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Effects of Density Stress on Growth Indices and Survival Rate of Goldfish (*Carassius auratus*)

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Abstract: To evaluate the effect of stocking density on survival and growth rate of goldfish (*Carassius auratus*), after 2 weeks adaptation to the new condition, 126 of fish with initial body weight 3.50±0.27 g were distributed randomly among 12 aquaria (76 liter) to stocking densities 6, 9, 12 and 15 fish per aquarium, respectively. The experiments were continued for 5 months in triplicates. Fish were hand-fed two times daily up to 3% body weight/day. Biometric characteristics of fish including total length (cm), weight (g), growth rate (g), specific growth rate (g/day), feed conversion ratio (FCR) and condition factor were determined every two weeks. After the end of the trial period, the average weight of fish in each group was recorded 14.79±2.61, 15.98±0.32, 33.15±1.47 and 14.2±0.30 g, respectively. With the increasing density of goldfish, growth indices, including secondary weight, weight gain, specific growth rate and feed conversion ratio showed a significant difference ($P<0.05$), but condition factor did not show significant different ($P<0.05$). Survival rate were not significantly different among different densities ($P>0.05$). The results of this study indicate that density is significantly affected the growth, but had no significant effect on survival.

Key words: Goldfish · Stress · Density · Growth · Survival

INTRODUCTION

The goldfish (*Carassius auratus*) is a freshwater fish in the family Cyprinidae that is similar to common carp (*Cyprinus carpio*) in biological and nutritional aspects [1]. The goldfish associated with the culture and beliefs of people around the world and is also an important fish aspects of research and economically. The goldfish is the most extensively studied species with respect to reproductive physiology and hormonal control [2].

Stress is a physiological cascade of circumstances that happens when the organism is trying to reestablish homeostatic norms or resist death against an insult [3]. Under natural situation fish often experience short periods of stress, inbreeding about a provisional disturbance of homeostasis [4]. Different taxa of fish have different tolerances to stress [3]. This mentions that for a particular stressor, intensity may vary depending on the fish species [3].

Potentially, the growth of fish affect by several biotic factors including fish population density, potential competitors and availability of food [5].

In aquaculture, increasing in stocking density is one of the solutions for the problems of lack of land for cultivating. A lot of farmed species, growth is inversely correlated with the stocking density and this is especially due to social interactions during competition for food or living space, creating a kind of chronic stress which can affect on the growth of fish [6].

Stocking density is an important factor that affect on growth, efficiency and reproductive performance in fish. Specific stocking density can have positive and negative effects on fish growth [7]. Variation stocking density of fish may change growth and survival rates [8]. Fish larvae have slow growing and low survival rates at high density [9]. Several studies have investigated the effects of stocking density on different farmed species growth including rainbow trout (*O. mykiss*) [10], common carp (*Cyprinus carpio*) [11], endangered mahseer (*Tor putitora*) [12] and Thai climbing perch (*Anabas testudineus*) [13]. On the other hand, studies on Giant gourami (*Osphronemus goramy*) [6] showed that density had no significant effect on the growth indices of the fish.

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High stocking densities in many species indicate that this technique can have negative effects on growth indices and survival in some species [11]. At different densities, food availability is different. Competition for intake food is an important and limiting factor on fish growth and fish competition and aggregation behavior increase in conditions of food deficiency [14]. Survival, growth indices and water physical and chemical factors affect by density [11]. This study investigates the effects of different stocking density on the goldfish growth and survival indices to achieve healthy fish and obtain rearing optimal conditions.

MATERIALS AND METHODS

This study was done for 5 months in the Research Center of Aquaculture of Barabadi Fazli martyr, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. At the beginning of trial, one hundred and twenty-six goldfish (*Carassius auratus*) (averaged weight 3.5±0.27g) were obtained from ornamental fish reproduction center in Golestan, Iran. Then, after 2 weeks adaptation to the new condition, fish were divided into 12 groups (three replicates each of four stocking densities) of similar mean weight. Experimental stocking densities (groups 1, 2, 3 and 4) comprised 6, 9, 12 and 15 fish per aquarium, respectively. Fish were hand-fed two times daily up to 3% body weight/day by energy concentrate food diet (made in Thailand). During rearing period, environmental parameters (pH, oxygen) were measured two times daily. Oxygen saturation varied between 8.15 to 8.71. Also, pH varied between 8.02 to 8.27. Water temperature (21.70±0.14) was constant in all aquaria. Fish biometric were performed every two weeks to survey growth indices. The growth parameters were including body weight increasing (BWI), condition factor (CF), Specific growth rate (SGR), Food conversion ratio (FCR) and Survival rate (SR). To measure body weight increasing and condition factor were used the following formulas:

Where W is the fish weight (g), Wt_1 is the initial weight of fish (g), Wt_2 is the final weight of fish (g) and L is the total length of fish (cm).

To measure specific growth rate was used the below formula:

$$SGR= ((LnWt_2-LnWt_1)/ t_2-t_1) \times 100$$

Where $LnWt_1$ and $LnWt_2$ are respectively the natural logarithm of initial weight of fish and natural logarithm of final weight of fish and t_2-t_1 is the growing period.

For measure Feed Conversion ratio was used the following formula:

$$FCR= \text{eaten food rate (g)}/ \text{increased weight gain (g)}$$

The following formula was used to measure the percentage of survival:

$$\text{Survival Rates} = (\text{initial number-casualties number}) \times 100$$

Statistical Analysis: Data from growth indices and survival in four treatment 6, 9, 12 and 15 fish per aquarium were determines by one-way analysis of variance (ANOVA), followed by Duncan's test in level 95 percentage (α=0.05). Statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

Monthly mean water temperatures ranged was 21.70±0.14. Dissolved oxygen content did not show much variation ranging from 8.15 to 8.71 (Table 1). Because of water replacement no significant differences were found in water quality parameters so possible variation in growth can be correlated with stocking density.

Variance analysis results and data comparison (mean±SD) in the densities are presented in table 2 and figures 1, 2, 3 and 4. As shown in table 2, significant differences were not found between weight mean and length mean of fish in different treatments during 5 months the experimental period ($p<0.05$).

Table 1: Changes in water temperature, pH and oxygen concentration recorded in different treatments during the experiment of period

Variable	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Density (fish/aquarium)	6	9	12	15
Temperature (°C)	21.70±0.14[a]	21.70±0.14[a]	21.70±0.14[a]	21.70±0.14[a]
pH	8.02±0.64[a]	8.05±.78[a]	8.12±0.65[a]	8.27±0.24[a]
DO (mg/l)	8.71±0. 21[a]	8.52±0.31[a]	8.32±0.33[a]	8.15±0.52[a]

Simillar superscript letters in the same row indicate no significant differences among the experimental groups ($p<0.05$).

Table 2: Analysis of variance and comparison of data (mean±SD) of weight (g) and length (cm) of goldfish in different treatments

Variable	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Density (fish/aquarium)	6	9	12	15
Initial weight (g)	2.90±0.90[a]	3.89±0.18[a]	3.83±0.30[a]	3.41±0.48[a]
Final weight (g)	14.79±2.61[a]	15.98±0.32[a]	15.33±1.47[a]	14.20±0.30[a]
Initial length (cm)	5.96±0.30[a]	6.31±0.59[a]	6.22±0.31[a]	5.94±0.35[a]
Final length (cm)	8.57±0.60[a]	8.68±0.03[a]	8.43±0.31[a]	8.31±0.09[a]

Simillar superscript letters in the same row indicate no significant differences among the experimental groups (p<0.05).

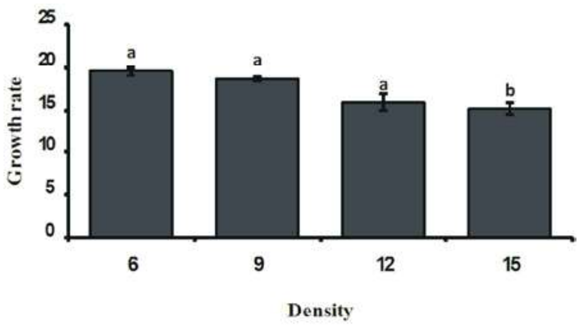

Fig. 1: Changes of growth among different treatments (Different English letters indicate significant difference among treatment at 0.05).

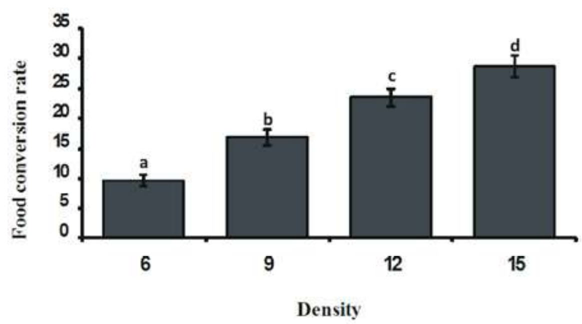

Fig. 3: Changes of feed conversion ratio among different treatments (Different English letters indicate significant difference among treatments at 0.05).

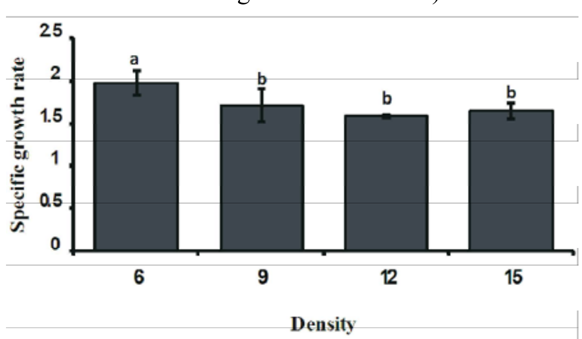

Fig. 2: Specific growth rate among different treatments (Different English letters indicate significant difference among treatment at 0.05).

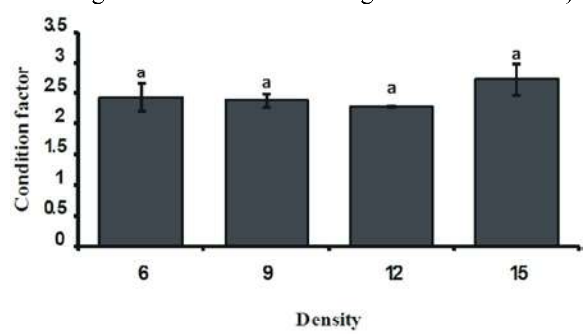

Fig. 4: Condition factor among different treatments (Different English letters indicate significant difference among treatments at 0.05).

Statistically significant difference was observed between growth rate of different densities, as with increasing density (density of 15), growth rate was significantly lower (p<0.05, 15.15±0.67), but significant difference were not found between low density of 6, 9 (control) and 12 (p<0.05). On the other hand, the highest specific growth rate was observed in low-density (density of 6) (1.97±0.14) that statistically significant difference was from density of 9, 12 and 15 (p<0.05).

Contemporary with decreasing growth rate at the highest density (density of 15), feed conversion ratio was significantly increased (p<0.05, 27.58±1.8), indicating fish high stress in high density that leading to increased metabolism and so increased fish requirements of energy. However, feed conversion ratio was not shown significant difference between density of 9 and densities of 6 and 12 (p<0.05).

On the other hand, despite changes in some biomarkers in the present study, fish condition factor remained on desirable condition and was observed a slight increasing associated with condition factor in high density (density of 15) but no significant difference was observed between different treatments (p<0.05).

The growth is an important physiological parameter that is well studied in correlation with fish social interactions [11]. Density was known to be a potential source of stress [15-17] that has significant effects on fish growth rate [14, 5, 18]. Environmental stressors are the main factors that limit fish performance under aquaculture conditions [19, 20]. Under stressors conditions, fish need to spend more energy for homeostatic processes [21]. In other words, this energy spends for stress than growth [22]. Thereby, the reduced feed intake might be an indicator of higher levels of stress that the fish encountered in higher densities [22].

Ellis *et al*. [20] expressed that stocking density is an important factor for fish welfare, but cannot be seen in separation from other environmental factors. To achieve desirable size fish at harvesting must regulate stocking density [23]. These factors can be easily measurable and can be useable as an indicator of population stress [24].

Depending on the species of farmed fish, stocking density may affect or not affect on the fish growth indices and survival [25].

One of the effects of increasing density is the growth prohibition [26] that Rahman *et al*. [27, 28] and Rahman and Verdegem [29] stated its reasons decreased food utilization and increased energy demand.

In the present study, the obtained results showed that growth rate was significantly decreased with increasing the density (density of 15), on the other hand, specific growth rate was significantly increased with decreasing density (density of 6). Also, Trenzado *et al.* [30] and Imanpoor *et al.* [11] obtained similar results in rainbow trout (*Oncorhynchus mykiss*) and in common carp (*Cyprinus carpio*), respectively. These results are consistent to findings of Mohseni *et al*. [31] on *Huso huso* fry, Bolasina *et al.* [32] on Paralichthys and Mirza and Chakraborty [33] on endangered bata (*Labeo bata*). If deficiency of farmed space affect on the population, the fact is well known that the growth and survival of fish is negatively correlated with stocking density [11]. Mollah and Nurullah [34] found that decreasing stocking density raises the growth rate in *Clarias macrocephalus.* Also, the growth in lower density is better than higher density that was similar with findings of Papoustoqlou *et al*. [35]. Many studies have examined the effects of density on growth, but the results do not always agree. Ebrahimi *et al.* [6] and Hardy and Audet [36] reported that density was not affected on growth rate in Giant gourami (*Osphronemus goramy*) and Brock charr, respectively.

During experiments on three different densities on Nile tilapia fingerlings (*Oreochromis niloticus*), El-sayed [37] concluded that increasing growth and specific growth rates had a negative correlation with stocking density and was significantly decreased with increasing density. Khatune-Jannat *et al.* [13] and Gholipour *et al.* [10], in Thai climbing perch (*Anabas testudineus*) and rainbow trout (*Oncorhynchus mykiss*) respectively, concluded that increasing density has a negative effect on growth and specific growth rates.

Also, in the present study, feed conversion ratio significantly increased to increasing density. So that the highest feed conversion ratio was observed in the density of 15. Such as process of changes of feed conversion ratio in different densities show a significant positive correlation between increasing density and feed conversion ratio. The results of the present study in terms of feed conversion ratio were similar with the findings of Imanpoor *et al.* [11] in common carp (*Cyprinus carpio*), Papoustoqlou *et al.* [35] in trout (*Salmo gairdneri*) and Moradyan *et al*. [22] in trout alvines (*Oncorhynchus mykiss*). Also, Gholipour *et al*. [10] to investigation on rainbow trout at four levels of density found that feed conversion ratio at lower densities was better than higher densities. Davy and De silvia [38] stated that digestibility to food adequate intake play an important role in reducing the amount of feed conversion ratio. Initially, digestibility depends on daily feed rate, feeding frequency and type of food given [39]. Furthermore, in this study, reducing the amount of feed conversion ratio indicate better efficiency of food intake in lower densities. Aminur Rahman *et al*. [12] also found similar results in endangered mahseer (*Top putitora*). On the other hand, Papoutsoglou *et al*. [40] obtained the opposite results with the results of the present study in European sea bass (*Dicentrarchus labrax*). They found feed conversion ratio was lower at higher densities.

In another studies, Ebrahimi *et al*. [6] and Saoud *et al.* [41], respectively, to study on Giant gourami (*Osphronemus goramy*) and rabbit fish (*Siganus rivulatus*) concluded that feed conversion ratio had not significant difference at different densities.

In the present study, despite changes in some biomarkers due to density, there was no significant difference between condition factor at different treatments that were comparable to the findings of Tolussi *et al.* [42] on species piabanha (*Brycon insignis*). Also, Saoud *et al.* [41] to study on rabbit fish (*Siganus rivulatus*) concluded that condition factor has increased over the growing period but seen no significant differences at different densities. Moreover, high density has no significant effect on survival rate that is consistent with the findings of saoud *et al*. [41] on rabbit fish (*Siganus rivulatus*).

In conclusion, with increasing density per unit area, increased food interactions between fish due to the reduced amount of food per fish and created weight different levels that leads to unequal contribution of fish to get food and will follow increasing vibration of weight and reducing food efficiency [31]. In the present study, we observed that stocking density affected the growth process through chronic effects on goldfish but not on survival rate. The results of this study can be useful in evaluating the effects of density on some biological parameters. Of course at the end state that effects of stocking density in regarded to the growth stages is different and it is necessary to evaluate effects of density at every stage of growth.

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