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Bhuneshwari Usandi
Department of Aquaculture, College of Fisheries, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

VP Saini
Aquaculture Research & seed Unit, Directorate of Research Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

ML Ojha
Department of Aquaculture, College of Fisheries, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

HK Jain
Department of Agriculture Statistics, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Correspondence
Bhuneshwari Usandi
Department of Aquaculture, College of Fisheries, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Effect of larval rearing density on growth and survival of koi carp, *Cyprinus carpio*

Bhuneshwari Usandi, VP Saini, ML Ojha and HK Jain

Abstract
The present study was conducted to optimize the larval rearing density for a freshwater ornamental fish 'koi carp'. For this purpose, five different rearing densities i.e., 2500 (T_1), 5000 (T_2), 7500 (T_3), 10000 (T_4) & 12500/m^3 (T_5) were tested using static water holdings (aquarium tank of 2'x1'x1' size). Koi larvae having an initial mean length and weight of 6±1 mm and 1±0.1mg respectively were stocked at five different rearing densities in triplicate. They were fed with live food (mixed zooplankton) and dry larval feed (crude protein 22%, crude fat 3%, fibre 8% and moisture 11%). The average maximum values in terms of net weight (0.0644g), length (22.7 mm) and specific growth rate (13.44) were observed in T_1. The highest survival was recorded in T_1 (62.67%) and lowest in T_5 (21.67%). The results revealed that stocking density had an inverse relationship with growth performance (length gain, weight gain, and specific growth rate) and survival. A rearing density of 7000m^3 was calculated using the second order polynomial regression analysis between stocking density and total numbers of fry harvested.

Keywords: Koi carp, *Cyprinus carpio*, density, growth, survival

1. Introduction

Koi carp (*Cyprinus carpio*) is one of the popular and economically important cultivable freshwater ornamental fish in the aquaculture industry. This fish is most famous for its beautiful colours that have been developed via selective breeding programmes. There are over 20 different varieties of koi that differ in colour, patterns and type of scales. Koi originated from Eastern Asia and live in a wide temperature range, the minimum is 40 ºF. The optimal range is 60 to 75 ºF. The average life span of a koi fish is 15 to 20 years. Some can live upto 30 years or more.

A gradual evolution towards intensive systems for fish production has been observed in countries having large scale operations and adopted according to local conditions [1]. This involves higher stocking densities, hatchery production of seed where feasible, greater human control of environmental conditions, supplementary feeding and higher yields per unit area [1]. Stocking density plays an important role in the survival and growth of fish larvae and hence it is essential to determine the optimum level in larval rearing. Specific stocking density can have positive and negative effects on fish growth and survival, knowing the optimal stocking density is one of the basic factors influencing intensive fish culture. Fish stocking density is the most sensitive factor determining the productivity of a culture system as it affects growth rate, size variation and mortality [2]. In comparison to food fish, the densities at which ornamental fishes have been kept are rather low.

In aquaculture, increasing stocking density is one of the solutions for the problems of lack of rearing resources. Variation in stocking density of fish may change growth and survival rates. Fish larvae have a slow growth and low survival rates at high density. Several studies have investigated the effects of stocking density on different farmed species growth including rainbow trout (*O. mykiss*) [3], common carp (*Cyprinus carpio*) [4], endangered mahseer (*Tor putitora*) [5] and Thai climbing perch [6]. On the other hand, studies on giant gourami (*Osphronemus goramy*) showed that density had no significant effect on the growth indices of the fish [7].

The rate of rearing density is further decided based on the expected growth increment of individual fish and survival level in production systems. Rearing at higher densities not only results in higher production but also minimises the total land requirement and water usage. The high rearing density, however, may exert adverse effects on growth [8] and survival [9].

Therefore, it is necessary to predetermine and standardise the optimum rearing density for each species in order to obtain the best possible output. In view of this, the present study has been proposed to standardize the larval rearing densities of koi. The main aim of this study is to understand the growth and survival of koi, *Cyprinus carpio* larvae at different rearing densities.

2. Materials and Methods

This experiment was conducted in the wet laboratory of Aquaculture Department, College of Fisheries, Udaipur (Rajasthan) during March-April of 2016. The experiment was conducted in fifteen glass tanks of 2'x1'x1' size. Before initiating the experimental trial on koi larval rearing, all the tanks were cleaned and filled with 40 litres of water. The water of each aquarium was aerated 3-4 hrs daily in the early morning hours. Healthy larvae of koi (average length 6±1 mm and average weight 1±0.1 mg) were procured from the Aquaculture Research & Seed Unit, Directorate of Research, MPUAT, Udaipur and stocked in five different rearing densities i.e. 2500(T_1), 5000(T_2), 7500(T_3), 10000 (T_4) and 12500(T_5) larvae per m^3. The experiment was conducted in triplicate for a period of 30 days. During the rearing period, the larvae were fed adlibitum twice a day. Initially, during first three days the larvae were fed with boiled egg yolk two times a day at an interval of 8 hours. For the next eleven days they were fed infusoria and mixed zooplankton. Then for the rest of the study period larvae were fed with commercially available feed pellets feed (ABIS) containing crude protein 22%, crude fat 3%, fibre 8% and moisture 11%. The feed pellets were crushed before feeding to the larvae.

The selected physico-chemical water quality parameters viz., water temperature, pH, electrical conductivity (EC), dissolved oxygen and total dissolved solids (TDS) were analysed on initial day and subsequently at an interval of five days following standard methods as outlined in APHA [10]. At the time of termination of experiment (i.e. on 30th day) the length, weight and total number of fry from each treatment were noted. Growth parameters *viz.*, net weight gain, average length increment, survival percentage and specific growth rate (SGR) were calculated using following standard formula.

Net weight gain (g) = Final weight of fish (g)-Initial weight of fish (g)

Length increment (mm) = Final length (mm)-Initial length (mm)

$$\text{Specific growth rate (\%)} = \frac{\ln \text{Final weight (g)} - \ln \text{Initial weight (g)}}{\text{No. of days}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{No. of fry harvested}}{\text{No. of spawn stocked}} \times 100$$

The data recorded were statistically analysed using standard procedures for the analysis of variance following completely randomized design (CRD) technique as described by Panse and Sukhatme [11] in order to test the significance of experimental results.

3. Results

The results pertaining to water quality, larval growth and survival are presented in Tables 1-2 and Figs. 1-2. The physico-chemical water quality parameters in the rearing trial are summarized in Table 1. As such there was no significant difference in water quality of different treatments and all the studied parameters remained within the congenial levels for larval rearing. However, a significant impact of rearing densities was visible on growth and survival.

Table 1: Range and average (in parenthesis) of water quality parameters during the experimental period

Parameters	Treatments				
	T_1	T_2	T_3	T_4	T_5
Temperature (ºC)	23.3 – 27 (25.64)	23.3 – 27 (25.74)	25.3 – 27 (26.14)	25.3 – 27 (26.28)	25 – 27 (26.28)
pH	8.6 - 8.9 (8.75)	8.6 - 8.9 (8.82)	8.6 - 8.9 (8.82)	8.5 - 8.9 (8.75)	8.6 - 8.9 (8.8)
EC (mS/cm)	2.10 - 8.60 (5.63)	2.27 - 8.63 (6.22)	1.93 - 8.63 (6.19)	2.13 - 8.73 (6.24)	1.97 - 8.73 (6.25)
Dissolved oxygen (mg/l)	5.8 - 8.13 (6.46)	4.8 -7.8 (6.45)	5 - 8.9 (6.61)	5.5 - 8.2 (6.45)	5.5 - 8.2 (6.50)
Total dissolved solids (g/l)	0.5 - 1.2 (0.78)	0.5 - 1.06 (0.79)	0.5 - 1.2 (0.80)	0.5 - 1.2 (0.90)	0.6 - 1.4 (0.95)

3.1 Growth Parameters

3.1.1 Survival: The survival of koi larvae at five different rearing densities are given in Table 2. It shows highest survival in T_1 with 62.67% followed by 32.17, 29.53, 22.42 and 21.67% in T_2, T_3, T_4 and T_5 respectively. The treatment (T_1) with the lowest rearing density had the highest survival rate of koi larvae. ANOVA indicates a significant difference in the survival of koi larvae in different treatments. Statistically, it was revealed that treatment T_1 was significantly higher ($p<0.05$) than the other treatments.

3.1.2 Weight gain: The growth in terms of net weight gain of koi (*Cyprinus carpio*) larvae in different treatments has been shown in Fig. 1. The maximum weight gain of 0.0644 g was observed in T_1 whereas, T_4 showed the minimum weight gain of 0.0224 g. Statistically, ANOVA has revealed significant differences ($p<0.05$) in the average net weight gain of koi larvae reared at different densities. It is obvious from the Table 2 that the mean net weight gain was significantly higher in T_1 ($p<0.05$) than the T_2, T_3, T_4 and T_5, whereas, the net weight gain in T_2, T_3, T_4 and T_5 were found non-significant. The net percent gain in weight in different treatments has been shown in Table 1. The average per cent gain in weight for the 30 days culture period was 5906.9, 2764.8, 1313.9, 2165.4 and 2378.3% in T_1, T_2, T_3, T_4 and T_5 respectively. It shows that the stocking density affects the growth of the fish. The treatment with lowest stocking density (T_1) shows the highest percent gain in weight. Statistical analysis of variance has revealed significant differences ($p<0.05$) in the average percent weight gain of koi larvae in different treatments (Table 2).

Table 2: Summary of significant and non-significant difference in growth parameters

Treatments	Weight gain (g)	Percentage weight gain	Length gain (mm)	Percentage length gain	SGR (%)	Survival (%)	Total Fry Production (Nos/m^3)
T_1	0.0644^a	5906.97^a	22.70^a	324.37^a	13.44^a	62.67^a	1565^a
T_2	0.0281^b	2764.80^b	12.17^b	176.20^b	11.134^b	32.17^b	1610^a
T_3	0.0240^b	2245.79^b	10.30^b	147.13^b	10.510^b	29.53^b	2215^b
T_4	0.0224^b	2165.47^b	10.37^b	148.17^b	10.42^b	22.42^c	2242^b
T_5	0.0258^b	2378.33^b	10.23^b	146.65^b	10.65^b	21.67^c	2710^c
SEm	0.0099	34.38	3.90	55.79	1.044	1.086	63.06
Figures with same superscripts are not significantly different							

3.1.3 Length gain: The growth in terms of net length gain is shown in Fig.1. The maximum average length gain of 22.7 mm was observed in treatment T_1 and minimum (10.23 mm) in treatment T_5. The average length gain was 12.17, 10.3, and 10.37 mm in treatments T_2, T_3 and T_4 respectively. A decreasing trend in length gain was found with increasing rearing density. The results of the statistical analysis ANOVA shows no significant difference between length gains in treatments ($p>0.05$) except treatment T_1 with other treatments. The percent gain in length in different treatments has been shown in Fig.1. In the treatment T_1 it was highest (324.37%) and in T_5 was lowest (146.65%). The percent gain in length was 176.2%, 147.13% and 148.17% in T_2, T_3 and T_4 respectively. The total percent gain in length was highest in the treatment (T_1) having lowest rearing density and lowest in T_5 having highest rearing density.

3.2 Specific growth rate: During the 30 days of the experimental period, the maximum average specific growth rate of 13.44% was observed in T_1 and minimum (10.42%) was found in treatment T_4 (Table 2). The average specific growth rate was 11.13, 10.51 and 10.65% in T_2, T_3 and T_5 respectively. There was no significant difference in the specific growth rate among the treatments. However, a significant difference was found between the treatment means and which was significantly higher in T_1 ($p<0.05$) than that of T_2, T_3, T_4 and T_5.

3.3 Fry Production: The data pertaining to total numbers of fry harvested from individual tank/treatment are presented in Table 2. The minimum (1565 Nos) and highest (2710 Nos) were recorded from T_1 and T_5 respectively. The data recorded for total numbers of koi carp harvest were further processed by second order polynomial regression analysis to determine the optimum stocking or rearing density of koi carp larvae. As such the results obtained are presented in Fig.2. From the second order polynomial regression curve (Fig.2), it is evident that a stocking density of 7000 larvae per m^3 is most suitable for koi carp rearing.

4. Discussion

The results of the present investigation (Tables 1 & 2) have revealed a significant impact of rearing densities on survival and growth of koi larvae. However, no significant impact of rearing density on water quality was visible. On comparing the initial and final day values of different water quality parameters, obviously the levels of dissolved oxygen were found to have decreased because of high stocking density. On the other hand, EC and TDS showed increased trends (Table 1). Still the observed water quality parameters in different treatments remained within the congenial levels for seed rearing [12]. The variations in fish growth and survival could be due to rearing densities and not the water quality.

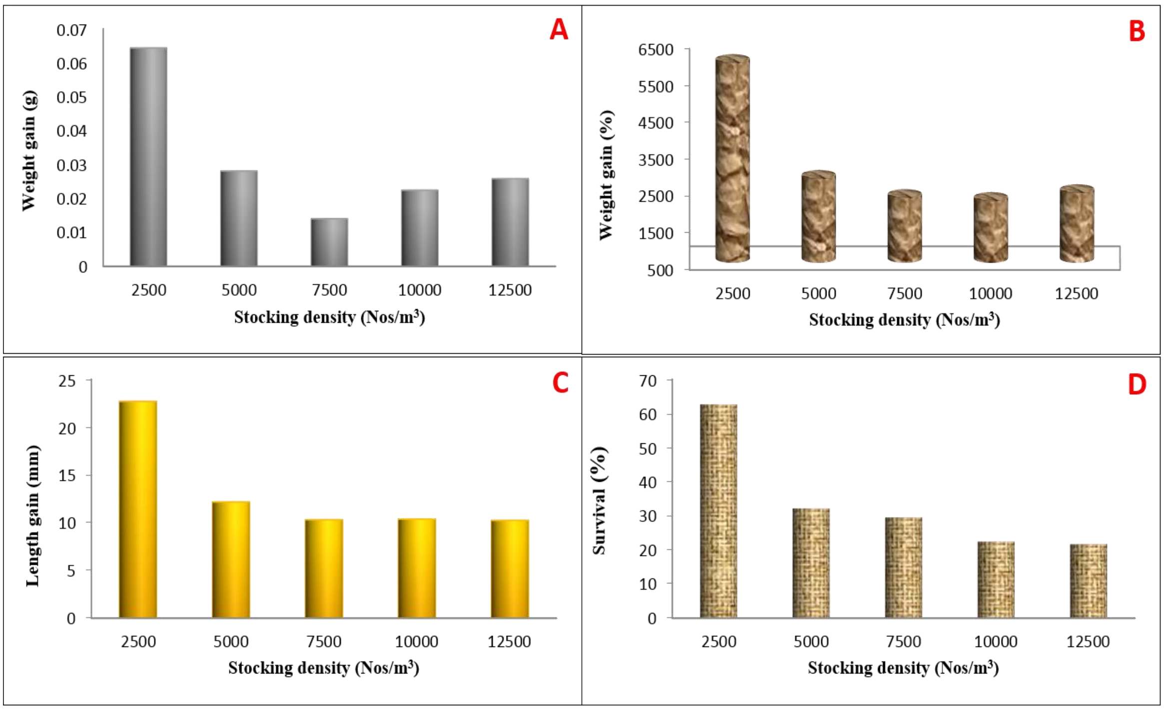

Fig 1: Growth performance (A. weight gain, B. weight gain in percentage & C. length gain) and survival (D) of koi carp in relation to stocking density

The effect of rearing density on survival and growth of early life stages of different fish species has been the focus of research for many years. For larval stage, rearing density experiments are more relevant when reported as number of fish per volume of water rather than as biomass per volume [13]. During the larval stages relationship between rearing density, survival, growth and FCR has been reported to be positive [14], negative [15, 16] density independent [17, 18] or dependent on different experimental density ranges. Rearing density might vary as a function of behavioural adjustments, food availability and water quality. However, the mechanisms linking rearing density and growth are not fully understood, but it is generally accepted that when water quality is not affected by the increased number of fish per cubic meter and sufficient food is provided, differences in growth performances could be attributed to the onset of hierarchies and dominant relationship [19].

In an experiment aimed at studying the effect of rearing density on koi, common carp and gold fish, the sensitivity of common carp to stocking density was shown to be highest whereas koi could be stocked up to 2 million fry per ha without any negative density effects, goldfish was recommended to be stocked at low densities (50000 million fry per ha) in spring and at higher rates (2 million fry per ha) in summer. These authors speculated growth reduction to be a result of crowding of fish leading to decreased feeding rates. Sayed [20]. reported survival of 90-100% for the fry of *Oreochromis niloticus* over a wider stocking density range of 3-20 fish/litre but optimum stocking density in terms of growth rate was found to be 5/litre. Studies on several fish species have revealed that the stocking density had a significant impact on growth and survival, growth rates increase by reducing densities and vice versa. It has been reported that the growth of many species is density dependent and that there was an inverse relationship between stocking density and individual size of fish produced, primarily because the food supply has to be shared between individuals [21-26].

In the present study of rearing larvae to fry in aquarium tanks, mean length and weight observed was 22.70, 12.17, 10.30, 10.37, 10.23 mm and 0.0644, 0.0281, 0.0240, 0.0224, 0.0258 g in T_1, T_2, T_3, T_4 and T_5 respectively. Among all the mean length and weight observed, the highest gain was observed in the stocking density of 2500 larvae m^3 in 30 days rearing period and these values are 22.70 mm and 0.0644 g, respectively. Similar results were found in rearing of koi in concrete tanks and reported that the weight gain for koi carps stocked at lower density was significantly higher than that of fish in the other treatments [27].

Niazie *et al*., [28] reported that 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 difference (p<0.05). Survival rate were not significantly different among different densities (p>0.05). The results of this study indicate that density significantly affected the growth, but had no significant effect on survival. Ronald *et al*. [18] observed weight gain of 555, 605, 846.4, 1073.81, 1647, 1349.76 g in stocking density of 1000, 1330, 2000, 2670, 4000, and 5330 fry m^{-3} of *Oreochromis niloticus* for 23 days.

During the present study the maximum specific growth rate of 13.44% was observed in rearing density of 2500 larvae m^3 (T_1) followed by 11.13% (T_2), 10.51% (T_3), 10.42% (T_4), 10.165% (T_5) in stocking density of 5000, 7500, 10000 and 12500 larvae m^3 respectively. Thus, the SGR values showed an inverse relationship with stocking densities. More or less similar results were also reported by Mensah *et al*. [29].They observed the specific growth rate of 11.126±0.308, 8.970±0.659, 7.404±0.294 in stocking density of 10000, 15000, 20000 numbers of *Oreochromis niloticus* fry in 10 m^2 hapa for 30 days. Hussain *et al*. [30] observed specific growth rate of rohu, silver carp, mirror carp were 4.95±0.10, 5.07±0.04, and 5.12±0.09% in treatment-I in stocking density of 2960, 2432, 2425 fry m^{-3} and 4.80±0.02, 5.70±0.13, 5.0±0.17 in treatment II in stocking density of 2368, 1945, 1940 fry m^{-3} during 90 days of rearing.

In the present study, survival observed was 62.67, 32.17, 29.53, 22.42 and 21.67% in T_1, T_2, T_3, T_4 and T_5 respectively. Among all the treatments, maximum survival was noticed in T_1 and lowest in T_5, which is significantly different (p<0.05). On comparing different rearing densities lower survival was noticed with higher rearing densities (i.e., 12500 and 10000 larvae m^3) than lower rearing densities (2500, 5000 and 7500 larvae m^3). While working on *Labeo bata* fingerlings, Chakraborty and Mirza [31] have also recorded reduced survival with increasing densities. Further, they suggested that the reduced survival in higher stocking densities was probably due to the competition for food and space in the experimental ponds. Similar results were also obtained by Rahman and Rahman [5] and Chakraborty *et al*. [32] for fry and fingerlings of various carp and barb species.

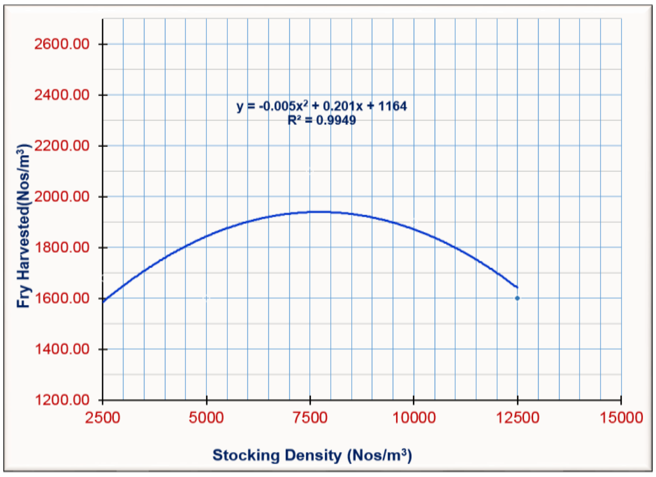

Fig 2: Relationship between koi carp larval rearing density with total harvest (Fry)-as described by second order polynomial regression

The results of the present study revealed that fish, koi, maintained at higher densities (>7500 m^3) showed significantly less specific growth rate (SGR) and survival as compared to lower rearing densities. The survival decreased with increasing rearing numbers. These results support the earlier findings that growth and survival of fish are optimum within a defined rearing densities [29, 33]. This study was to determine the optimum rearing density of koi larvae rearing in terms of growth and survival. Using a second order polynomial regression, the optimum rearing density of 7000 larvae/m^3 was computed in terms of net numbers of fry harvested (Fig. 2). In conclusion, rearing density of 7000 Nos/m^3 seemed to be the most effective for rearing of koi, common carp and hence the same is recommended. However, further field trials are also suggested, because the present study was conducted under fully controlled laboratory conditions.

5. Conclusion

The rearing density is one of the key to successful koi carp seed production which is a valuable freshwater ornamental fish. The aim of the present study was to evaluate the growth and survival rate of koi carp (reared with different stocking density). The data obtained for fry production from individual rearing density treatment suggested that koi carp can be reared at stocking density up to 7000 larvae per m^3 without negative effects on production. Hence it is recommended to stock koi carp larvae at 7000 /m^3 for growing up to fry stage. However, a further research needs to be conducted to evaluate the growth and survival in relation to stocking density under field conditions.

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