supplied on a regular daily basis; it was provided to attain the propotion of C/N at 1: 10, which is the proper rate for smtiulating the growth of bacteria **(Avnimelech, 1999)**. During the daylight, the starch solution was poured into the water culture tanks and stirred well to be prepared for spreading on the surfaces of the experimental tanks. Only the water lost via evaporation was replaced, hence there was no net transfer of water.

Water quality

The pH was monitored using a pH meter, while the temperature was tracked with a Senso Direct Oxi 200. (Hanna 981017, USA). The concentrations of nitrate (NO3-N) and nitrite (NO2-N) levels, as well as total ammonia nitrogen (TAN, nitrogen (NH3-N)) and total suspended solids were monitored every week **(APHA, 1998)**. After weekly sedimentation for 15–20 minutes, an Imhoff cone was used to assess biofloc volume **(Avnimelech & Kochba, 2009)**.

Chemical analysis

After the completion of the experiment, a representative sample of both whole fish fry and feed was collected. The fish fries were numbed with an anaesthetic solution containing 40mg/ L of clove oil. The fries were then sealed in containers and kept in the freezer for additional testing. Chemical analysis was then performed on the fry and feed samples using the Kjeltech auto-analyzer (Model 1030, Tecator, Hoganas, Sweden) to assess the proximate composition analysis of diets and fish, where dry matter (DM) and crude protein were evaluated according to the Kjeldahl technique **(Bligh & Dyer, 1959)**. Both ash and ether extract (EE) concentrations were measured. Fish and food were analyzed for their chemical make-up using **AOAC** protocol **(1995)**.

Growth performance

Every 15 days, we recorded the length/ weight measurements for the samples of fish fry to determine their average body weight and length. Constant adjustments were achieved for the amount of feed given to the fries as their weight increased.

The following equations of **Castell and Tiews (1980)** were adjusted to calculate the total weight gain (g), average daily body weight increase (g), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (Kc):

TWG = [final body weight – initial body weight].

ADG= TWG/ duration of the experiment.

SGR % = 100 X (Ln FBW - Ln IBW) / (t);

Where, Ln: natural log; t is the duration of the experiment.

FCR= feed intake/weight gain.

Kc= W (weight) /L^3 (length) X 100.

The survival rate (SR) and protein efficiency ratio (PER) were evaluated in accordance to the study of **Hung *et al*. (1993)** as follows:

SR (%) = (No. of fish at the end of the experiment/ No. of fish at the beginning of the experiment) X 100.

PER= weight gain/ protein intake.

The protein productive value (PPV) was measured according to the equation of **Nose (1971)** as follows:

PPV= (protein gain/total protein intake) X 100

Histological studies

For the analysis of histology, 5 randomly chosen *Siganus rivulatus* specimens were collected from each duplicate. They were anesthetized with (40mg/ L) clove oil, where 2ml of clove oil was introduced to 5 litres of water, and samples were left for 10 minutes **(Simoes *et al*., 2011)**. Samples were cut open to see the internal organs in general. After that, little sections from the intestines, liver and gills were taken out and preserved in 10% neutral buffered formalin. Tissues were dehydrated, cleaned and then fixed in paraffin before sectioning at a thickness of 5µm. Hematoxylin and eosin stains were applied on the serial sections **(Bancroft & Layton, 2013)**.

Statistical analysis

The experimental data were subject to a one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences software **(SPSS, 2013)**. Significance was set at $P < 0.05$. Duncan's multiple range test was used to determine the existence or non- existence of significant differences among the groups under study **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Water quality

The readings for water quality parameter are shown in Table (1). The use of starch as a carbon source facilitated the development of biofloc in the BFT tanks. Floc volume (FV) values rose steadily by time. Direct uptake of dissolved nitrogenous materials from meals and fish excrements by heterophic bacteria was connected to the production and growth of bioflocs in BFT water **(Avninelech, 1999; Ebeling *et al*., 2006; Khalil *et al*., 2016)**. During the whole trial, the BFT tanks received no additional water. Water temperature average was between 25.6 and 28.8 degrees Celsius. The DO concentrations were determined at 8.2mg/L. The range of pH was 7.7 to 8.2. Total ammonia (NH3-N) levels varied between 0.28 and 0.34mg/ L, while salinity was between 32.67 and 43.6mg/ L. According to previously published research, the values recorded for the parameters under study are suitable for *Siganus rivulatus* fries **(ANZECC, 2000; EPA, 2003; Saoud *et al*., 2007; Saoud *et al*., 2008**).

Table 1. Water quality profile of the biofloc system containing different densities of *Siganus rivulatus*

Variable	400 fry /m^3	600 fry /m^3	800 fry /m^3
Temperature (ºC)	27.3±0.57 (25.6-28.1)	27.7±0.13 (27.5-28.1)	27.9±0.49 (26.6-28.8)
pH	7.9±0.06 (7.7-8.1)	7.8±0.06 (7.7-8. 1)	8.0±0.08 (7.9-8.2)
TAN (mg/L)	0.3±0.01 (0.28-0.31)	0.3±0.01 (0.29-0.33)	0.3±0.01 (0.28-0. 34)
DO (mg/L)	8.3±0.2(7.9-9.0)	8.1±0.1(7.8-8.4)	8.2±0.1(8.1-8.4)
Salinity (mg/L)	35.9±1.5 (32. 67-43.6)	35.2±1.3 (33.67-38.50)	35.5±1.5 (33.15-43.6)
Floc volume (mg/L)	17.1±5.9(3.3-30.0)	17.3±6.1(3.2-31.5)	17.7±6.2(3.5-32.0)

Values represent mean ± SE; SE: standard error of means.

Growth performance

Table (1) displays the present study's parameters of growth performance. Specific growth rate, weight increase and average daily gain reached their highest values (2.32, 2.3, 0.04, respectively); these values were associated with the medium stocking density condition (600 fry /m^3); however, no significant difference was deteted with the high stocking density treatment (800 fry/ m^3).

The relation between the fish stocking densities and it's growth performance is controversial. It was reported that the stocking density (up to 770 fish/m^3) under clear water system had no effect on growth dispensation of *S. rivulatus*; however, the schooling behavior might explain the previous finding **(Saoud *et al.*, 2007)**. Growing African catfish in a biofloc system at a medium stocking density of 6 fish/ L may be preferable than growing them at a low (4 fish/L) or high (8 fish/L) density, presumably because of the less agonistic behavior **(Kaiser *et al*., 1995; Van de Nieuwegiessen *et al*., 2009; Fauji *et al.*, 2018)**. Stocking density may impact the developmental performance of the tilapia fish reared in a clear water system, as stated in the studies of **Ayyat *et al*. (2011)** and **Abdel Tawwab (2012)**.

The survival rate of *Siganus rivulatus* ranged between 75.0 & 80.3%, with no significant differences among the experimental treatments. **Ghanawi *et al.* (2010)** evaluated the survival rate of *S. rivulatus* juvenile culture stocked under size grading and clear water system, recording values higher than the current findings (97.8–100%), with no significant differences among treatments. Under a clean water system, **Krummenauer *et al.* (2011)** observed that, the survival rate varied from 75.0 to 92.0 percent depending on the stocking density of 150- 450 shrimp/ m^3. On the othr hand, the survival rate of the Nile tilapia was not remarkably affected by the density of breading according to the studies of **Huang and Chiu (1997)** and **Abdel- Tawwab (2012)**. While, **Fauji *et al.* (2018)** discovered that the biofloc system improved the feed utilization efficiency and growth performance of the nursery production for the African catfish, compared to the clear water system.

Table 2. Growth performance parameters and survival rate of *Siganus rivulatus* grown under biofloc system with various densities

Parameter \ stocking densities	400 fry/ m^3	600 fry/m^3	800 fry/ m^3
IBW	0.76±0.006	0.76±0.007	0.75±0.003
FBW	2.81±0.063[b]	3.07±0.011[c]	2.05±0.017[a]
WG	2.05±0.061[b]	2.3±0.007[a]	1.29±0.02[a]
ADG	0.033±0.003[b]	0.04±0.0001[a]	0.02±0.000[a]
SGR	2.17±0.033[b]	2.32±0.0097[a]	1.66±0.036[a]
SR	80.33±2.31[a]	75.00±2.89[a]	77.78±2.22[a]

Values designate mean ± SE; SE: standard error of means (n=3). The values in the same row with various superscripts show statistical significant difference (P< 0.05).

Feed and nutrient utilization

The correlation between feed intake and feed utilization is shown in Table (3). Both the greatest FCR and highest PER were determined in the medium stocking density treatment of 600 fry/ m3, recording values of 1.22 and 327, respectively. It's possible that the fish fry's increased energy and effort consumption are due to stress brought on by the greater densities. *Oreochromis niloticus* FCR and feed intake were also strongly influenced by rearing density **(Abdel-Tawwab *et al.*, 2010)**. The present investigation found that the maximum PER was achieved in the bioflc system with a medium stocking density treatment (600 fry/m3). Feed utilization efficiency may be impacted by fish density, and hence, the more fish are supplied, the less the food is available for each fish

(Chang, 1988). Conversely, PER and PPV decreased with increasing the Nile tilapia density **(Abdel-Tawwab, 2012)**.

Table 3. Values recorded for nutrient utilization and feed of *Siganus rivulatus* raised under biofloc system, with different densities

Parameter \ stocking densities	400 fry /m^3	600 fry /m^3	800 fry/m^3
FI	2.82±0.006^a	2.82±0.002^a	2.8±0.002^b
FCR	1.38 ±0.038^b	1.22 ±0.004^c	2.16 ±0.034^a
PER	1.81 ±0.05^b	3.27 ±0.56^a	1.74 ±0.081^b
PPV	83.15 ±0.18^b	83.31 ±0.06^b	83.8 ±0.06^a

Values illustrate mean ± SE; SE: standard error of means (n=3). The values in the same row with different superscripts designate statistical significant difference at $P< 0.05$.

Body length and condition factor

Siganus rivulatus samples were raised in a biofloc system at varying stocking densities, and their body length and condition factor (KC) are shown in Table (4). The medium stocking density treatment (600 fry/ m^3) recorded the highest significant final body length. The conditional factor differed significantly among the experimental treatments, where the high stocking density (800 fry/m^3) treatment recorded the highest significant condition factor, followed by the medium stocking density treatment (600 fry/m^3) and the lower stocking density treatment (400 fish/m^3) that did not show any significant difference. The *Liza carinata* final conditional factor under biofloc system conditions was significantly improved from 30 fry/m^2 to 40 fry /m^2 then decreased by increasing the stocking density to 50 fry/ m^2 **(Khalil *et al*., 2016)**.

Whole body chemical composition

Table (5) displays the study results of the *Siganus rivulatus* whole-body chemical composition. It's important to note that, the stocking density has a major impact on the overall chemical makeup. Moreover, the rearing density recorded a substantial effect on the moisture, crude protein, crude lipid and ash levels of the Nile tilapia raised in clear water **(Abdel-Tawwab, 2012)**. The rate at which proteins and lipids are synthesized and deposited in fish muscles may be related to overall body variations in protein and lipid composition **(Fauconneau, 1984; Abdel-Tawwab *et al.*, 2006)**. Lower and medium stocking densities (400 and 600 fry/ m^3) showed the greatest significant crude protein and crude fat levels. Whereas, the high stocking density treatment (800 fry/ m^3) displayed the most significant moisture and ash levels.

Table 4. The conditional factor (K_C) and starting/ final body lengths of *Siganus rivulatus* reared under biofloc systems with varying densities

Parameter \ stocking densities	400 fry /m^3	600 fry /m^3	800 fry/m^3
Initial body length (IBL)	3.9±0.021	3.9±0.025	3.9±0.022
Final body length (FBL)	6.56±0.035^b	6.87±0.06^a	5.69±0.094^c
K_C	0.99±0.007^b	0. 95±0.021^b	1.12±0.048^a

Values clarify mean ± SE; SE: standard error of means (n=3). The values in the same row with different superscripts indicate statistical significant difference at *P*< 0.05.

Table 5. The chemical composition of *Siganus rivulatus* whole fish fry reared under biofloc systems with different densities

Parameter \ stocking densities	400 fry /m^3	600 fry /m^3	800 fry/m^3
Moisture	72.46±0.36^b	71.74±0.22^b	75.95±0.01^a
Crude protein	57.31±0.13^a	55.23±0.23^b	56.89±0.047^a
Crude lipid	15.07 ±0.024^c	10.73 ±0.40^a	12.45 ±0.37^b
Ash	14.45 ±0.18^b	16.42 ±0.36^a	16.02 ±0.13^a

Values designate mean ± SE; SE: standard error of means (n=3). The values in the same row with different superscripts illustrate statistical significant difference at *P*< 0.05.

Histological studies of selected organs of *Siganus rivulatus*

The gills of the lower stocking density treatment (400 fry/ m^3) showed normal histological structure, with normal primary and secondary gill lamellae (Fig. 1A). However, the gills of the medium and high stocking density treatments (600 and 800 fry/ m^3, respectively) showed some histopathological alterations, such as hyperplasia (Fig. 1B), swelling (Fig. 1B, C) and edema of the secondary lamellae (Fig. 1C). It is obvious that the stocking density, mainly the high stocking density, adversely affects the gills histology as mentioned in the study of **Wang *et al.* (2019)**. Such histopathological alterations coincide with those reported in the work of **Paswan *et al*. (2022)** upon rearing *Labeo rohita* under high stocking densities. This is considered as a stress response mechanism during hypoxia as mentioned by **Sollid *et al.* (2003)** regarding the curcian carp.

The liver of *Siganus rivulatus* of the lower and medium stocking density treatments (400 and 600 fry/ m^3, respectively) showed normal hepatic histological structure that consists of a compact mass of hepatocytes radiating from central veins and interrupted by blood sinusoids (Fig. 2A, B). On the contrary, the high stocking density treatment (800 fry/ m^3) showed mild histopathological alteration, including a mid-zonal area of liquefactive necrosis (Fig. 2C). It is worthy to mention that, the overcrowdness of fish in the tanks of the high stocking density treatment (800 fry/ m^3) may have triggered bacterial, viral or fungal infections, causing such histopathological alteration. The hepatocytes necrosis in the Nile tilapia can be attributed to ammonia exposure **(Benli *et al*., 2008)**. While, in channel catfish, it may be related to hypoxia **(Harper & Wolf, 2009)** or high stocking density as in the case of the largemouth bass **(Wang *et al*., 2019)**.

The intestines of the lower and medium stocking density treatments (400 and 600 fry/ m^3, respectively) showed normal histological architechture with normal gastric glands (Fig. 3A, B); while for the high stocking density treatment, marked degenerative changes of the gastric glands were associated with mononuclear inflammatory cells infiltration (Fig. 3C). As a result, cells in the intestine were negatively affected by stocking densities that were extremely high. This histopathological change is consistent with the findings for Nile tilapia **(Abdalqadir, 2014)** and channel catfish **(Refaey *et al*., 2018)**, showing that the increased stocking densities cause a decline in the length and intensity of the intestine's villi, which in turn indicates a smaller absorption area of intestine, resulting in weak growth and low efficiency of nutrients.

Similarly, high stocking densities reduced the thickness of the muscosal layer in the intestines of the Nile tilapia **(Abdalqadir, 2014)** in addition to the quantity & size of goblet cells in the intestines of channel catfish **(Refaey *et al*., 2018)**. The intestinal muscle thickness, intestinal goblet cell size and intestinal goblet cell quantity decreased in fish raised at high stocking densities **(Wang *et al.*, 2020)**.

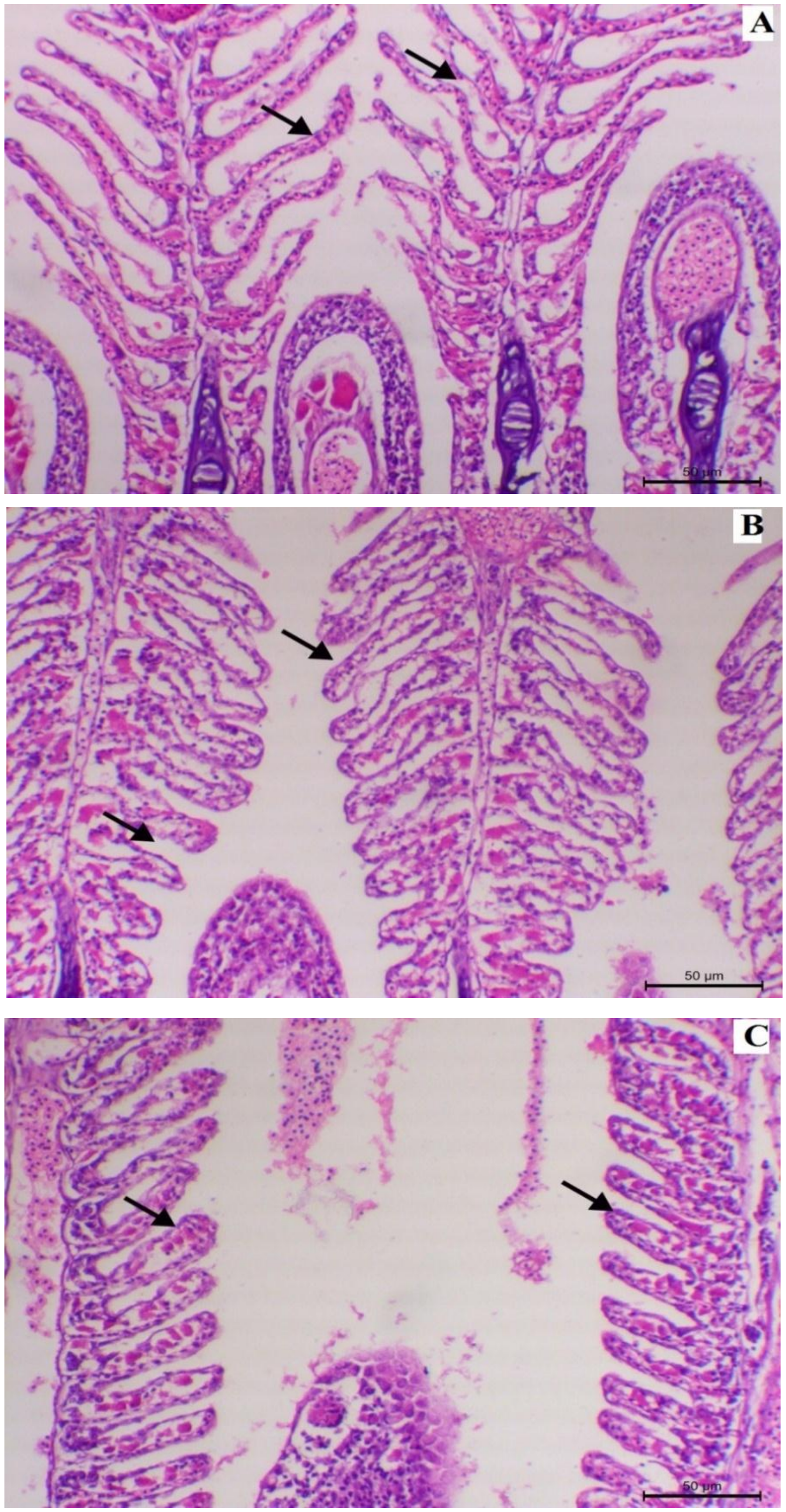

Fig. 1. Photomicrographs of sagittal sections of the gills of *Siganus rivulatus* reared under biofloc systems with different stocking densities. (A) 400 fry/m^3 showing normal gills architechture with primary (arrow) and secondary (arrows) lamellae. (B) 600 fry/m^3 showing swollen secondary lamellae. (C) 800 fry/m^3 showing swelling and edema of the secondary lamellar (arrow). HX & E stain.

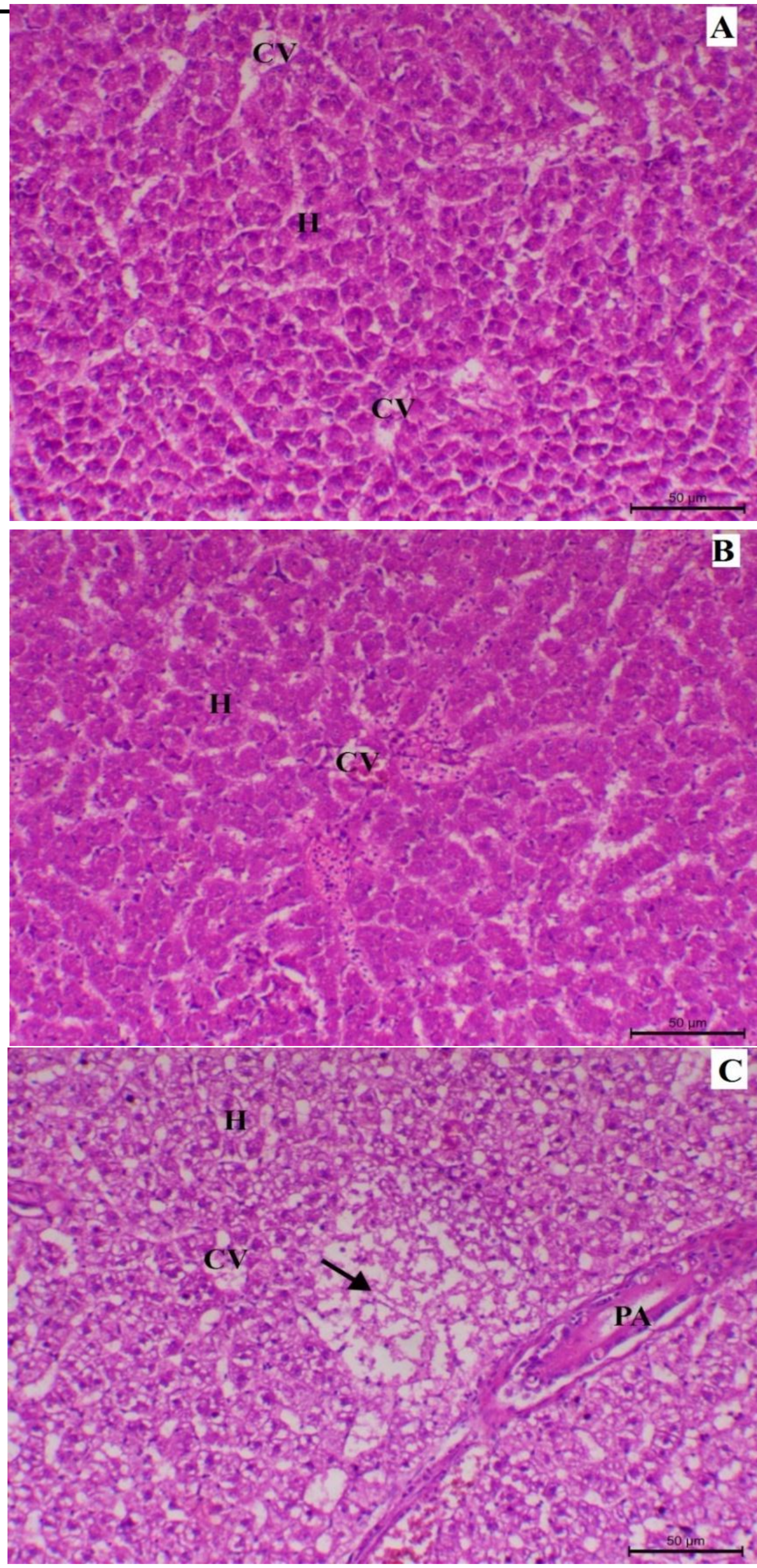

Fig. 2. Photomicrographs of S. of liver of *Siganus rivulatus* reared under biofloc systems with different stocking densities. (A) and (B) 400 fry/m^3 and 600 firy/m^3 , respectively, showing normal liver architechture. (C) 800 fry/m^3 showing liquefactive necrosis (arrow). HX & E stain. Abbreviations: C.v, central vein; H, hepatocyte. HX &E stain

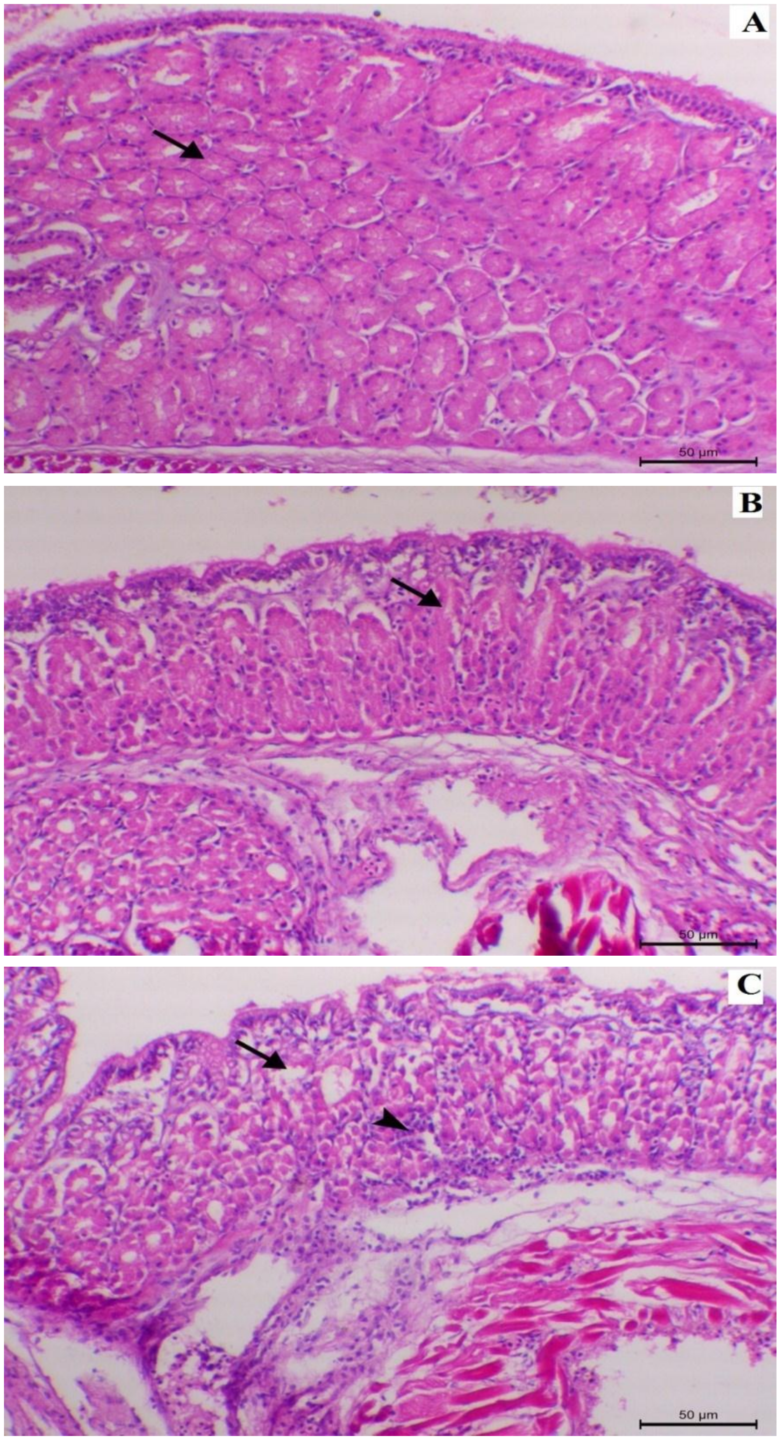

Fig. 3. Photomicrographs of T.S. of the intestines of *Siganus rivulatus* reared under biofloc systems with different stocking densities. (A) and (B) 400 fry/m^3 and 600 fry/m^3, respectively, showing normal intestinal architechture with normal gastric glands (arrows), (C) 800 firy/m^3 showing gastric glands degeneration (asterisk) and mononuclear inflammatory cells infiltration (arrow head). HX &E stain

CONCLUSION

It could be concluded that, *Siganus rivulatus* cultivation gives the best production and growth performance for fish group stocked at 600 fry/ m^3 during the nursery phase under biofloc system condition. The medium stocking density (600 fry /m^3) improves the water quality and provides nutrients for fish, as well as continuously minimizing the competition and stress. Moreover, its safe impact on the health state of the cultured fish was proven, as evidenced by the histological sections.

ETHICS STATEMENT

All procedures of animal experiment were achieved according to the Ethics Care and Animals/ Aquatic Animals of National Inst. of Oceanography and Fisheries (NIOF), Egypt (NIOF- AQ1- f- 23- R- 021).

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