Effect of Stocking Density Difference with the Same Amount of Feed on the Growth and Survival of Koi Fish (*Cyprinus rubrofuscus*) at the Balai Riset Budidaya Ikan Hias (BRBIH), Depok, West Java, Indonesia

Muhamad Gilang Permana[1*], Rafika Dinillah[1], Siti Nurlaela[1], Rusdi Rusdi[1], Fitria Pusparini[1], Nina Meilisza[2], and Joni Hariyadi[2]

[1]Program Studi Pendidikan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Jakarta, Jakarta, Indonesia
[2]Balai Riset Budidaya Ikan Hias, KKP, Jl. Perikanan Raya No. 13, Pancoran Mas, Kec. Pancoran Mas, Kota Depok, Jawa Barat, Indonesia
*laaangstudies@gmail.com

Abstract. Koi fish (*Cyprinus rubrofuscus*) have high aesthetic appeal and promising economic value, making them highly sought after by fish farmers. However, the primary challenge in koi fish farming is determining the most efficient and effective conditions, particularly regarding stocking density and optimal feed amounts. This study aims to evaluate the effect of different stocking densities on koi fish larval survival. An experimental method with a simple random sampling design was used, where research subjects were randomly selected and divided into three treatment groups. Each treatment was repeated three times to ensure the accuracy and consistency of results. Three different stocking densities were tested, namely 250, 500, and 750 fish per aquarium with a water volume of 48 liters. The main parameters observed included length, weight, specific growth rate, and larval survival rate. The data obtained were statistically analyzed to determine the significance of differences between treatments. Data were analyzed using ANOVA to test for variations between treatments, followed by Tukey's test for post-hoc comparisons. The results showed that different stocking densities did not significantly affect the length and weight of the fish, but significantly affected larval survival. Treatment with a stocking density of 250 fish per aquarium resulted in the highest survival rate of 30.13%, indicating that a lower stocking density provided better conditions for koi larvae to survive. These findings provide important guidance for cultivators in optimizing koi fish cultivation efficiently and sustainably.

Keywords: *Cyprinus rubrofuscus*, cultivation, stocking density, survival

1 Introduction

Indonesia possesses abundant natural resources that are ideal for supporting aquaculture development, particularly in the ornamental fish sector. Freshwater ornamental fish encompasses around 400 species out of 1,100 ornamental fish species found worldwide. One of the leading commodities in the freshwater ornamental fish category that remains a favorite in international markets is koi fish (*Cyprinus rubrofuscus*). This fish belongs to the high-value ornamental fish group, with prices remaining relatively stable despite

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market fluctuations (Kusrini et al., 2015). Koi fish (*Cyprinus rubrofuscus*) is highly sought after due to its aesthetic appeal and high economic value. Koi fish have long been bred in Japan for generations and are considered the national fish of the country (Iskandar et al., 2021 in Amelia et al., 2024). The beauty of its color and unique body patterns make koi fish a prime choice among ornamental fish enthusiasts in various countries, including Indonesia. According to the Directorate General of Aquaculture (2022), koi fish production reached 484,391 fish. This figure encourages koi fish breeders to scale up their businesses.

The breeding process of koi fish is relatively easy, which attracts many fish farmers to cultivate this species. Koi fish have a high reproductive rate, and their spawning cycle can be well-regulated. This facilitates breeders in producing koi fish in large quantities and meeting the growing market demand. According to Khasanah (2016 in Lembang & Rahman, 2022), the breeding method plays an important role in ensuring the quality of the koi fish produced. This process includes broodstock maintenance, broodstock selection, spawning, egg and larva care, as well as water quality management, all of which determine the success of koi fish cultivation. Spawning is one of the key stages in fish breeding techniques. The primary goal of aquaculture activities is to obtain fish in large quantities and high quality, which heavily depends on the effectiveness of the spawning process (Lembang & Rahman, 2022). Koi fish spawning can be conducted through three methods: natural spawning, semi-artificial spawning, and artificial spawning. The natural method involves spawning without external intervention or the addition of any substances to the koi broodstock (Lukmantoro, 2018). Semi-artificial spawning is carried out by administering hormone stimulation to the broodstock, while ovulation still occurs naturally (Yuatiati et al., 2015). In the artificial spawning method, hormone stimulation is administered to the broodstock, followed by ovulation with human assistance through the technique of stripping or manually pressing the broodstock's abdomen (Meilala, 2018).

One of the main challenges in koi fish farming is determining the most efficient and effective cultivation conditions, including stocking density and feed quantity. Stocking density and feeding are two important factors that affect the growth and survival of the fish. Excessively high stocking densities can lead to intense competition for resources such as oxygen and space, which can ultimately hinder growth and increase mortality. On the other hand, stocking densities that are too low may not optimally utilize the pond's capacity. Therefore, this study aims to explore the effects of different stocking densities with the same feed quantity on the growth and survival of koi fish. Additionally, the research aims to provide practical guidelines for small-scale breeders in optimizing the use of their cultivation ponds and identifying the optimal feed value in the context of varying stocking densities to improve feed efficiency. Thus, the results of this study are expected to provide new insights and useful guidelines for small-scale breeders in managing more efficient and sustainable koi fish farming practices.

2 Method and Materials

2.1 Location and Time of the Study

This research was conducted at the Balai Riset Budidaya Ikan Hias (BRBIH) in Depok, West Java, from February 21 to March 1, 2024.

2.2 Materials and Equipment

The equipment used in this study included breeding ponds, aquariums, larval rearing ponds, nursery ponds, natural feed culture ponds, siphoning hoses, brushes, aerators, aeration hoses, measuring cups, water pumps, blowers, syringes (1 cc and 3 cc), rulers, towels, flashlights, egg shelters (raffia string and water hyacinth), millimeter blocks, catheter hoses, lamps, container boxes, weights, scissors, scoops, packing plastic, rubber bands, buckets, nets, scales, petri dishes, and microscopes.

The materials used included koi broodstock, artificial feed, natural feed (Artemia sp. and Moina sp.), salt, methylene blue, water, Ovaprim hormone, phenoxyethanol, organic fertilizer (compost), and rice husks.

2.3 Research Procedure

Spawning

Broodstock Selection:
Koi broodstock was selected from Parung with a male-to-female ratio of 4:1.

Hormone Injection:
Female and male broodstock were injected with Ovaprim hormone at a dose of 0.3 ml/kg body weight for males and 0.2 ml/kg body weight for females. The injection was administered at 16:00 with a latency period of 8-12 hours.

Spawning Process:
Spawning took place in a single tank with fecundity of 34,000 eggs, a fertilization rate of 27,642 eggs, and a hatching rate of 16,538 larvae.

Larval Stocking Density Treatment:
A total of 4,500 larvae were used. Stocking density treatments were 250, 500, and 750 larvae per aquarium, with three replicates for each treatment.

Larval Rearing

Larval Placement:
Larvae were placed in aquariums measuring 40 x 60 x 30 cm with a water volume of 48 liters.
Feeding:

Age 0-14 days: Artemia sp. was provided at 3 liters per day, with a frequency of 600 ml every 2 hours (148,200 Artemia sp. per 600 ml).
Age 15-40 days: Moina sp. was provided at 25 grams per day, with a frequency of 5 grams every 2 hours.

Plotting and Sampling
Plotting:
Plotting was conducted using simple random sampling by drawing lots.

Data Collection Frequency:
Data were collected every 10 days.

Observed Parameters:

1) Larval Length: Measured daily using a millimeter block.
2) Larval Weight: Measured every 10 days using an analytical balance.
3) Larval Survival Rate: Calculated using the formula (Muchlisin et al., 2014):

$$SR = \frac{Nt}{N0} \times 100\%$$

Where:
SR = Survival rate (%)
Nt = Number of fish at time t (individuals)
N0 = Number of fish at the start of the study (individuals)

Data Analysis
The data were analyzed using ANOVA to test the differences between treatments. If significant differences were found, Tukey's test was performed to determine which treatments differed significantly.

3 Results

Table 1. Parameters of Absolute Length, Absolute Weight, and Survival Rate

Parameter	Treatment		
	250 larvae/aquarium	500 larvae/aquarium	750 larvae/aquarium
Absolute Length (cm)	1 ± 0.17[a]	0.87 ± 0.08 [a]	1.17 ± 0.34 [a]
Absolute Weight (grams)	0.44 ± 0.16 [a]	0.33 ± 0.15 [a]	0.52 ± 0.29 [a]
Survival Rate	30.13 ± 5.62[b]	13.33 ± 5.99[a]	6.94 ± 3.40[a]

Note: The same superscript letters in the same row indicate no significant difference (P>0.05).

3.1 Length Growth

The maintenance of koi fish during the study lasted for 40 days, and measurements of absolute length growth and variance analysis showed that the stocking density treatment had no significant effect (P>0.05) on the absolute length parameter of koi fish. The length growth of koi fish over the 40-day study period (Figure 1) showed an increase in length for each treatment. The increase in length continued steadily over the maintenance period, indicating that there was length growth in koi fish kept at different stocking densities with the same amount of feed. Length growth can be seen in Figure 1. In this study, the highest daily length growth rate in koi fish was found in treatment A, which had the lowest stocking density of 3 fish per liter, reaching 2.85% per day.

This differs from a study by Putri et al. (2023), which stated that high stocking density had a significant impact on fish growth rate. This is due to competition for space, feed utilization, and dissolved oxygen among the fish.

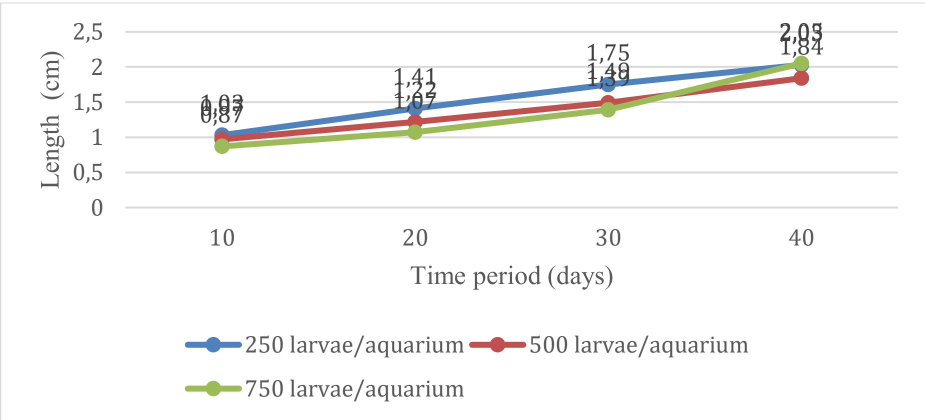

Fig. 1. Length Growth of Koi Larvae

3..2 Weight Growth

According to the research results, the measurement of the average absolute weight of koi larvae (*Cyprinus rubrofuscus*) during the 40-day maintenance period by treatment is shown in Figure 2 below.

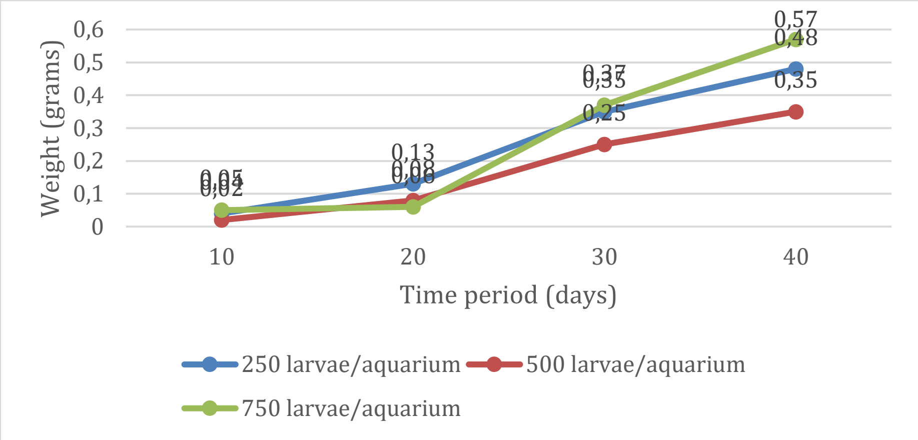

Fig. 2. Weight Growth of Koi Larvae

Different stocking density treatments with the same amount of feed on the growth and survival of koi larvae (Cyprinus rubrofuscus) showed varying results in terms of average absolute weight. The highest absolute weight was shown by the green line on the graph (750 fish/aquarium), which was 0.57 g, and the lowest absolute weight was shown by the orange line (500 fish/aquarium), with an average absolute weight of 0.35 g. Based on the graph, it can be concluded that stocking density did not significantly affect the absolute weight of koi fish.

3.3 Survival Rate (SR)

The survival rate (SR) of fish refers to the percentage of fish that remain alive at a certain time compared to the initial number of fish at the start of the maintenance period. Survival is closely related to mortality, which is the death occurring within a population of organisms, leading to a decrease in the number of individuals. Survival is measured as the percentage of the organism population that survives during a given maintenance period. The survival rate is expressed as a percentage of the organisms alive at the beginning and end of the study and is the inverse of the mortality rate. Fish tend to experience higher mortality rates when subjected to stress, especially due to lack of food and poor environmental conditions, making them more susceptible to disease (Muhar, 2022).

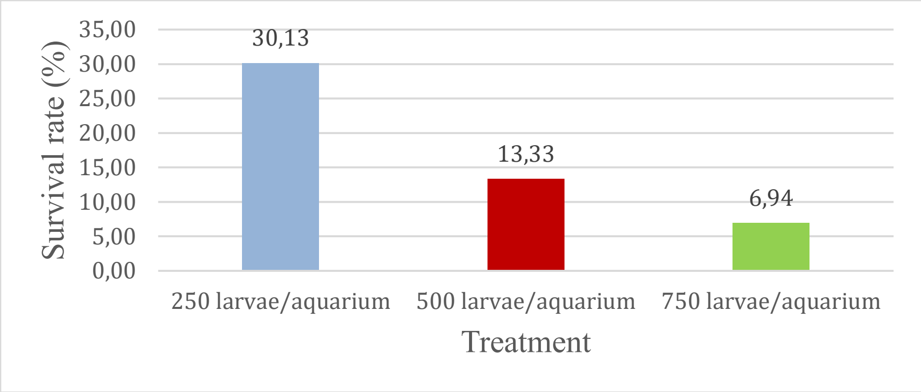

Fig. 3. Survival Rate of Koi Larvae

Based on the survival rate graph in Figure 3, it is shown that each aquarium treatment had different survival rates. The first treatment, with 250 larvae per aquarium, had a survival rate of 30.13%, the second treatment, with 500 larvae per aquarium, had a survival rate of 13.33%, and the third treatment, with 750 larvae per aquarium, had a survival rate of 6.94%.

4 Discussion

Based on observations conducted over 40 days, the survival rate showed a significant difference due to the influence of different stocking densities with the same amount of feed. After analysis of variance (ANOVA), it was found that the survival rate showed a significant difference due to the influence of different stocking densities with the same amount of feed ($p<0.05$). Therefore, the Tukey post-hoc test was conducted. The results showed that the optimal survival rate occurred in the first treatment, with 250 fish larvae per aquarium, supported by low stocking density, allowing optimal fish growth with a high survival rate. This contrasts with the second and third treatments, which resulted in low survival rates due to high stocking densities, affecting competition for food, oxygen availability (competition for oxygen in a limited medium), space for movement, and metabolic byproducts (CO2, feces) that can affect water quality. Fish mortality also impacts water quality degradation, leading to more polluted water as more fish die. This is consistent with Mutia et al. (2020), who stated that an increase in population density leads to increased ammonia and carbon dioxide concentrations in the living medium. This condition causes stress in koi fish and increases mortality rates. This finding is also in line with Satyani (2001), who argued that deteriorating environmental conditions can disrupt the normal functioning of fish, ultimately becoming a factor that contributes to high mortality rates.

In addition, the low survival rate in high stocking densities is suspected to result from larval death due to insufficient feed. This is supported by Maiyulianti (2017) in Pratama et al. (2021), who stated that during the larval stage, fish require ample space for movement and more food, as larvae tend to be highly active. Furthermore, the absence of competition for oxygen and food allows for optimal growth and a high survival rate.

Under high stocking density conditions, fish larvae face difficulties utilizing feed optimally due to limited movement space, high competition for food, and low oxygen levels, leading to increased larval mortality. The higher the stocking density, the lower the larval survival rate, and vice versa. Fish survival depends greatly on the fish's ability to adapt to food, health status, stocking density, and water quality, which supports growth. Providing the same amount of feed causes competition among larval individuals for food. The larvae that successfully obtain food will survive, while those that do not will starve, leading to death. Wijayanti (2010) stated that mortality can occur due to prolonged starvation, caused by an inability to meet the energy needs for growth and mobility due to inadequate nutritional content in the feed as an energy source. Starving fish larvae can be observed from their behavior. Hungry fish will eat other larvae that have already died to meet their nutritional needs.

Regarding absolute length and weight parameters, the calculation results showed no significant difference in the stocking density treatments. High stocking density can increase fish weight due to various factors. According to Rauw (2017), fish in high stocking density conditions show a greater coefficient of variation in body weight, indicating that growth and feed intake are influenced by dominance relationships and competition for food. Additionally, Kapinga (2014) stated that high stocking density can cause stress and competition for food and living space, affecting fish growth performance. Furthermore, Lee (2022) added that high stocking density significantly affects the growth characteristics and stress levels of fish. This statement is also supported by Costas (2007), who found that high stocking density causes stress due to crowding, affecting amino acid metabolism and energy requirements. Based on these explanations, all of these suggest that high stocking density can increase fish weight due to stress and competition for resources, which can trigger compensatory growth and feed efficiency.

For the specific growth rate of fish, the ANOVA calculation showed that the specific growth rate of fish did not differ significantly between different stocking density groups. This differs from previous research by Amin (2023), which found that the specific growth rate in Cyprinus rubrofuscus fish showed a significant difference. It is suspected that this is due to complex interactions between environmental factors such as water quality, temperature, and other conditions that can affect the overall growth rate of fish. The effects of stocking density and feeding may be offset by other factors, resulting in no significant difference (Ritonga, 2020).

5 Conclusions

This study demonstrates that variations in stocking density with the same amount of feed have a highly significant effect on the survival rate of koi larvae. However, variations in stocking density did not significantly affect the growth in length and weight of koi larvae. The treatment with a stocking density of 250 fish per aquarium resulted in the highest survival rate. Therefore, the lower the stocking density, the higher the survival rate of koi larvae.

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