Modern Environmental Science and Engineering (ISSN 2333-2581)
March 2022, Volume 8, No. 3, pp. 186-194
Doi: 10.15341/mese(2333-2581)/03.08.2022/006
Academic Star Publishing Company, 2022
www.academicstar.us

Growth Performance of Life (*Trichomycterus punctulatus*) Raised in an Intensive Recirculating Aquaculture System

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Abstract: This research was conducted to assess the effect of stocking density on the growth performance of Life (*Trichomycterus punctulatus*) reared in an intensive recirculating system. For this purpose, an increasing stimulus experimental design was carried out with a control and three treatment groups: 3.68 fish/L (control), 4 fish/L (treatment 1), 4.5 fish/L (treatment 2) and 5 fish/L (treatment 3). Experimental fish were raised in recirculating aquaculture tanks for six months and fed a commercial pellet feed of 50% protein, the first and second month, and 45 % protein the four remaining months of the experiment. Fish biometry was performed monthly, taking a sample of 15 fish from each tank. The physico-chemical parameters of the rearing water were recorded fortnightly. The results revealed that fish growth was significantly ($p < 0.05$) affected by stocking density, positively. The highest growth performance in terms of final body weight and length was observed at a stocking density of 5 fish/L (treatment 3): 11.81 cm and 18.6 g. The highest total production (2.75 kg), net production (91.76 kg/m^3), and the best feed conversion ratio (FCR): 1.49, were also noted in treatment 3. The used recirculating system provided good water quality as the physico-chemical parameters were found to be suitable for fish growth throughout the study.

Key words: aquaculture, intensive recirculating system, trychomycterus punctulatus, stocking density

1. Introduction

Aquaculture has become a leading animal production industry with a remarkable growth rate in the last 20 years [1], with an estimated meat production of 82 million tonnes in 2018 [2]. In developing countries, this industry is considered by governments as a mean for fighting poverty and hunger; therefore, policies are being implemented to encourage aqua-farmers to develop sustainable aquaculture by farming either introduced or cultivable local fish species under non-conventional culture systems [3].

Life (*Trichomycterus punctulatus*) is a freshwater fish species of great economic and nutritional importance, widely distributed in the north coastal regions of Lambayeque, Piura, and La Libertad in Peru. This species has great potential for aquaculture and as a promising marketable fish species because of its tolerance to high stocking densities [4, 5], gregarious behaviour, meat quality, excellent taste and body shape. Moreover, Life (*T. punctulatus*) is characterized as an omnivorous species with a considerable preference for insect larvae [6], suggesting that this species needs a high protein requirement necessary to sustain normal growth under culture conditions.

Fish growth under farmed conditions is affected by stocking density, a major factor that, if not considered seriously, might prevent fish from getting reasonable weight and length for market [7, 8]. The density factor effects may be mainly divided into two categories: the density dependent and the density independent. The former category affects significantly negatively the

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growth of fish [9-11], and the latter affects significantly positively this feature.

Rearing fish at high stocking densities, if measures are not taken, leads to stress and immune competence issues due to factors such as social interactions and deterioration of water quality [12], causing ultimately reduced growth and food utilization [13]. Consequently, in the aquaculture industry, it is critical to identify the optimum stocking density for a given species besides environmental conditions, genetics and food supply quality [14, 15].

In intensive aquaculture, water quality is a big concern since tanks are stocked with high densities of fish. To tackle this issue, new ways to farm fish have been developed such as recirculating aquaculture systems designed to filter, clean, and recycle the water through fish culture tanks. This system is well known for its technology to minimize water replacement, compensate for an insufficient water supply, and ultimately to maintain water quality when farming fish at high densities, things that certainly can not be achieved by traditional methods of growing fish outdoors [16]. Furthermore, recirculating systems will work more effectively if they are at least provided with aeration, mechanical filters to remove particulate matter, and biofilters to remove waste ammonia and nitrite [17].

Although little research has been done to assess the farming potential of Life fish in recirculating systems, studies carried out under monoculture, semi-intensive and intensive polyculture systems, feeding low protein diets, found this species present slow growth rates; however, they have tolerated high stocking densities as fish reared at higher densities were found to grow better than those reared at low densities [4, 5]; therefore, the growth of this species may be favoured by the stocking density positively. The aquaculture research of Life (*T. punctulatus*) should undergo continuous evaluation, addressing different issues such as, among others, stocking density and water quality to broaden the knowledge base of this species in an effort to make this economic activity sustainable and profitable.

This situation led to conduct this research to evaluate growth performance (body weight and length) of Life (*T. punctulatus)* cultured at four stocking densities in an intensive recirculating aquaculture system and select the treatment that provides the best growth and production performance. Based on the gregarious behavior of the fish, the study hypothesized that growth performance will be significantly affected by stocking density positively, whereby fish reared at higher stocking densities will attain the best final body weight and length.

2. Material and Methods

The experiment consisted in a 6-month rearing trial from December, 2018 to May, 2019 and was conducted indoors in a private facility located at 6°41'46" S, 79°54'40.1" W in the region of Lambayeque, Peru.

Four recirculating plastic tanks measuring 60 L volume each were filled with 30 litres of chlorine-free water and provided with aeration (SOBO Air Pump SB-548A). Furthermore, water treatment systems, which consisted of 12-liter buckets equipped with a mechanical filter at the top and a biological filter at the bottom, were placed at a higher position with respect to the tanks. A submersible pump was also placed inside each tank to lift the culture water to the water treatment systems, and the treated water would return by gravity to the rearing tanks (Fig. 1).

The experimental system consisted of four groups: one control and three treatment groups. A set of 515 Life juveniles with initial mean average weight and length (± standard error) of 3.53±0.37 g and 0.8±0.37 cm, respectively, were obtained from the Piura River, Peru and transported in 20-liter plastic buckets to the facility where the study was carried out. On the arrival at the facility, all fish juveniles collected from the river were gradually acclimatized for about 20 minutes to adjust to the water temperature of the plastic tanks. The culture tanks properly labelled were stocked with 110 (control), 120 (treatment 1), 135 (treatment 2), and 150 fish (treatment 3) giving a stocking density of 3.68, 4, 4.5, and 5 fish/L, respectively. The stocking density of the control was based on the results of a previous study [4].

The experimental fish were fed a commercial pellet feed of 50% protein (1 mm diameter pellet), the first and second month at a rate of 2% of fish biomass; and 45% protein (2 mm diameter pellet) at 1.5% of fish biomass, the third and fourth month, and at 1.25% of fish biomass the fifth and sixth month of the experiment. The fish were fed manually twice daily at 06:00 and 16:00 h.

Fig. 1 Recirculating tanks and water treatment systems for intensive culture of fish at different stocking densities.

Biometry of fish was performed monthly, taking a random sample of 15 fish from each tank. Body weight and total length were recorded with a 25-ml graduated cylinder and a 0.5 kg/0.1 g digital scale, respectively.

The environmental and water temperature were monitored daily at 07:00 and 13:00 h, with a digital thermometer (BOECO, model SH-144, Germany). Dissolved oxygen, pH, ammonium (NH^{4+}), nitrates (NO^{3-}), nitrites (NO^{2-}), and total alkalinity were determined every two weeks using a reliable colorimetric kit (JBL ProAquaTest®, Germany).

To determine the effect of stocking density and time on fish growth, analysis of variance was applied for a two-factor fixed factorial design [18]. The Duncan's multiple rang test was used to determine which treatment showed the best growth performance. Office Excel 2010 was used for data handling and Statistical analysis was performed using SPSS statistics package version 22.0, at confidence interval of 95%.

3. Results and Discussion

3.1 Growth Performance of Fish

At the end of the experiment, fish growth was found to be affected significantly ($p < 0.05$) by stocking density positively (Table 1) since fish cultured at high densities grew better than those cultured at low stocking density. In our experiment, the highest growth was achieved at a stocking density of 5 fish/m^2 (treatment 3): 11.8 cm and 18.6 g. The better average weight and length of fish seen in treatment 3 group was observed from the first month of the experiment with respect to the control and treatment 1 groups, and from the second month in relation to treatment 2 group (Fig. 2). Fish weight was affected by stocking density and time ($p < 0.05$) but not by the interaction of both factors ($p > 0.05$); however, fish length was affected by both factors and their interaction ($p < 0.05$) as seen in Table 1.

Table 1 Analysis of variance to determine the effect of stocking density, time and their interaction on the growth of Life (T. punctulatus) reared in an intensive recirculating system.

	Weight		Length	
Source of Variation	F	P value	F	P value
Treatments (T)	29.16	0.000*	25.58	0.000*
Time (t)	119.59	0.000*	125.19	0.000*
Interaction (T x t)	2.10	0.666	1.46	0.000*

* Significantly different. Treatments refers to the different stocking densities.

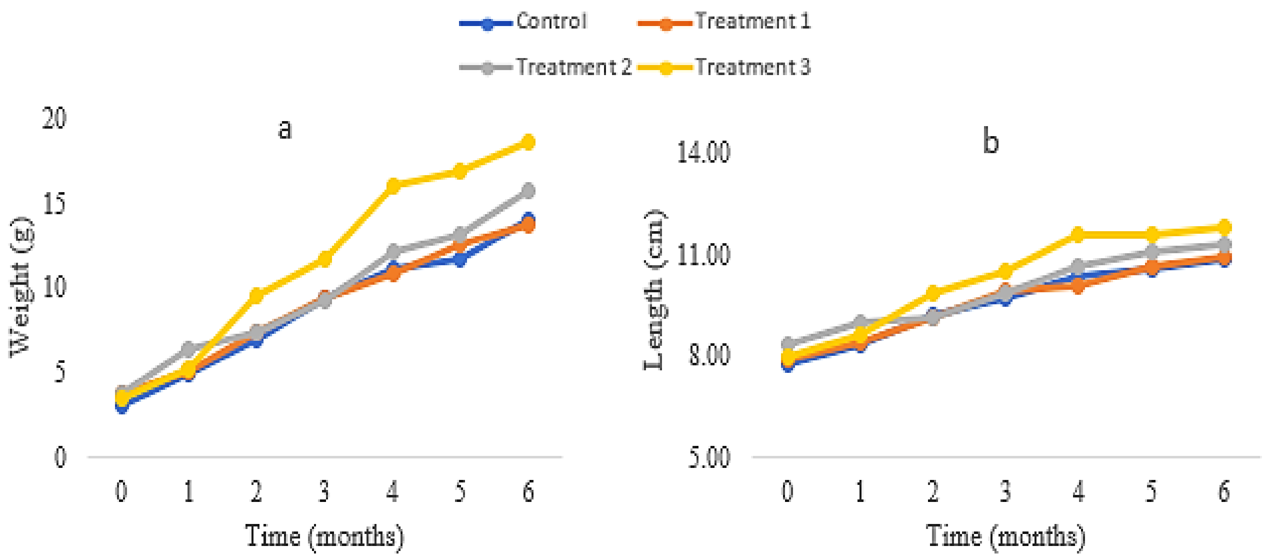

Fig. 2 Weight (a) and length (b) of fish against time in months reared at different stocking densities in an intensive recirculating system.

These results confirm the hypothesis proposed earlier, since, in the present study, the best final weight and length of fish was obtained at a stocking density of 5 fish/L. This can be explained by the fact that *T. punctulatus* is a gregarious species and thus the stress on fish caused by social interactions and crowdedness may have had little or none impact on this species as it happens for other fish species [19, 20].

Our results correspond with those by López and Lora (2013) [4], who cultured this species in an intensive recirculating system at stocking densities of 2.21, 2.94 and 3.68 fish/L, fed 32% protein diet and chopped earthworm (*Eisenia foetida)*, finding that the fish grew better at the highest stocking density (7.45 cm; 4.38 g); likewise, Correa and Guevara (2013) [5], working with the same culture system and stocking densities of 1, 1.5 and 2 fish/L, supplementing with 32% protein diet, found the highest growth at the two higher stocking densities (9.9 cm; 5.95 g); however, the growth in weight and length achieved in this study are greater than obtained by the aforementioned authors, which is partly explained by the higher protein levels used in the current experiment (45-50%) and the higher stocking densities as well.

The observed growth performance from the present study is greater than those reported from a series of semi-intensive monoculture on-farm trials conducted in earthen ponds [21, 22]. The former study recorded a final weight and total length of 16.22 g and 11.5 cm, respectively, after rearing Life (*T. punctulatus)* for six months at a density of 4 fish/m^2 using a formulated feed containing 22% protein. The latter reported similar findings (17.23 g and 11.9 cm of final weight and length, respectively) for the same species and density, but fish were fed 35% protein feed.

Therefore, the best growth performance of Life (*T. punctulatus)* found in this experiment compared to the above studies could be associated with the higher dietary protein level (45-50%) used throughout the experimental period. This, however, may not be the only reason for the obtained results, as in the current study a different aquaculture system that provides good water conditions was used.

3.2 Production Performance

The total production (kg) and net production (kg/m^3) determined at harvest showed a strong trend to increase with increasing stocking density (Fig. 3). The highest total and net productions were obtained at a stocking rate of 5 fish/m^3 (treatment 3) with 2.75 kg and 91.76 kg/m^3, respectively. This trend may be attributed to both the higher stocking density and the individual weight of the fish at the end of the experiment. These results are in agreement with previous studies on the same fish species and culture system, reporting that production of Life (*T. punctulatus)* is generally dependent on the stocking density [4, 5].

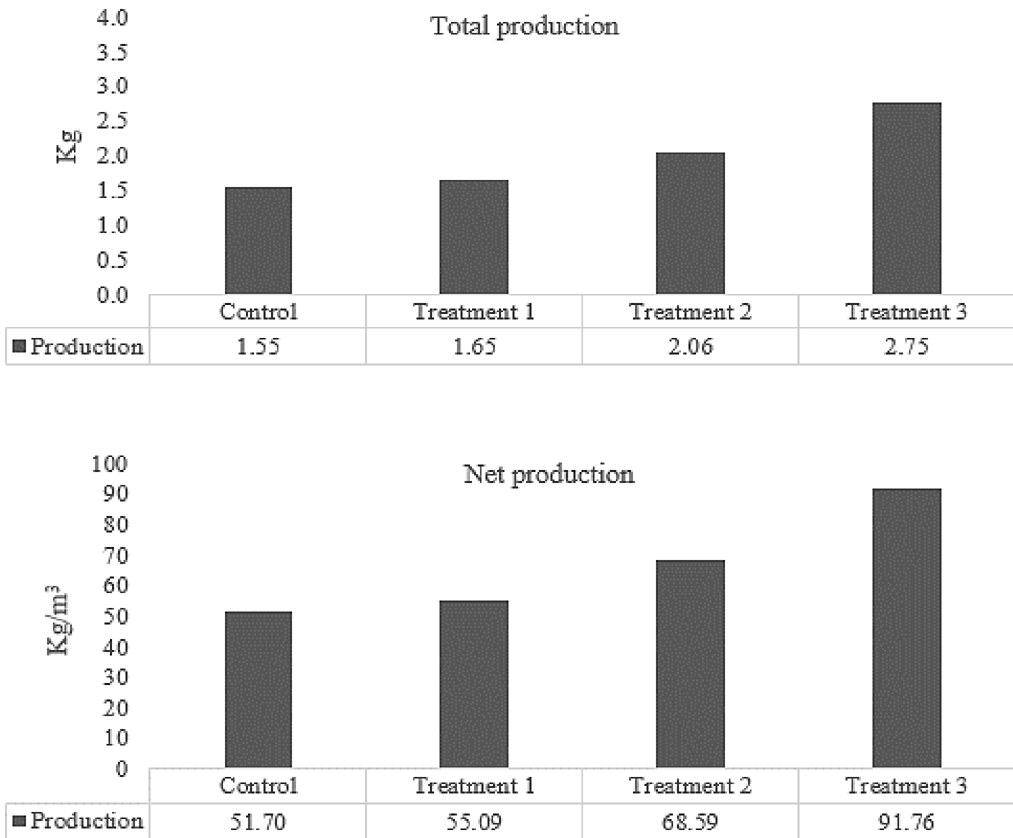

Fig. 3 Total and net productions of fish reared at different stocking densities in an intensive recirculating system.

3.3 Feed Conversion Ratio and Feed Efficiency

The feed conversion ratio (FCR) was found to decrease with increasing stocking density in all treatments except for the control group. The opposite trend was observed with the feed efficiency (FE) values (Fig. 4). The best FCR and FE were observed at a stocking density of 5 fish/L: 1.49 and 67.26%, respectively; which is mostly because of the higher growth and production of fish.

The FCR, in the present study, was lower to those reported by López and Lora (2013) [4] with an FCR value of 5.27 and Correa and Guevara (2013) [5] with 3.52. Although these studies were also conducted with the same fish species, the different results obtained could probably be due to the high protein level used in this study which would have better satisfied the nutritional requirements of *T. punctulatus*.

3.4 Fish Mortality

Mortalities during the experiment were of low significance and only occurred at stocking densities of 4.5 and 5 fish/L with 3.05% and 1.35%, respectively. This fact might be explained by the great tolerance of this species to high stocking densities as well as by the good water quality conditions provided by the recirculating culture system, which proves the importance of this method as a safe and efficient mean for rearing fish at high densities.

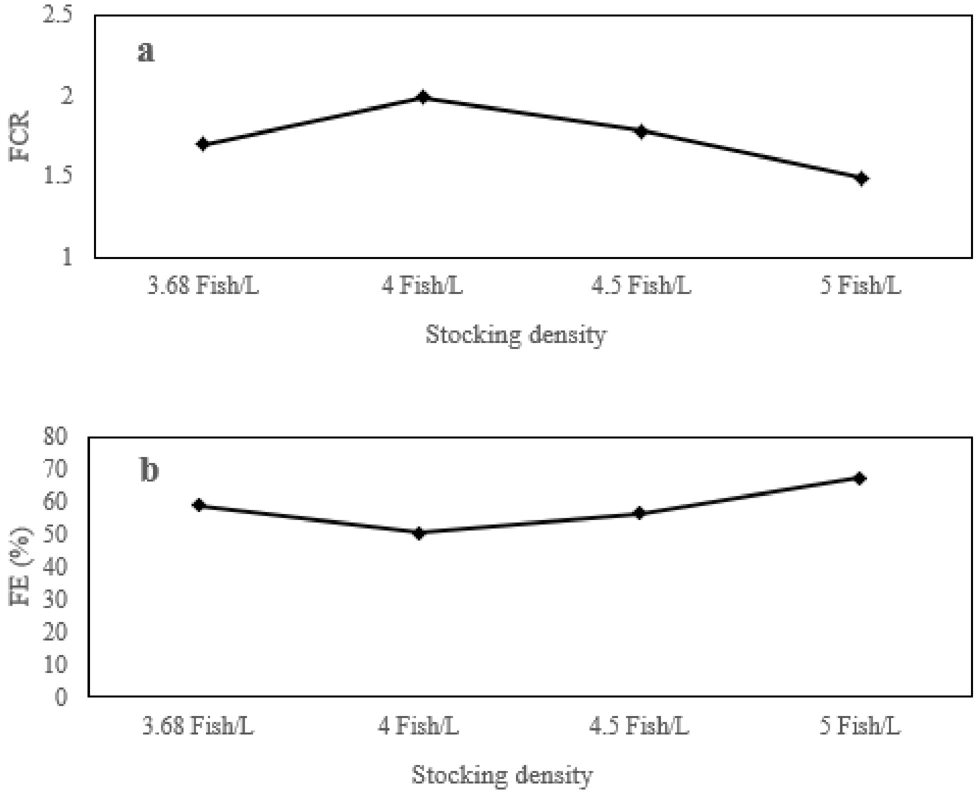

Fig. 4 The relation between stocking density and feed conversion ratio (FCR) (a), and feed efficiency (FE) (b).

3.5 Water Physico-Chemical Parameters

Beside stocking density, water quality plays a critical role in aquaculture. In this study, the water temperature of the recirculating tanks remained above 27°C until the fourth month, then a decrease was seen in the last two months of the study. On the whole, this parameter ranged from 25.22°C to 28.35°C due to the changing seasons while the study was being conducted. These values are considered suitable for farming warm-water fish [23] and for siluriformes culture [24], as it is the case of Life (*T. punctulatus*). The environmental temperature showed a similar behaviour, ranging from 26.13 to 28.93°C.

The water dissolved oxygen slightly decreased with increasing stocking density. Values varied from 4 to 8 mg/L. Miao (1992) [25] reported that higher stocking density led to lower dissolved oxygen and suggested that the resulting changes in this parameter might have a detrimental impact on growth and survival of fish, however, no negative effects on fish were observed in all groups. Furthermore, in this study, the oxygen concentrations are in agreement with Kubitza (2006) [26], who recommends values above 4 mg/L for recirculating systems.

As for the pH parameter, very similar levels in all treatments were observed, ranging from 6.2 to 7.5. Although, these values were not suitable as considered by Kubitza (2006) [26] for recirculating systems (pH:7 to 8); the recorded range has been reported as optimum for fish growth and yield [27], and no signs of strange behaviour in the fish due to this parameter was noted, which probably indicates their good tolerance to changes of this abiotic factor in the rearing water.

One of the principal nitrogenous waste in intensive aquaculture is ammonium (NH^{4+}), whose levels were found to increase as the stocking density increased. A variation from 0.02 mg/L to 0.15 mg/L was observed, which could be explained because of a larger population of fish will generate higher amounts of nitrogen-containing excreta since fish are not able to assimilate all the ingredients of the diet, including protein [28]. Nevertheless, the levels registered in the present study are suitable for fish well-being, since Kubitza (2006) [26] states that they should be less than 0.2 mg/L for recirculating systems.

Two other nitrogen compounds are known to be of importance in aquaculture: nitrate (NO_{3^-}) and nitrite (NO_{2^-}). As for nitrates, the values in this experiment showed the same trend as the ammonium. In general, they were between 30 mg/L and 350 mg/L, beyond the optimum range for fish rearing (less than 50 mg/L). Nevertheless, the recorded values did not reach a dangerous level of more than 400 mg/L, considered by Kubitza (2006) [26] for recirculating systems. The increase in nitrate concentration might be due to the reasons already explained for ammonium.

Nitrites (NO^{2-}) in the water of the culture tanks showed a similar behaviour to ammonia and nitrates, increasing in direct relation to the stocking density as well as with the course of the experiment. The concentrations ranged from 0.4 mg/L to 5 mg/L, which were found to be above the suitable level (less than 0.3 mg/L) for keeping fish under intensive culture conditions [26] since high nitrite concentrations in water, and therefore in fish blood, can impair physiological functions like the capacity of haemoglobin to transport oxygen [29]; however, no evidences of anoxia and occurrence of diseases were observed throughout the experimental period in all groups.

Finally, alkalinity levels reached very close values among all groups, ranging from 35.80 mg/L to 89.50 mg/L, which, as reported by Boyd (1990) [23] and Stickney (1994) [30], are within the range of normality (30 to 300 mg/L) for aquaculture.

Overall, in all treatments including the control, water conditions did not fluctuate considerably throughout the experiment, which probably could be attributed to a proper working of the recirculating aquaculture system used in the current study, an advantage of this fish-farming method that has been previously reviewed [31].

4. Conclusion

The results of this study suggest that growth performance of Life (*Trichomycterus punctulatus*) reared in an intensive recirculating system is positively affected by the stocking density. The best final body weight and length, production, feed conversion ratio (FCR), and feed efficiency (FE) were found at the highest stocking density of 5 fish/L. Furthermore, the recirculating system used provided fairly good water quality conditions as the physico-chemical parameters were suitable for rearing this fish species.

We recommend further research, stocking fish at densities higher than 5 fish/L in intensive recirculating systems and perform economic analysis, so based on results, the Peruvian aquaculture industry will be encouraged to rear this species under this ecofriendly and sustainable culture system.

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