



RESEARCH ARTICLE

The Influence of Varying Stocking Densities on the Behavioral Indices, Growth Performance, and Welfare of Grass Carp (Ctenopharyngodon idella) Fish

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ABSTRACT

The aim of this research was to determine how varying stocking densities affected the behavioral traits and the biological performances of grass carp (Ctenopharyngodon idella) larvae. Larvae weighted 3.00 ± 1.00 g were randomly distributed in three groups (G1-G3; 2 replicate/group) of low-density medium density, and high density (7, 14, and 21 fish/aquarium), respectively for 8 weeks. The findings revealed that high stocking density (21 fish/ aquarium) showed the lowest values in normal behavior with the highest values of aggressive behavior when compared to low and medium density. Low density raised fish were more active than medium and high stocking density raised fish when it came to the terms of midline crossing test, and finally there was an opposite relationship between average body weight and the stocking density, the lower the density the more weight gained and vice. We can conclude that, to achieve welfare for grass carp, stocking density should be carefully considered with suitable floor space for each fish in low stocking density as mentioned group 1 (7 fish/ aquarium).

Keywords: Carp, Management, Stress, Aggressive, and Crossing test.

Introduction

The aquaculture industry has experienced significant growth in recent decades, primarily driven by a series of innovations aimed at enhancing and competitiveness, operational control these innovations encompass a spectrum of approaches, ranging from pioneering developments conceptual to the adaptation of knowledge derived from terrestrial food production systems [1]. Nowadays, there increasing is an recognition of the significance of nutritious consuming foods, with fish gaining prominence due to its distinctive

nutritional advantages, fish meat encompasses a range of macros and trace elements in abundant amount; therefore, Fish always regarded as a nutritionally valuable constituent of the human diet since the beginning of time [2]. Carps derive their nomenclature from their geographical distribution. Within China, two principal classifications of carp exist: the first comprises the Chinese carps, notably including the grass carp (*Ctenopharyngodon* idella). silver carp (Hypophthalmichthys *molitrix*), and bighead carp (Aristichthys nobilis). The second category encompasses the Indian major carps, which include species such

(*Catla catla*), rohu (Labeo catla as rohita), mrigal (Cirrhinus mrigala), and common carp (*Cyprinus carpio*), the China's rivers are naturally habitat to grass carp. It was brought to numerous primarily for biological other nations aquatic weed and macrophytes in both natural and artificial ponds [3]. Common carp, silver carp, and grass carp are the commonly cultivated carp species [4, 5]. human Besides passing carp for consumption there are many reasons for their cultivation as carp can beneficially remove the weeds and algae by consuming it as natural source of food, this practice is very common in Egypt [6]. Stocking density, feeding methods, and management practices can significantly impact stress responses, subsequent stress tolerance, soundness of fish, and the incidence of aggression [7]. In numerous cultivated fish species, growth is inversely correlated with stocking density, due to There socialization [8]. is direct a relationship between water quality, intensive stocking density and growth changes Behavioral performance [9]. were directly linked to higher stocking density. It was also shown that at high densities, some of the fish presented aggressive domineering behavior and [10]. Countless stressors have been demonstrated to cause changes in fish, affecting feeding patterns, activity levels, and aggressive behavior [11]. One of the factors that can significantly affect a variety of behavioral responses including aggressive behavior stocking is the density [12]. For this reason, the purpose of this study is to demonstrate the

influence of various stocking densities on behaviour and management of Grass carp (Ctenopharyngodon *idella*) fish to identify the ideal stocking density to allow the fish to express normal behavior achieve optimum growth and to maximize productivity performance to and to achieve fish welfare

Materials and methods

Fish rearing conditions

Grass carp larvae weighted 3.00 ± 1.00 g with 4 ± 1 cm in length were taken from central laboratory for aquaculture research at El-abbassa. Abu Hammad. Al Sharqia Governorate. Fish were kept for 15 days for acclimation. Glass tanks with following dimensions (100 x 30 x 40 cm), each one was equipped by using an air supply, thermostat-controlled heater and mercury thermometer. About a quarter of the aquarium, water was changed day after day and the whole water changed each week by water free chlorine from reservoirs water tanks. During the acclimation and trial, fish were fed on a basal diet. It was formulated in the form of dry sinking pellets, to meet the nutrient requirements of grass carp larvae, the essential components along with the composition of chemical the ration according to Sweilum et al. [13] was presented in Table 1.

The daily ration was divided into three portions by hand and the fish were fed thrice a day at a rate of 5% of the total body weight Feed only as much as they can consume within a period of five min [14, 15].

Feed ingredients (g)					
Herring meal	20.0				
Soybean meal	19.0				
Yellow corn	23.0				
Wheat bran	33.0				
Soybean oil	3.0				
Vitamin and mineral premix*	2.0				
Chemical analysis					
Crude protein (%)	30.09				
Ether extract (%)	7.0				
Crude fiber (%)	5.60				
Nitrogen free extract (%)	48.91				
Ash (%)	8.40				
ME (kJg1) w**	10.5				
Protein/energy	118.30				

Table 1. Composition and chemical analysis of the basal diet for Grass carp larvae

Experimental design

Then fish was transported Fish to Management Behaviour and Research Department Unit. of Behaviour and Management of Animal. Poultry and Aquatic, and randomly divided into 3 groups (n = 84 fish; 28 fish/group) in two replicate for 8 weeks. Group one low density 7 (G1), group two medium density 14 (G2) and group three high density 21 (G3) fish/aquarium,

Behavioral observation

To record the different behavioral patterns the following was done: each glass tank aquarium was observed daily for 4 min at circularly predetermined time. Using scanning technique. Intervals of 1 h weekly throughout the weeks of experiment for all groups [16].

The observed behavioral patterns were recorded as the following:

1) Feeding behaviour: it means the actual consumption of food at time of feeding [17].

- Mean frequency of swimming was recorded / 4 min.

2) Swimming behavior: Swimming behavior can be defined as the act of rapid or slow swimming without showing any behavioral indices [18].

- Mean frequency of swimming was recorded / 4 min.

Body care behavior

Scratching: The act of using any hard surface as rubbing surface by fish [19, 20].

- Mean frequency of scratching was recorded / 4 min.

Aggressive behavior: That one fish fight another or starts to attack it. The following patterns was defined and recorded as previously described [19, 21-23].

- Mean frequency of aggressive was recorded / 4 min.

a) Approach: one fish moves straight forward toward another fish.

b) **Chasing:** The vulgar swimming of fish after another fish.

c) **Fleeing:** The escapism of one fish from the coming fish.

d) **Spreading of fins:** Exhibiting the whole length of the fins.

e) **Fighting**. Male fish will fight to protect their territory

Crossing test

A demarcated line was drawn to divide the glass tank from outside then the total number of crossing this line was recorded for five min according to the estimated calculation of Scott *et al.* [24].

Live fish performance

The entire population of each density was weighted biweekly; where the second weight is recorded after one month and the final weight is recorded at the end of experiment in each time the whole weight is divided by the total number to calculate the average body weight according to Khalil *et al.* [25].

Data handling statistical analysis

The numerical data were recorded. tabulated. and then analyzed using Analysis System package Statistical 28) [26]. Results version (SPSS are described as Mean ± SD. Onewav analysis of variance (ANOVA) test was applied to test behavioral parameters and the differences of average body weight of fish at each density. Tukey's honesty significant test was applied where the results with (P- value < 0.05) were considered statistically significance.

Results

The impact of different stocking densities on normal behavior a. Feeding behavior

Results in Table 2 demonstrated that the duration of the experiment resulted in significant difference when it came to the terms of frequency of feeding among groups (P<0.05). It showed that G₃ had the lowest values of frequency feeding behaviour while G₁ expressed the highest values.

b. Swimming behaviour

The findings in Table 2 indicated that experimental period led the to insignificant difference when it came to the terms of frequency of swimming among groups (P- value = .05). It showed that G₁ recorded the highest value of frequency swimming of behaviour compared to G_2 and G_3 , where the lowest values were recorded by G3.

c. Scratching behaviour:

It was evident from the results in Table 2 that the duration of the experimental phase resulted in significant difference when it came to the terms of frequency of scratching among groups (P<0.05). G₂ and G₃ had the lowest values of frequency scratching behaviour while G₁ expressed the highest values.

Consequence of different stocking densities on aggressive behavior

a) Approach

The outcomes of the experiment indicated illustrated in Table (2) that significant difference there was a regarding the frequency of approach among groups (P < 0.05). It showed that G₂ and G₃ almost had similar values of approaching frequency behaviour compared to G₁ that clearly showed the lowest values.

b) Chasing

When pointing to chasing behaviour results highlighted that the duration of the trial led to insignificant difference (Pvalue = 0.06) of frequency among groups. It showed that G₃ had the highest values of frequency chasing behaviour when compared to G_1 and G_2 , while G_1 recorded the lowest value.

c) Fleeing:

Concerning the frequency of fleeing behaviour, it was evident from the data in Table 2 resulted in insignificant difference of frequency among groups P- value (0.8), it showed that G_2 and G_3 almost had similar values of frequency fleeing behaviour when compared to G_1 that recorded the lowest value.

d) Spreading of fins:

Empirical evidence obtained from Table 2 revealed a significant difference regarding the frequency of spreading fins among groups (P < 0.05). G₃ had the highest values of frequency spreading of fins when compared to G₁ and G₂. G₁ recorded the lowest value.

e) Fighting:

The frequency of fighting behaviour data demonstrated insignificant difference among groups (*P*- value = 0.6). G_2 and G_3 almost had similar values of frequency fighting behaviour when compared G_1 that recorded the lowest value (Table 2).

Crossing test:

Significant difference between the groups (P < 0.05) regarding the frequency of midline crossing as obtained from the data in Table 2. G_1 and G_2 almost had values similar of frequency midline crossing behaviour compared that G_3 lowest nearly showed the values.

Behaviour		G1	G2	G3	P value
Normal behaviour	Feeding	11.1 ± 3.6^{a}	9.8 ± 2.9^{ab}	$7.8 \pm 1.5^{\text{b}}$	0.04
	Swimming	$15.1\pm3.1^{\mathrm{a}}$	7.5 ± 2.3^{ab}	$5.2\pm2.4^{\text{b}}$	0.05
	Scratching	1.2 ± 0.4 a	$0\pm 0^{\ b}$	0 ± 0 ^b	0.03
Aggressive behaviour	Approach	2.3 ± 0.2^{b}	3.3 ± 0.2^{ab}	3.6 ± 0.4^{a}	0.045
	Chasing	3.6 ± 0.7	4.7 ± 0.7	4.7 ± 0.7	0.061
	Fleeing	0.9 ± 0.2	1 ± 0.2	1.1 ± 0.1	0.875

 Table 2. Effect of different stocking densities on the frequency of the observed Grass carp

 larvae behavior during 8 weeks of experiment.

	Spreading of fins	$2.3\pm0.2^{\text{b}}$	3.6 ± 0.3^{ab}	4.6 ± 0.6^{a}	0.012
	Fighting	0.19 ± 0.1	0.3 ± 0.1	0.3 ± 0.15	0.671
Midline crossing	Crossing test	6.6 ± 1.04^{a}	6.4 ± 0.3^{a}	$2.3\pm0.4^{\text{b}}$	0.001

*The values are mean \pm SD; * G1=Group one (7 fish/aquarium), G2= Group two (14 fish/aquarium) and G3= group three (21 fish/aquarium). * Letters in the same rows with different superscripts are significantly different at P < 0.05.

Table 3 illustrated the relationship between different densities and average body weight per gram. It resulted in significant difference in the second and final weight among groups (P> 0.05). It showed that G₁ recorded the highest values in the terms of weight gain while G₃ recorded the lowest.

 Table 3. Effect of different stocking densities on the average body weight of Grass carp larvae during 8 weeks of experiment.

Criteria	G1	G2	G3	P value
Initial weight	3.02 ± 0.8	3.1 ± 0.6	3.01 ± 0.7	0.966
2 nd weight	3.7 ± 0.7 a	3.4 ± 0.5 a	2.7 ± 0.3 b	0.011
Final weight	4.4 ± 0.4 a	3.7 ± 0.9 a	$2.8\pm0.7~^{b}$	0.003

*The values are mean \pm SD; * G1=Group one (7 fish/aquarium), G2= Group two(14 fish/aquarium) and G3= group three(21 fish/ aquarium). *Letters in the same rows with different superscripts are significantly different at *P* <0.05.

Discussion

Stocking density can be described as the measurement of fish weight in a given amount of water [27]. In order to exhibit their natural behavioral patterns with minimal pain, stress, and fear fish need abundant space [28].

Taking consideration into the aggressive behaviour high stocking density recorded the highest values of frequency aggressive of all patterns (approach, chasing, fleeing, spreading of fins, and fighting). Aggressive patterns can be split into two phases the first one during competition for food resources and sheltering phase sites this include activities like approach, chasing and

fleeing while the second phase is directly dominant and subordinate linked to relationship including more fighting and spreading of fins that eventually led to formation of the dominance hierarchy. These results were compatible with previous studies that behavioral changes were directly linked to higher stocking density and with increasing densities the frequency of aggressive behavior increased [10, 29]. At high densities, some of the fish presented aggressive and domineering behavior. Our findings also agreed with those obtained by Whiteman and Cote [30] those higher densities competitive behaviour, associated with aggression, and result in physical harm. Moreover, Manley et al. [31]. declared 341 that high stocking density can either increase cannibalism by increasing the possibility of encounters between aggressors and prey or decrease cannibalism by interfering with normal territorial or aggressive behavior

Regarding the crossing test the results demonstrated crossing that test (frequency) significantly affected by stocking density, as G_1 recorded the highest values while the lowest one was recorded in G₃. This result is related to high stocking density where swimming activity decreased with decreased and space which allowed for one fish. This result agreed with the results of Bjornsson [9] who reported that water quality limits the growth of fish in higher densities; these findings agree with the findings stated by Martins et al. [32] who found that water quality parameters have a direct effect on swimming and activity of fish.

The findings of this study demonstrated the impact of different stocking densities on average body weight, which significantly was influenced, where final body weight was the greatest in G_1 and the lowest in G_3 . These results agreed with the findings of Moniruzzaman et al. [33] that high stocking densities lead to problems of high mortality and poor development. It also goes hand in hand with the results of previous study [34] that competition for space and food is the main reason why the stocking density influences the development of the fish. High density limits access to food, thereby leading to poor development. Also, high stocking density can negatively affect the growth performance [35].

Conclusion

This study revealed numerous changes in the behavior of grass carp. These changes were directly linked to rearing density, high density came along with decrease in normal behavior frequency with increasing in aggressive behavior and fighting for food, which eventually led to decrease in body weight and poor welfare. Furthermore, low and medium densities recorded decrease in aggressive behavior and higher body weight. To achieve welfare for grass carp, stocking should be carefully considered density with suitable floor space for each fish in low stocking density as mentioned group 1 (7 fish/ aquarium).

Conflict of interest

The authors declare that there is no conflict of interest.

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الملخص العربي تأثير كثافات التسكين المختلفة علي سلوك وكفاءة نمو ورعاية أسماك مبروك الحشائش (Ctenopharyngodon idella)

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تم إجراء هذه الدراسة للكشف عن تأثير كثافات التسكين المختلفة على السلوك والأداء البيولوجي لأصبعيات سمك مبروك الحشائش (Ctenopharyngodon idella). تم تربية اليرقات الصغيرة (3 ± 1 جم) عند 3 كثافات تسكين مختلفة وبتكرارين ؛ كثافة منخفضة 7 (المجموعة 1)، وكثافة متوسطة 14 (المجموعة 2)، وكثافة عالية 21 (المجموعة 3) سمكة / حوض، في أحواض زجاجية بأبعاد 30 × 40 × 100 سم لمدة 8 أسابيع. أظهرت النتائج أن الكثافة العالية أظهرت اقل قيم في السلوك الطبيعي مقارنة بالكثافة المتوسطة والمنخفضة التي أظهرت أعلى معدل للسلوكيات الطبيعية. كما أظهرت النتائج أن الكثافة العالية أظهرت النتائج أن السلوك الطبيعي مقارنة بالكثافة المتوسطة والمنخفضة التي أظهرت أعلى معدل للسلوكيات الطبيعية. كما أظهرت النتائج تربيتهابكثافة منخفضة ومتوسطة. أظهر اختبار التقاطع أن الأسماك التي تمت تربيتها بكثافة تسكين عالية مقارنة بالأسماك التي تمت تربيتهابكثافة منخفضة متوسطة. أظهر اختبار التقاطع أن الأسماك التي تمت تربيتها مكثافة منخفضة كانت أكثر نشاطًا من تربيتهابكثافة منخفضة ومتوسطة. أظهر اختبار التقاطع أن الأسماك التي تمت محموطة في الوزن النهائي للأسماك التي تمت تربيتهابكثافة منخفضة ومتوسطة. أظهر اختبار التقاطع أن الأسماك التي تمت تربيتها مدوطة في الوزن النهائي للأسماك التي رأبيت بكثافة منخفضة متوسطة وعالية. وقد خلصت الدراسة بملاحظة زيادة ملحوظة في الوزن النهائي للأسماك التي رأبيت بكثافة منخفضة، بينما أظهرت الأسماك التي رأبيت بكثافة تسكين عالية انخفاضاً ملحوظة في الوزن النهائي على مدار أسابيع الدراسة لذلك لتحقيق رعاية الأسماك التي رأبيت بكثافة تسكين عالية انخفاضاً ملحوظة في الوزن النهائي على مدار أسابيع أشرنا في المجموعة 1 (7 أسماك/ الحوض) .