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The Effect of Different Stock Densities on the Growth of Bristlenose Pleco, *Ancistrus multispinis* (Regan, 1912) Fry

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ABSTRACT

This study examined the growth performance of bristlenose pleco (*Ancistrus multispinis*) fry raised at five different stocking densities. For this purpose, five experimental groups were formed, each consisting of three replicates: 1 fry/L (d1), 2 fry/L (d2), 4 fry/L (d4), 6 fry/L (d6), and 8 fry/L (d8). The experiment used fry produced in the laboratory, with average initial live weights of 0.0275 ± 0.004 g and average total lengths of 1.46 ± 0.049 cm. The study lasted a total of 90 days. At the end of the experiment, the number of live fry in each group was recorded, and weight and length measurements were taken. According to these measurements, the average length of the fry in the 1 fry/L group (d1) was 3.1 cm, and the average weight was 0.321 g, while in the 8 fry/L group (d8), the length was found to be 2.8 cm, and the weight was 0.249 g. The size differences between groups were examined, and it was found that the d1 group showed statistically significant differences when compared to the other groups. The data obtained indicate that fry of *A. multispinis* grew more in lower stocking densities, such as 1 fry/L, compared to other groups. Based on these results, it can be said that utilizing lower stocking densities, such as 1 fry/L, is advantageous in the growth of *A. multispinis* fry.

KEYWORDS: *Ancistrus*, stock density, growth, aquarium.

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1. Introduction

Stock density is one of the critical parameters in aquaculture and directly affects the growth performance, health, welfare, and economic sustainability of fish production (Liu et al., 2014; Abe et al., 2019; Liu et al., 2021). Particularly in intensive farming systems, determining and implementing optimal stock density is vital. Inappropriate high stock densities can adversely affect growth, feed intake, feed conversion ratio, behavior, and health (Ellis et al., 2002; Ashley, 2007). High stock density can exert pressure on the living space of fish and water quality, thereby increasing stress levels. This condition elevates cortisol levels in fish, suppressing the growth hormone (GH) and insulin-like growth factor-I (IGF-I) axis (Liu et al., 2013). Furthermore, high stock density increases the formation of reactive oxygen species (ROS), leading to oxidative stress (Braun et al., 2010). In stressed fish, blood glucose is considered a reliable indicator of stress, while serum protein levels reflect the metabolic, nutritional, and immune status of the fish (Ruane et al., 2001; Ni et al., 2014; Tahmasebi-Kohyani et al., 2012). For these reasons, stock density must be well-regulated in aquaculture. Optimal stock density varies by fish species. For instance, it has been reported that stock densities exceeding 50 kg/m^3 in Atlantic salmon farming lead to deterioration in water quality (Liu et al., 2017). In some species, fish larvae raised at high stock densities have shown increased cannibalism, reduced feed intake, and decreased growth performance (de Barros et al., 2019; Santos et al., 2020).

While low stock density can lead to inefficiencies in space utilization, high stock density can result in production losses and economic damage. Therefore, it is essential to determine and implement optimal stock density specific to each species for sustainable aquaculture (Can et al., 2023). This approach allows for the optimization of both the economic sustainability of production and the welfare of the fish (Refaey et al., 2018; Lupatsch et al., 2010).

This study investigates the growth performance of juvenile bristlenose pleco (*A. multispinis*), a popular aquarium fish species with high economic value, at different stock densities.

2. Materials and Methods

In this study, the broodstock fish available in our laboratory were used to obtain juveniles. Our laboratory infrastructure is equipped with all the machinery and equipment necessary to produce this species until they reach broodstock size. At the beginning of the study, the juveniles to be used in the experiment were produced. After successfully completing the production process, newly hatched juveniles were kept in larval rearing tanks (40 cm X 30 cm X 40 cm) until their yolk sacs were depleted and they began to consume artificial micro-particle feed. Approximately on days 30-35 post-hatching, the total lengths and weights of the juveniles were measured, and they were transferred to experimental tanks of 5 liters each. The experiment lasted for 90 days.

The research was conducted in the following general order:

- Enough juveniles were obtained from the broodstock.
- Experimental setups were established.
- Live weight and length measurements of the juveniles were taken at the beginning of the experiment.
- No anesthetic was applied during the measurements.
- The experiments were initiated.

- Three replicates were created for each group.
- At the end of the experiment, length and weight measurements were taken.
- The weight and length data obtained at the end of the experiment, along with survival rates, were statistically compared and analyzed.
- For statistical analyses, after checking the assumptions for differences in length among the experiments, the Kruskal-Wallis test was conducted due to the failure to meet the assumptions. The Dunn test was performed to identify which experiments showed differences.
- All groups were fed with the same brand of commercial fish feed (0.5-0.8 microns, ALLTECH) in the same amounts and number of feedings. At the end of the experiment, the survival and growth rates among the groups were compared. Water quality conditions were maintained within similar ranges across all groups.

This study was designed to determine the effect of stock density on growth; the number of individuals stocked in each group varied. The stock densities and live counts applied in the experimental groups are as follows:

- In Group 1 (d1), 1 juvenile was stocked per liter, totaling 15 juveniles, with 5 juveniles used in each replicate tank.
- In Group 2 (d2), 2 juveniles were stocked per liter, totaling 30 juveniles, with 10 juveniles used in each replicate tank.
- In Group 3 (d4), 4 juveniles were stocked per liter, totaling 60 juveniles, with 20 juveniles used in each replicate tank.
- In Group 4 (d6), 6 juveniles were stocked per liter, totaling 90 juveniles, with 30 juveniles used in each replicate tank.
- In Group 5 (d8), 8 juveniles were stocked per liter, totaling 120 juveniles, with 40 juveniles used in each replicate tank.

Each of the replicate tanks used in the experiment had a water capacity of 5 liters. A total of 0.02 grams of feed per fish per liter was provided, which amounts to a total of 0.1 grams of feed per meal for 5 fish in 5 liters.

3. Results and Discussion

In the experiment testing the effects of stock density on the growth and survival rates of juvenile bristlenose pleco, the stock densities were applied as shown in the table below. The average total length of the juveniles at the beginning of the experiment was measured at 1.46 ± 0.049 cm, and the average live weight was 0.0275 ± 0.004 g.

In the dataset testing the effect of stock density on juvenile growth, five experiments were established based on the number of juveniles (Table 1). Descriptive statistics and graphs for total length (cm) and live weight (g) were obtained from the collected data, and the Kruskal-Wallis test was conducted to assess differences among the experiments (Table 2, Table 3).

To examine whether there were differences in length among the experiments, the assumptions were checked for compliance, and due to the failure to meet these assumptions, the Kruskal-Wallis test was conducted. According to the test results, the null hypothesis that there is no difference among the experiments was rejected, and the differences among the experiments were statistically significant ($p < 0.05$). The Dunn test was performed to identify which experiments showed differences. The Kruskal-Wallis chi-squared = 18.321, df = 4, p-value = 0.001068.

Based on the results from the Dunn test, the differences between Experiment 1 (d1) and Experiments 4 (d4), 6 (d6), and 8 (d8) were statistically significant.

Table 1. Descriptions of the groups and the number of juveniles in the stock density experiment.

Experimental Groups	**Stock Density** (juvenile /L)	**Number of Juveniles in Each Replicate Tank (fish / 5L)**	**Total Number of Juveniles Used in Each Group (individuals) (= 3 replicates)**
d1	1	5	15
d2	2	10	30
d4	4	20	60
d6	8	30	90
d8	10	40	120

Table 2. Descriptive statistics of the end-of-experiment data for total length (cm) across groups.

	Groups	**n**	**min**	**max**	**median**	**iqr**	**mean**	**sd**	**se**	**ci**
1	d1	14	2,8	3,5	3,1	0,2	3,129	0,202	0,054	0,116
2	d2	30	2,1	4	3	0,5	2,973	0,383	0,07	0,143
3	d4	57	2,4	3,4	2,8	0,4	2,816	0,249	0,033	0,066
4	d6	82	2,2	3,5	2,9	0,3	2,859	0,255	0,028	0,056
5	d8	117	2,1	3,7	2,8	0,4	2,834	0,305	0,028	0,056

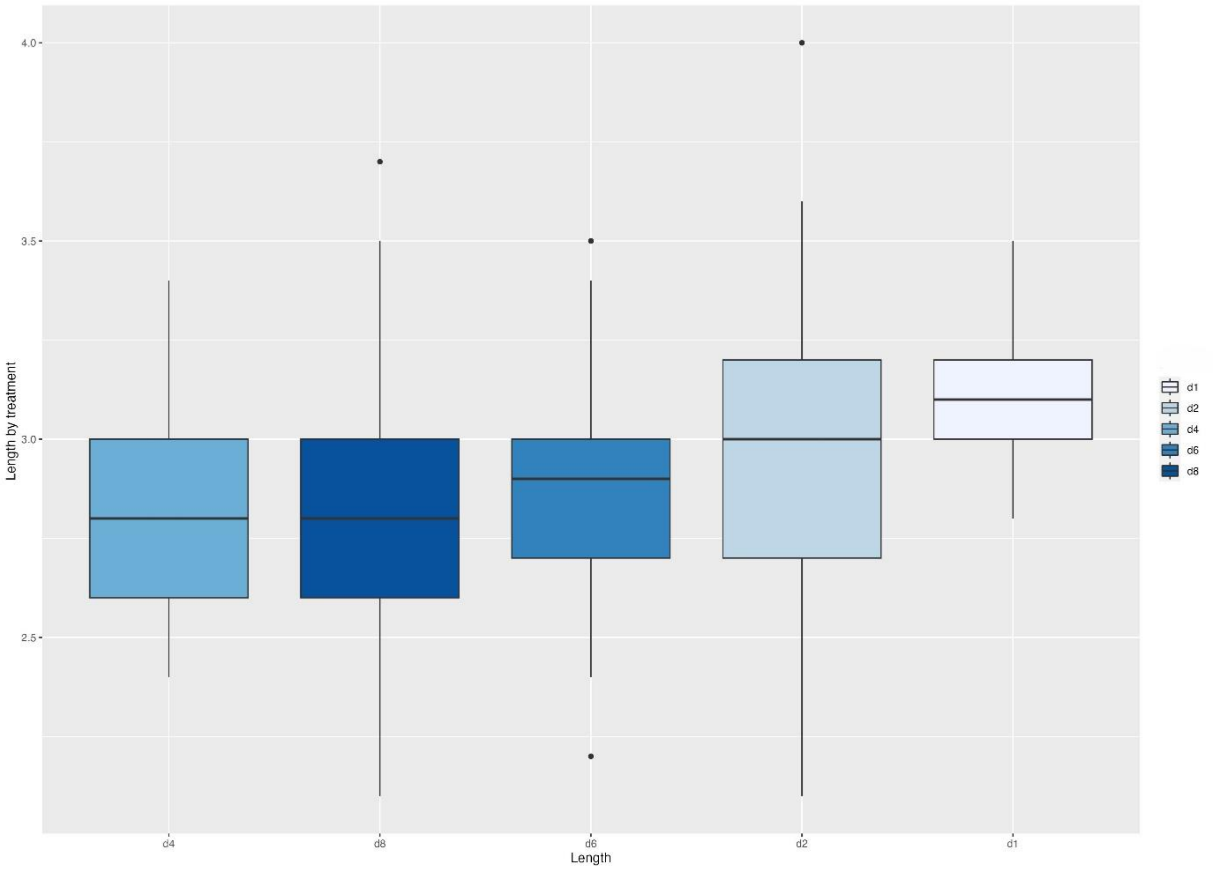

Figure 1. Differences in total length (cm) among groups at the end of the experiment.

To examine whether there were differences in weight among the experiments, the assumptions were checked for compliance, and due to the failure to meet these assumptions, the Kruskal-Wallis test was conducted (Table 3, Figure 2). According to the results of the Kruskal-Wallis test, the null hypothesis that there is no difference among the experiments was rejected, and the differences among the experiments were statistically significant ($p < 0.05$). The Dunn test was performed to identify which experiments showed differences. The Kruskal-Wallis chi-squared = 20.157, df = 4, p-value = 0.0004649.

Based on the results from the Dunn test, the differences between d1 and d4, as well as between d6 and d8, were statistically significant. The differences between d2 and d1, d6, and d8 were not significant. Differences were observed in the pairwise comparisons.

Additionally, a regression model was conducted to analyze the relationship between weight and length (Figure 3). The logarithm of weight and length was taken to perform the regression analysis. Below are the scatter plots for the weight-length relationship for this experiment.

To interpret the scatter plots of the weight-length graphs in more detail, it is important to examine the data distribution, trends, and possible outliers in each graph.

In the d1 graph, a clear positive relationship between weight and length is observed. As length increases, weight also appears to increase. The data generally exhibit a normal distribution, although some points may be more distant than others. Outliers may represent a few data points with lengths significantly higher than normal but with low weights.

In the d2 graph, there is a strong positive relationship between length and weight. It is understood that longer individuals are generally heavier. The data align well with a linear model overall. However, some points distinctly fall outside the distribution. Outliers may represent juvenile individuals that fall within a certain length range but have unexpectedly low weights.

In the d4 graph, a positive relationship between weight and length is observed, but there is more dispersion. This suggests that some individuals may be lighter or heavier than expected. The data are spread over a wider range, which may reflect a mixture of different samples. Outliers may represent a few data points with lengths significantly higher than normal but with low weights.

Table 3. Descriptive statistics of the end-of-experiment data for live weight (g) across groups.

Groups	n	min	max	median	iqr	mean	sd	se	ci
d1	14	0,21	0,44	0,315	0,088	0,321	0,062	0,017	0,036
d2	30	0,13	0,58	0,29	0,14	0,301	0,102	0,019	0,038
d4	57	0,13	0,39	0,23	0,08	0,243	0,061	0,008	0,016
d6	82	0,11	0,45	0,25	0,075	0,255	0,065	0,007	0,014
d8	117	0,07	0,49	0,24	0,12	0,249	0,078	0,007	0,014

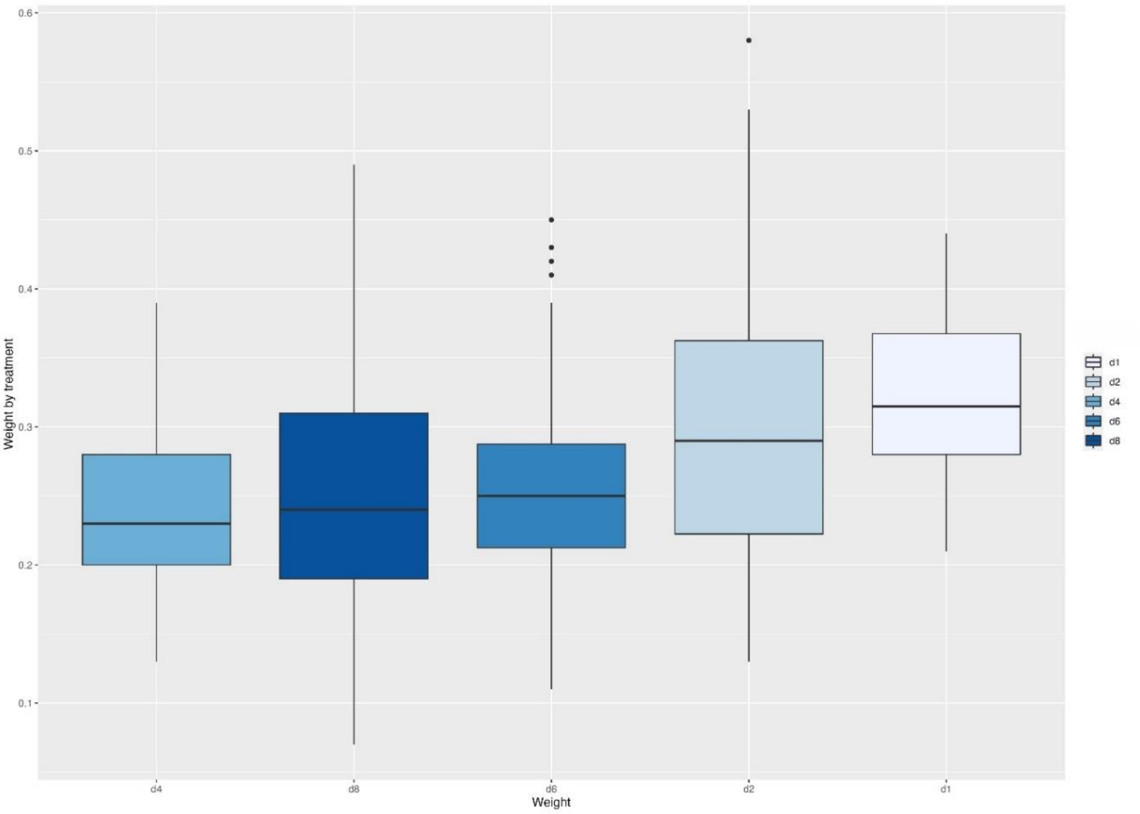

Figure 2. Differences in live weight (g) among groups at the end of the experiment.

In the d6 graph, there is again a positive relationship between weight and length. However, some points are noticeably distant from the others. Although the data generally align with a linear model, some points distinctly fall outside the distribution. Outliers may represent individuals within a certain length range but with unexpectedly low weights.

In the d8 graph, a strong positive relationship between weight and length is observed. As length increases, weight also appears to increase. The data generally exhibit a normal distribution, although some points may be more distant than others. Outliers may represent a few data points with lengths significantly higher than normal but with low weights.

Overall, a positive relationship between length and weight is observed in all graphs. Outliers may reflect abnormal conditions of specific samples. The normality of the distribution indicates the homogeneity or heterogeneity of the dataset. A heterogeneous distribution may reflect a mixture of different samples.

In Figure 4, five different scatter plots illustrate the relationships between "logL" (the logarithm of length) and "logW" (the logarithm of weight).

In the d1 graph, a weak relationship is observed; the data points are scattered and do not show a clear linear trend. Some points in this graph fall outside the general distribution, indicating the presence of outliers.

In the d2 graph, a strong positive relationship is evident; the data points exhibit a distinct linear trend, and there is an overall normal distribution, clearly illustrating the relationship between length and weight.

In the d4 graph, a positive relationship is observed, but there is more dispersion compared to the d2 graph. Some points in this graph also fall outside the general distribution, which may reflect abnormal conditions for certain species.

In the d6 graph, a strong positive relationship is present; the data points show a clear linear trend, and outliers are less pronounced, indicating that the dataset is more homogeneous.

In the d8 graph, a strong positive relationship between logL and logW is observed; the data points exhibit a distinct linear trend and generally show a normal structure. Outliers are less pronounced compared to other graphs.

Overall, strong positive relationships between length and weight are observed in the d2 and d6 graphs. The d1 and d4 graphs show more dispersion and outliers, while the d8 graph presents a more stable relationship due to the logarithmic transformation.

When examining the box plot comparing the lengths of juvenile bristlenose pleco (*A. multispinis*) across different experimental groups (d1, d2, d4, d6, d8) (

Figure **1**), the median line within the boxes for the d1 and d2 groups indicates that the lengths of juveniles in these groups are more homogeneous. This suggests that the growth conditions for juveniles in these groups are similar, resulting in comparable growth performance.

The d4 and d8 groups have higher median length values. Notably, the median of the d8 group is significantly higher than that of the other groups, indicating that the juveniles in these groups are generally longer and have better growth conditions.

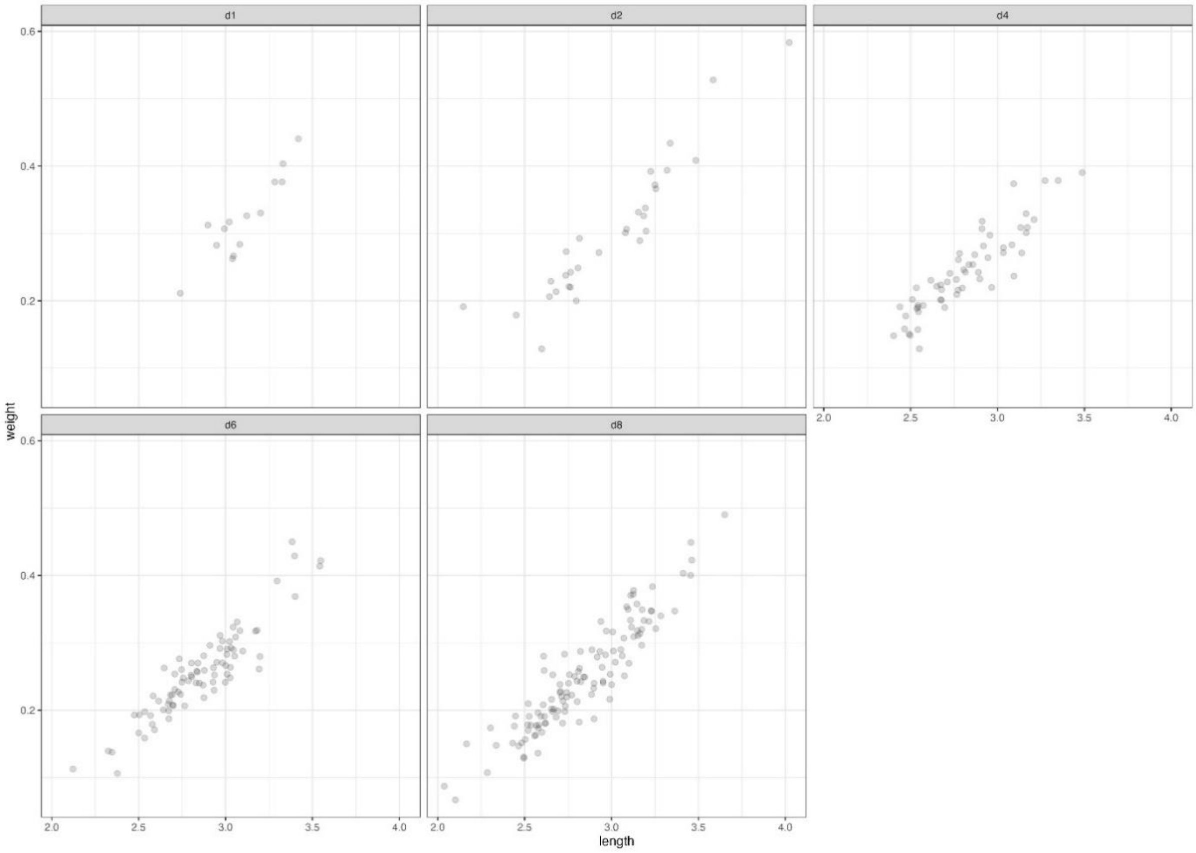

Figure 3. Scatter plots of weight-length relationships from the experiments conducted to test the effect of stock density on growth.

The d6 group has the lowest median length value, suggesting that the growth performance of juveniles in this group is weaker compared to the other groups and that they may have been exposed to potentially adverse environmental or nutritional conditions.

The boxes for the d1 and d2 groups have a narrower range, indicating that the lengths of juveniles in these groups exhibit less variability. This may suggest that the growth conditions for juveniles in these groups are more stable and that they face fewer stress factors.

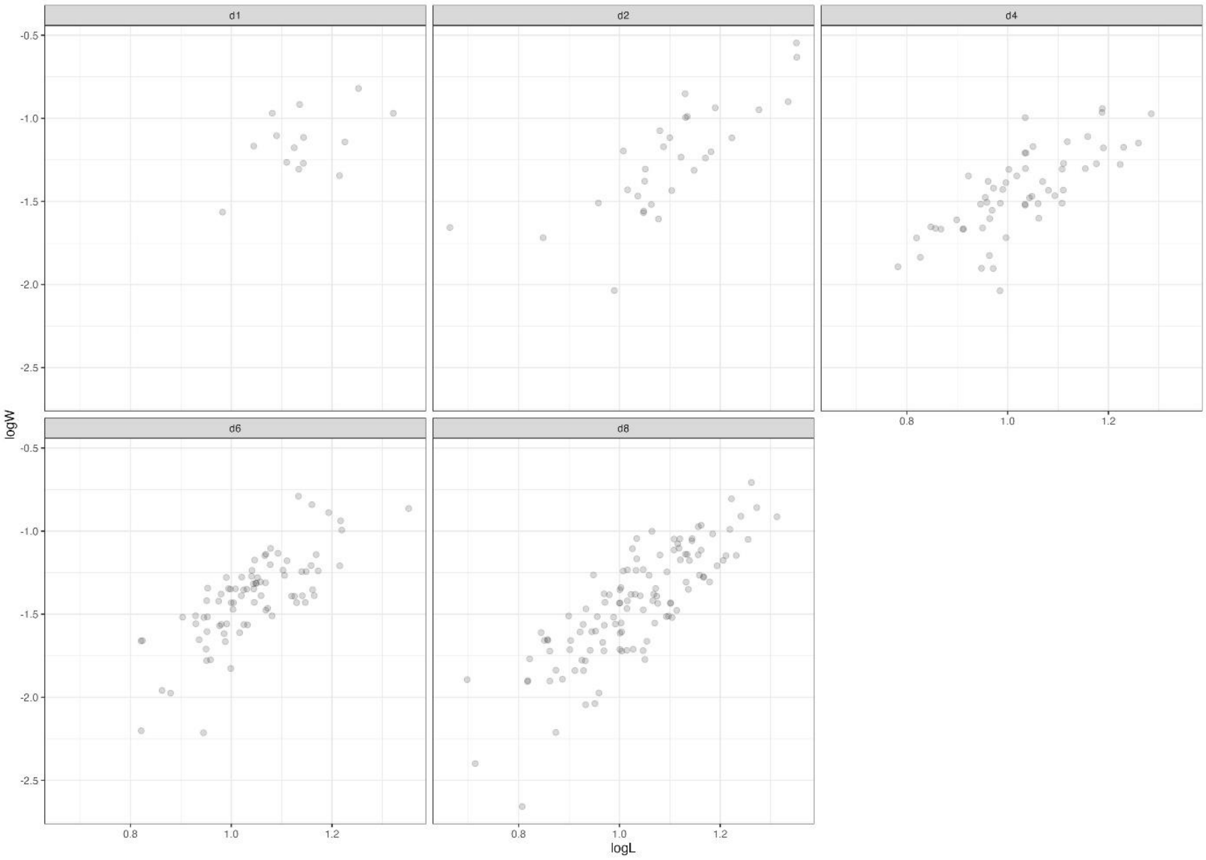

Figure 4. Scatter plots showing the relationship between the logarithm of length ("logL") and the logarithm of weight ("logW") at the end of the experiment across groups.

The d4 and d8 groups have a wider range of lengths, indicating greater variability among the lengths of juveniles. This may suggest that juveniles in these groups have different growth rates and may have been exposed to varying nutritional or environmental conditions.

In the d6 group, one or more outliers are observed. These outliers indicate that some juveniles in this group are significantly shorter than expected. This may suggest that the health or growth conditions of these juveniles are more unfavorable compared to others. The presence of outliers could negatively impact the overall growth performance of this group and is a situation that producers should be aware of.

This graph (

Figure **1**) clearly illustrates how the lengths of juvenile bristlenose pleco vary across different experimental groups. Notably, the d4 and d8 groups have longer juveniles, while the d6 group exhibits a shorter and more variable length distribution.

These findings provide important insights into understanding the effects of different stock densities on juvenile length.

As clearly seen in the graph (

Figure **1**), it can be understood that the fish in the d1 group, stocked at 1 fish per liter, and the fish in the d2 group, stocked at 2 fish per liter, may differ in total length from the other groups. It is observed that there are no differences among the other groups (d4, d6, and d8). This suggests that using stocking rates of 1 fish per liter or 2 fish per liter for juvenile bristlenose pleco may be somewhat more advantageous. However, this situation will ultimately be determined by analyzing multiple parameters, considering operational cost calculations in intensive production tanks and the specific conditions of the operation.

This study investigates the effects of different stock density ratios on the growth of juvenile bristlenose pleco (*Ancistrus multispinis*). The findings indicate that existing aquaculture practices need to be reviewed and improved. The research results reveal that stock density has a significant impact on the growth of juvenile bristlenose pleco. It was observed that lower stock densities enhance the growth performance of the fish without negatively affecting survival rates. This finding is supported by the work of Bolasina et al. (2006), which states that lower stock density increases growth performance by reducing stress levels. It is known that high stock density increases competition among fish, leading to a decrease in food resources and an increase in stress levels. A study by Karakatsouli et al. (2008) indicated that high stock density negatively affects the body moisture content of fish and results in adverse outcomes for growth. Therefore, determining the optimal stock density in bristlenose pleco aquaculture is critical for economic efficiency.

Determining the optimal stock density can affect not only growth performance but also the overall health of the fish. It should be noted that fish raised at high densities are more susceptible to diseases, which can lead to economic losses for producers. For example, the increase in stress-related diseases in fish raised at high densities can raise costs for producers (Jorgensen et al., 1993; Lambert and Dutil, 2001). Stock density is a critical parameter that significantly impacts the growth performance, health, and welfare of fish in aquaculture. As one of the many parameters to consider in fish farming, stock density directly affects the availability of food resources and water quality. Therefore, this study investigates the growth performance of juvenile bristlenose pleco, a commercially valuable aquarium fish species, at different stock densities. High stock density can increase social interactions and competition among fish, leading to elevated stress levels. Stress negatively affects the growth hormones and insulin-like growth factors in fish, adversely impacting their growth performance (Liu et al., 2017). For instance, a study conducted on goldfish (*Carassius auratus*) (Niazie et al., 2013) examined the growth and survival rates of fish stocked at densities of 6, 9, 12, and 15 fish per aquarium. Another study on guppies (*Poecilia reticulata*) reported that high stock density negatively affected growth performance (Khan et al., 2022). As a result, it was found that increasing stock density adversely affected the growth indices of fish, although survival rates did not show significant differences among different stock densities. Similar results were observed in our study, indicating that the increased stress levels due to social interactions and competition among fish held at high densities negatively affected their growth performance.

The effects of stock density vary among species (Foster and Vincent 2004). For example, a study on *Siganus rivulatus* found that experiments conducted at densities of 10, 20, 30, and 40 fish per aquarium showed no negative effects of high stock density on growth and survival rates (Saoud et al., 2008). This suggests that the social structure and behavior of this species may reduce competition even at high densities. The effects of stock density on fish growth performance are also related to stress and energy requirements (High density increases the energy requirements of fish, which can negatively impact growth and feed utilization (Leatherland and Cho, 1985). Additionally, high stock density can lead to the deterioration of water quality and the accumulation of metabolic waste, adversely affecting fish growth performance (Braun et al., 2010). Regular monitoring of water quality and the implementation of appropriate water change rates can positively influence the health and growth of fish (Ebeling and Timmons, 2012). For example, a study by Santos et al. (2020) observed that increasing water change rates improved water quality, thereby maintaining fish health and enhancing growth performance. Similarly, a study conducted on goldfish (Shete et al., 2013) reported that low stock density potentially allowed for improved water quality. The effects of stock density also vary according to the age groups of the fish. Larval stages are more sensitive to high stock density, which can lead to negative outcomes such as cannibalism, reduced feed intake, and decreased growth performance (de Barros et al., 2019; Santos et al., 2020). Therefore, determining the optimal stock density should take into account the ecological characteristics and life strategies of the species.

In this study with juvenile bristlenose pleco, it was observed that the juveniles grew better at low stock density (1 juvenile/L). This finding is consistent with studies on goldfish (*Carassius auratus*) and guppies (*Poecilia reticulata*) (Khan et al., 2022). Additionally, it is known that social behaviors also influence growth, alongside stock density. The social hierarchy and interactions among fish become particularly pronounced at high densities. For example, a study on clownfish (*Amphiprion percula*) found that changes in social interactions under high stock density conditions negatively affected growth performance. The effects of stock density vary according to the age groups of the fish. Larval stages are more sensitive to high stock density, which can lead to cannibalism, reduced feed intake, and consequently affect growth performance (de Barros et al., 2019; Santos et al., 2020). Studies have shown that determining the optimal stock density, especially at this stage, is vital. In this study, experiments were conducted during the early juvenile period to determine the optimal stock density for the bristlenose pleco species. From this perspective, it is evident that a method supported by scientific studies was applied.

The effects of stock density can also have significant implications for the overall health of the fish; it should be noted that fish raised at high densities are more susceptible to diseases, which can lead to economic losses for producers. In particular, the stress conditions caused by high stock density weaken the fish's immune system, leading to increased susceptibility to diseases (Björnsson et al., 2006). Therefore, considering these factors, it is essential to regularly monitor and improve water quality. If appropriate water quality cannot be maintained, aquaculture practices conducted at high densities can jeopardize fish health and increase production costs.

Findings on how to optimize species-specific stock density in fish farming are also important for future research. As demonstrated in this study, low stock density practices provide better growth conditions for fish. However, attention must also be paid to other factors such as water quality management, feed quality, feeding frequency, and water change strategies, in addition to stock density. For example, a study on goldfish showed that the combination of low stock density and highwater change rates improved growth performance. Furthermore, considering stock density not only as an economic factor but also acknowledging its environmental impacts is crucial for sustainable aquaculture.

In Türkiye, it appears that traditional methods commonly used in bristlenose pleco farming are not supported by modern scientific findings. It seems that current producers are hesitant to transition to new methods based on their past experiences. However, this study emphasizes the importance of implementing new methods. Optimizing stock density ratios can enhance production efficiency and economic returns. Recent research findings clearly indicate that stock density has multifaceted effects on fish species. For instance, studies on discus fish (*Symphysodon aequifasciatus*) have shown that high stock density negatively affects growth performance and leads to changes in social behavior (Chong et al., 2002). It has also been highlighted that high stock density should be considered, especially in conjunction with water change rates.

4. Conclusion

In conclusion, stock density is a multidimensional parameter that must be considered in fish farming, yielding significant biological and economic implications. Low stock density is generally associated with better growth performance, while high stock density can lead to stress and growth pressures, resulting in negative outcomes. Therefore, determining the optimal stock density in aquaculture is critical for both fish welfare and economic sustainability. Future research should provide more data on optimal stock density for different species, and this data should contribute to the development of sustainable aquaculture practices.

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Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All procedures involving fish were approved by the Local Ethics Committee for Animal Experiments of Çanakkale Onsekiz Mart University (Approval date: 23.12.2022; Approval number: 2022/12-01), and were conducted in accordance with national and institutional guidelines for the care and use of animals.

Data availability
Not applicable.

Consent for publication
Not applicable.

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